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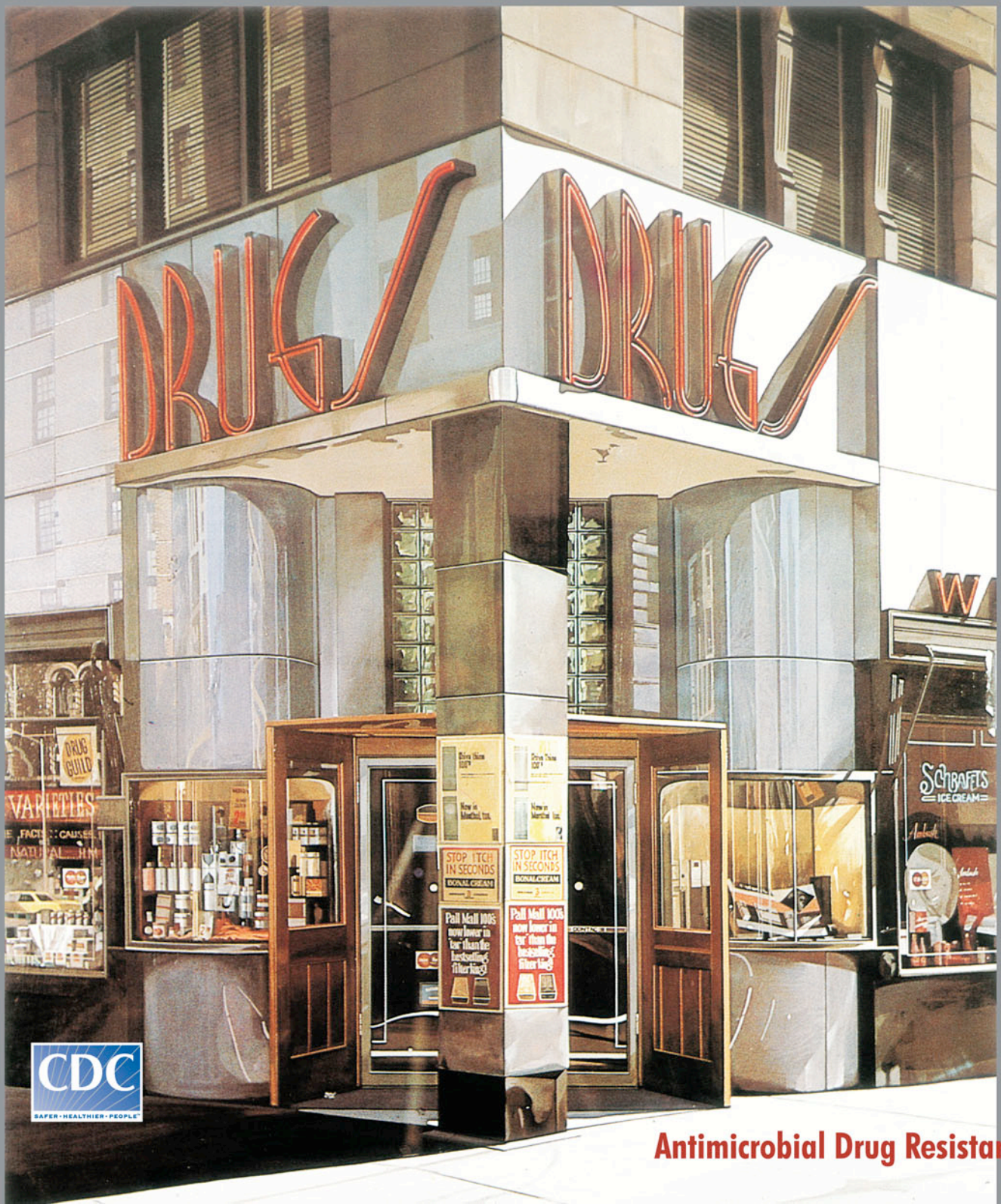

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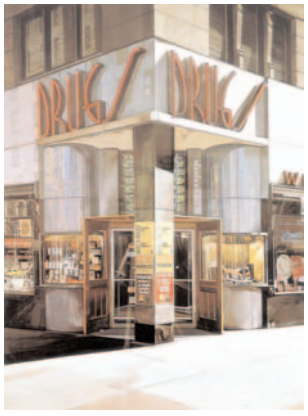

On the Cover

Richard Estes (b 1932)
DRUGS (1970)
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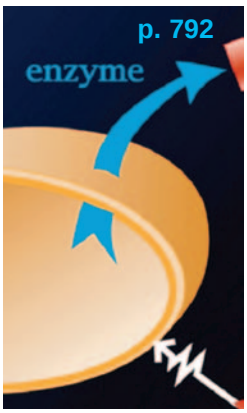

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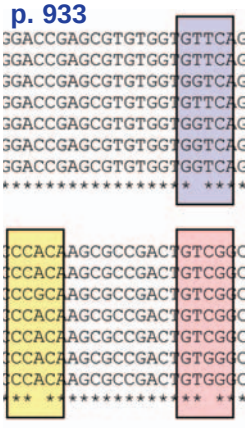
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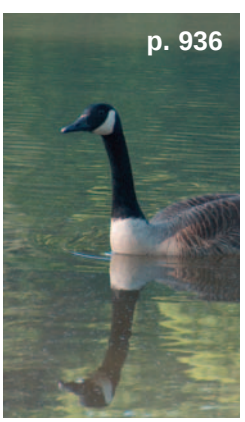
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News & Notes

An Emptying Quiver: Antimicrobial Drugs and Resistance

J. Todd Weber* and Patrice Courvalin†

Since the dawn of the antimicrobial drug era, resistance has shadowed the success of infectious disease therapy. In his 1945 Nobel Prize acceptance speech, Alexander Fleming noted the danger of resistance: "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body…. Moral: If you use penicillin, use enough" (1). Sixty years later, our understanding of resistance has grown vastly more sophisticated and the proliferation of new antimicrobial drugs has engendered an equally varied collection of resistance mechanisms (Figure). Resistance is now an important problem in virtually all areas of infectious diseases, including viral, bacterial, fungal, and parasitic diseases.

In a 2003 Institute of Medicine report, Microbial Threats to Health, antimicrobial resistance was noted as a paramount microbial threat of the twenty-first century (2). Some strains of pathogenic bacteria are now resistant to essentially all available antimicrobial drugs, and some remain susceptible to only one. At the same time, what once was an apparent deluge of antimicrobial drug development is now barely a trickle. The lack of new drug classes is a consequence of difficulties in discovery of new compounds that has persisted for many years. In addition, pharmaceutical companies are finding in industrialized nations more potent markets for other disease treatments and lower profit in nonindustrialized countries (3,4). This trend is reflected in the absence of any novel class of antibacterial drug approved for use in the United States between 1968 and 2000. Indeed, most of the new drugs approved since 1968 have been chemical modifications of existing drugs. However, since 2000, two new drug classes have been approved by the U.S. Food and Drug Administration (5,6). Whether this trend will continue is unclear and does not obviate the need for more new classes.

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Barring the arrival in the near future of new antimicrobial drugs that are effective against disparate organisms, we are left with imperfect tools to control drug resistance. With a notable exception, vaccines have not been produced that address the problem of resistance (7). Infection control in healthcare settings, which is essential for preventing transmission of susceptible and resistant microorganisms alike, remains imperfect. Reducing the discretionary use of antimicrobial drugs when possible is helpful, but even if we use these drugs with exquisite precision, resistance will continue to evolve and spread. Ensuring adherence to multidrug regimens to prevent emergence of resistance requires uninterrupted drug supplies and is vulnerable to human inconstancy. Finally, efforts to modify behavior

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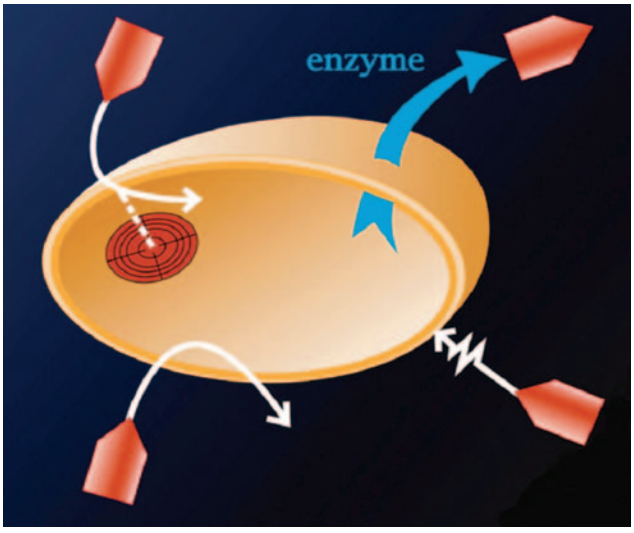

Figure. Schematic representation of mechanisms of resistance to antimicrobial agents.

(sexual and otherwise), will always have limitations in a free society. All of these shortcomings emphasize the critical role of research and dissemination of information. For that reason, this issue of Emerging Infectious Diseases is devoted to antimicrobial resistance and highlights both burgeoning and neglected areas.

Articles address antimicrobial resistance in pathogens from the community, healthcare settings, and agriculture, among children and adults, and in several countries. In the case of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA), articles cover outbreaks in Uruguay and in a US hospital nursery and maternity unit, emergence of a particular clone in Canada, prevalence in US emergency department patients, characteristics of patients admitted to a Swiss hospital, and the severity of this infection in pediatric patients. One article estimates hospitalizations associated with MRSA infection. These articles show some of the changing spectrum of disease and populations affected by this pathogen. The success of a relatively new vaccine against resistant *Streptococcus pneumoniae* and its impact on resistant infections is described. Resistance towards macrolides and structurally related drugs is the subject of several articles. Antimicrobial-resistant foodborne infections are addressed in articles on *Salmonella* spp. and *Campylobacter jejuni*. Several articles discuss resistant organisms that are largely problems in healthcare settings or among persons with underlying illness, such as extended-spectrum β-lactamase (ESBL) producing *Escherichia coli*, vancomycin-resistant *Enterococcus faecium*, and gram-negative bacilli. Multidrug-resistant tuberculosis, perennially transmitted inside and outside healthcare and other institutional settings, is also discussed in 2 articles. An article on *Trypanosoma brucei gambiense* describes the importance and difficulty in determining resistance in parasitic infections, which can have countrywide implications for treatment, control, and use of resources.

This issue does not cover resistance in malaria, gonorrhea, and HIV infection. An estimated 300–500 million clinical cases of malaria occur each year, and resistance exists to some extent to nearly all available antimalarial drugs. Efforts are under way to produce new drugs, but one of the most efficacious candidates has run into economic and manufacturing obstacles (8,9). Gonorrhea treatment and control are becoming increasingly difficult because of increases in resistance to multiple classes of drugs and the discontinued production of a preferred oral medication (10,11). Antiretroviral drug resistance has been well demonstrated in countries in which the standard of care mandates treatment of HIV-infected persons. The imminent widespread use of antiretroviral drugs in countries with large populations infected with HIV, but for whom treatment has not been provided, will carry with it the specter of global resistance, thereby threatening the intended benefits (12). Finally, in the absence of vaccine, the long-anticipated arrival of a global influenza pandemic, caused by H5N1 or another strain, would have potentially devastating consequences with respect to resistance. The potential for antiviral drug resistance to develop during treatment is manifest. The likely increased use of oral antibacterial drugs for patients with possible influenza and for prophylaxis and treatment of secondary bacterial pneumonia would enhance the existing evolutionary pressure toward resistance. The predictably large numbers of secondary bacterial pneumonias may be caused by established resistant pathogens (e.g., pneumococcus) and emerging ones (e.g., community-associated MRSA).

Fleming's warning about inappropriate use has resonance today. Several articles describe the importance of the appropriate use of antimicrobial drugs as well as the difficultly of enforcement. The hope of preserving the effectiveness of existing drugs through appropriate use as well as the urgent need for the development of new drugs are both represented by the artwork featured on the cover of this issue. We hope to promote greater awareness among our readers of the strong link between antimicrobial drug use and the development of resistance and to make clear that improving use in community, healthcare, and agriculture settings, combined with other strategies, is imperative if we are to confront effectively the further development and spread of antimicrobial resistance.

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Antimicrobial Resistance Determinants and Future Control

Stephan Harbarth* and Matthew H. Samore†

At the beginning of the 21st century, antimicrobial resistance is common, has developed against every class of antimicrobial drug, and appears to be spreading into new clinical niches. We describe determinants likely to influence the future epidemiology and health impact of antimicrobial-resistant infections. Understanding these factors will ultimately optimize preventive strategies for an unpredictable future.

> "Antibiotic therapy, if indiscriminately used, may turn out to be a medicinal flood that temporarily cleans and heals, but ultimately destroys life itself."
>
> Felix Marti-Ibanez, 1955

For more than 5 decades, the problem of how to contain antimicrobial resistance has preoccupied policy makers and members of the academic community. Nor is this preoccupation surprising, since antimicrobial resistance has become a public health concern throughout the world.

Pessimistic viewpoints about the low chances of success to stop the development of antimicrobial resistance have repeatedly been reported (1). The fundamental predicament is that antimicrobial drugs are a nonrenewable resource. Their duration of benefit and availability appears limited at the biological level, a constraint not seen with therapies for other disease conditions. As pointed out by the commentary in this issue of Emerging Infectious Diseases (2), the emergence of antimicrobial resistance is unavoidable from an evolutionary perspective. Moreover, for most microorganisms, it is unlikely that fitness costs of antimicrobial resistance will reduce their spread and clinical impact, since subsequent evolution commonly results in the amelioration of these costs (3).

This paradigm, which has been framed from a microbiologic perspective and could be summarized in the slogan "antibiotic therapy: use it and lose it," prompts questions about potential interventions that could slow down the dissemination of antimicrobial resistance and reduce its health impact in the next 2 decades. What will influence the demand and use of antimicrobial drugs in the near future? Which obstacles towards more judicious use and decreased transmission may get circumvented? How much will healthcare regulation affect antimicrobial resistance and our ability to control its spread? In short, we need to complement analysis of molecular biology with an examination of other determinants that are likely to influence the future epidemiology and health impact of antimicrobial-resistant, bacterial infections. That is the purpose of this article. For space reasons, we will not discuss the problem of viral, protozoal, or fungal resistance, and the controversial use of antimicrobial drugs in animal growth promotion, but certain analogies may be drawn from the ideas presented here.

Potential Determinants of the Future Dissemination and Control of Antimicrobial Resistance

Factors that drive uncertainty regarding the future dissemination and control of antimicrobial resistance are numerous and diverse. These determinants can be grouped into 4 categories (Table 1) (4,5). The first group is related to the molecular characteristics of pathogens, such as virulence, transmissibility, and survival fitness, which are issues beyond the scope of this article. Moreover, progress in microbiologic detection and identification of infectious pathogens is likely to influence diagnostic uncertainty and prescribing patterns of antimicrobial drugs. The second group of determinants is linked to prescribers of antimicrobial drugs, physicians, who may change their prescription patterns. Recent data from different parts of the world show promise in this area. The third group is related to characteristics of patient populations and host-related factors. Not only does this include infection rates and case-mix characteristics, but also consumer attitudes and global migration patterns. A fourth group of determinants is linked to macro-level factors related to the healthcare environment. These factors include regulatory policies that may influence use of antimicrobial drugs, infection control practices, technologic development, and drug discovery.

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Table 1. Potential determinants influencing future dissemination and control of antimicrobial resistance

Dimension	Determinant	Potential control measures and interventions
Pathogen and microbial ecology	Evolution	Evolutionary engineering
	Survival fitness	Inhibition of microbial gene expression
	Virulence	Antibodies, antipathogenicity drugs, biologic response modifiers
	Commensal flora	Probiotics
	Laboratory detection and identification	Improved rapid diagnostic tests
Physician's prescribing practice	Antimicrobial drug usage pattern	Multimodal interventions
	Diversity of antimicrobial drug prescribing	Decision support tools
	Training and knowledge	Academic detailing and educational campaigns
Population characteristics	Migration, travel, and globalization	Screening and improved surveillance
	Case mix and host susceptibility to infections	Immunization; better control of chronic diseases
	Antimicrobial demand and health beliefs	Public information campaigns
	Transmission and infection rates	Hand hygiene and barrier precautions
Politics and healthcare policy	Healthcare policy	Change in reimbursement patterns
	Promotional activities by industry	Regulation
	Technologic development	New prevention and treatment approaches

Diagnostic Uncertainty and Progress in Laboratory Detection

Diagnostic uncertainty is a key driver of drug misuse and overuse, which can lead to antimicrobial selection pressure and increased rates of resistant microbes (5). The risks associated with untreated microbial infection and the lack of accurate clinical or laboratory prediction methods result in a low threshold for initiating empirical antimicrobial drug therapy, especially if infection could be life-threatening (6).

In the future, diagnosis of microbial infection may be improved at several levels, allowing reduction of antimicrobial selection pressure. First, new diagnostic tests will facilitate initiation or withdrawal of antimicrobial therapy soon after onset of symptoms, especially in the hospital setting. Several new biological markers, such as procalcitonin and soluble triggering receptors expressed on myeloid cells, have been proposed to serve either goal (7,8). Second, molecular diagnostics may increase diagnostic accuracy and enable more prudent antimicrobial drug use in the future. Amplification technology with DNA microarrays and simplified automation opens the potential for rapid testing. Dunne et al. described a scenario in which by the year 2025, sophisticated laboratory platforms with real-time amplifiers will automatically obtain and analyze clinical samples and be able to detect any potentially pathogenic microbe within 30 minutes (9). The threat of bioterrorism may also foster research about rapid molecular diagnostic tests that may be used at the bedside. Third, new diagnostic tools may be available to rapidly distinguish between bacterial and viral infections in the ambulatory setting. Fourth, profound changes will be seen in the techniques used to perform molecular identification and antimicrobial susceptibility testing. In summary, there are several lines of evidence suggesting that a number of molecular, immunologic, and microbial techniques will change the way infectious diseases are diagnosed and reduce diagnostic uncertainty in the next 2 decades (10).

Prescribing Antimicrobial Agents

To most clinicians, the immediate risk for the patient outweighs the long-term disadvantages of liberal use of antimicrobial drugs. One of the most promising means of reducing antimicrobial selection pressure without impairing patient safety is cessation of antimicrobial drug therapy in patients who do not have a bacterial infection. Great progress has been made within the last 5 years to shorten the duration of treatment with antimicrobial agents (11). Prediction rules designed for the early discontinuation of antimicrobial agents have been validated by prospective trials and will further optimize antimicrobial drug use (12).

Although antimicrobial drug policies and guidelines may not have been of great help in individual decision making, they may have sensitized the medical community to the growing problem of antimicrobial drug overuse and resistance. Consequently, in many industrialized countries, either the number of antimicrobial agent prescriptions or the volume of antimicrobial use has decreased over the last 10 years, especially in the ambulatory setting (Table 2) (13,14). A plateau in worldwide antimicrobial consumption seems to have been reached, leading to a saturated market. As stated recently by representatives of the pharmaceutical industry, "The awareness of the relationship between use and emerging resistance has led to efforts to decrease, even restrict, antibiotic use, and therefore decrease the positive influence of resistance on the market and decrease market potential" (15).

Population Characteristics and Technologic Development

Case-mix characteristics and infection rates both inside and outside the healthcare setting will influence antimicro-

Table 2. Countries that have decreased either number of antimicrobial drug prescriptions or total volume of outpatient antimicrobial drugs used within the last 10 years

Continent	Country
Europe	France
	Belgium
	Spain
	Germany
	United Kingdom
	Sweden
Asian-Pacific region	South Korea
	Taiwan
	Australia
Americas	Canada
	United States
	Chile

bial drug use and resistance in the future. An increase in immunocompromised patients, the growing life-expectancy, and the susceptibility of older persons to infections could indirectly contribute to greater antimicrobial drug use and dissemination of resistant microbes. Moreover, infectious diseases are influenced by developments in other areas of patient care. New technologies and treatments can create new infectious diseases or eliminate existing ones. For instance, cancer chemotherapy led to new types of susceptible hosts and infectious disease problems, indirectly impelling the dissemination of antimicrobial resistance within hospitals. Key trends in clinical care and biomedical discovery that are likely to influence antimicrobial resistance are the increased use of medical devices and gene therapies, and better management of chronic diseases such as diabetes and cancer. These developments will likely reduce some types of resistance problems and help spawn others.

Global threats such as the next influenza pandemic may also affect prescribing of antimicrobial drugs by reversing the trend of decreasing antimicrobial drug consumption (16). Conversely, climate change may lead to a decrease in respiratory tract infections and antimicrobial drug use in the winter months (17).

Travel and Globalization

Globalization and migration into mega-cities has led to new possibilities of cross-transmission of antimicrobial resistance (1). Recent events such as the terrorist attack in Bali, the war in Iraq, and the tsunami in Southeast Asia have led to the transfer of patients infected with panresistant gram-negative bacteria such as *Acinetobacter* spp. to other parts of the world, causing outbreaks and public health concerns (18). Within the next 2 decades, global mixing, increased population density, and decreased travel times will facilitate the spread of a variety of antimicrobial-resistant pathogens such as fluoroquinolone-resistant pneumococci and enteric microbes.

Since antimicrobial resistance is influenced by international travel and globalization, resistance may, in turn, affect how nations respond to each other. Especially as surveillance systems improve in quality, international pressure may be applied to induce change in countries where antimicrobial agents are abused or where infection control policies are lax. The situation in antimicrobial resistance might become comparable to that which exists for other infectious problems such as mad cow disease: economic pressure may contribute to compliance and uniformity in control measures. Nevertheless, approaches to control the global spread of resistance will remain difficult to implement and will require intensive surveillance and screening efforts.

Health Beliefs and Antimicrobial Drug Demand

Although the interplay between health beliefs and demand of antimicrobial drugs is widely recognized, few, if any, systematic studies exist about the future influence of the cultural setting on antimicrobial drug use and related resistance rates (19). Social constraints and cultural views of infectious conditions that require antimicrobial treatment exert a strong influence on their use, particularly for community-acquired pathogens.

Several countries have recently taken the bold step of launching national campaigns to educate physicians and patients about antimicrobial misuse and the threat of resistance (Figure 1). These campaigns show promise in changing attitudes and behavior, among both the public and healthcare professionals (20). If repeated regularly, the campaigns are likely to reduce inappropriate patient requests for antimicrobial agents, which in conjunction with physician education models may reduce inappropriate antimicrobial prescription practices (21). Ultimately, they may slow the dissemination of certain antimicrobial-resistant pathogens (5). For instance, in several countries, such as France and Spain, which use a great amount of antimicrobial agents, a decrease in pneumococcal resistance rates among invasive isolates has been noted recently. This coincides with a decrease in antimicrobial drug use after nationwide campaigns and the introduction of a conjugate pneumococcal vaccine (22). Nevertheless, uncertainty persists about possible negative outcomes and countermeasures taken by the pharmaceutical industry to oppose these campaigns.

Vaccinology

Modern vaccinology (the development of new vaccines) is likely to contribute to the decreased transmission and impact of antimicrobial-resistant bacteria in the near future (23). More so than antimicrobial agents, vaccines have the potential to durably control infectious agents by blocking their ability to disseminate within a population. This expectation can be illustrated by the example of the new pneumococcal conjugate vaccine. Based on encourag-

Figure 1. Posters from nationwide educational campaigns against misuse of antimicrobial drugs.

ing results from countries with high prevalence of pneumococcal resistance such as Israel, France, Spain, and the United States, this vaccine will likely reduce the incidence of invasive disease due to resistant pneumococci (22,24). Further progress in pneumococcal vaccine development can be expected from conjugate vaccines that include more than 7 serotypes (25). Yet uncertainty remains regarding serotype replacement and the emergence of resistance in nonvaccine serotypes (25).

Other vaccines to prevent invasive, antimicrobial-resistant infections will be launched within the next 20 years (23). Potential candidates are vaccines against multidrug-resistant staphylococci and enterococci, but clinical studies need to confirm promising preliminary results.

Infection Control in the Healthcare Setting

While the intense selective pressure of antimicrobial drug use has been an important factor in the emergence of resistance, the inconsistent application of infection control guidelines by hospital personnel largely accounts for the dissemination of resistance in the healthcare setting. Infection control measures to limit the spread of antimicrobial resistance are being increasingly well defined. Despite the increase in the prevalence of resistance of several important pathogens, there has been some success in controlling its clinical impact. Several countries have recently reported a stabilization or decrease in infection rates due to multidrug-resistant *Staphylococcus aureus* (26).

The next 20 years will see an increase in infection control research and interventions to improve patient safety. Hand hygiene with alcohol-based hand rubs has been shown to decrease the transmission of resistant organisms (27). A campaign sponsored by the World Health Organization in 2005 is promoting its practice throughout the world. Early screening and isolation of patients carrying resistant organisms also appear to decrease the spread of resistant microorganisms and may be more widely implemented (28). Some experts have suggested that multimodal approaches that use a combination of different measures (for example, aggressive infection control with active surveillance cultures, hand hygiene, and possibly antimicrobial control) will effectively slow down and even halt the increasing trends of healthcare-associated antimicrobial resistance (29).

Healthcare Regulation

Antimicrobial use is affected by reimbursement policies, financial incentives, and healthcare regulation (19). Forecasting the political and regulatory development in this area presents a major challenge. There is always a short-term lack of predictability with regard to political decision-making after unexpected epidemiologic situations, such as the bioterrorist attacks in 2001 and severe acute respiratory syndrome in 2003, which quickly influenced perceived medical needs (30).

Looking at the future impact of healthcare regulation, many believe that political measures to control antimicrobial drug use have only a negligible short-term effect (1,31). We argue, however, that healthcare regulation will powerfully influence antimicrobial drug use in the future. To underline this hypothesis, we give 3 examples from different continents.

Interdiction of Over-the-Counter Sales of Antimicrobial Agents in Chile

Self-medication is an important driver of antimicrobial overuse in low- and middle-income countries. Therefore since 1999, the Chilean Ministry of Health has strictly enforced existing laws, which restricted purchase of antimicrobial agents without a medical prescription. These regulatory measures had a sustained impact on antimicrobial use in the outpatient setting: sales of orally used antimicrobial agents decreased by 43% from US $45.8 million in 1998 to US $26.1 million in 2002 (Figure 2) (32).

Restriction of Perioperative Antimicrobial Prophylaxis in Belgium

Inadequate and prolonged perioperative antimicrobial prophylaxis increases resistance to antimicrobial drugs (33). In 1997, a Royal Decree in Belgium limited reimbursement of antimicrobial drug prophylaxis to specific agents and a 24-hour period after surgery (34). Moreover, a fixed fee for antimicrobial costs was attributed to each type of intervention. As shown in Table 3, this regulatory restriction had a sustained effect on the use of antimicrobial prophylaxis in Belgium (34).

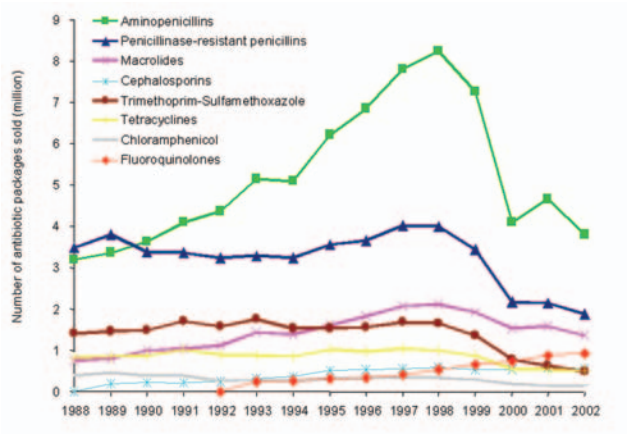

Figure 2. Number of antimicrobial drug packages sold in the outpatient setting in Chile, 1988–2002. Package is the term used to show sales figures of antimicrobial drugs from wholesalers or pharmacies. It is also used to calculate the number of daily defined doses for each marketed antimicrobial drug. Data are from Bavestrello et al (32). Unpublished data from 2001 and 2002 were provided by A. Cabello Munoz and L. Bavestrello (Viña del Mar, Chile).

Separation of Dispensing and Prescribing in South Korea

In Europe and North America, separation of antimicrobial prescribing and dispensing is a well-established system. In contrast, in many Asian countries, healthcare providers earn a significant proportion of their income from dispensing drugs, especially broad-spectrum antimicrobial agents (35). Consequently, physicians have traditionally compensated for relatively low medical service revenue by prescribing a high volume of antimicrobial agents. In 2000, against the strong opposition of physicians and the pharmaceutical industry, a new Korean government policy prohibited physicians from dispensing drugs and pharmacists from prescribing drugs (36). This new policy decreased overall prescribing of antimicrobial agents and selectively reduced inappropriate prescribing of them for patients with viral infections (36).

Future Directions

The uncertainty evolving around micro- and macrolevel determinants influencing antimicrobial resistance makes long-term prediction challenging. Although simulation studies may provide guidance about short-term trends (37), long-term predictions about the future of antimicrobial resistance are fraught with difficulties, as shown by a look back in history. When the antimicrobial drug era began, scientists were impressed by the milestones of antimicrobial agent discovery and issued predictions about the future of antimicrobial resistance that seem overly optimistic today (38). For instance, in 1952, a famous French microbiologist anticipated pneumococci, gonococci, and meningococci would not change their antimicrobial susceptibility profile in the future ("Pour une espèce qui au départ était entièrement sensible…, l'espèce sera toujours aussi sensible. C'est le cas des germes très sensibles à la pénicilline: gonocoques, pneumocoques, méningocoques") (39). Yet exactly 40 years later, we were rapidly progressing towards a "post-antimicrobial era" in which doctors may become helpless against even common infections (40).

In the last part of this article, we contemplate the possible status of antimicrobial resistance in 2025. Although the direction of a few major trends seems relatively easy, other factors that drive uncertainty present tremendous forecasting challenges. Therefore, we have developed 2 alternative scenarios about the future dissemination and control of antimicrobial resistance. These were extrapolated from the key determinants discussed earlier. The informed reader of 2025 may apologize for our lack of imagination.

What Will Be the Status of Antimicrobial Resistance in 20 Years?

The Bright Scenario

We will observe a change in prescribing habits and attitudes towards outpatient antimicrobial use, especially for respiratory infections. Policies and behavior change interventions contribute to a massive change in social norms around antimicrobial drug use, similar to what has happened with tobacco control. Intensive educational campaigns, aimed at optimizing antimicrobial drug use, combined with immunization programs for infants and children will lead to reduced spread and clinical impact of antimicrobial-resistant pneumococci.

Tools from information technology and progress in microbiology will reduce diagnostic uncertainty and improve antimicrobial dosing, selection, and treatment duration. Use of antimicrobial agents will, therefore, continue to decrease, not only in the outpatient setting, but also in the inpatient setting.

New therapies will be developed based on probiotic principles. Technologic advances will enhance the identification and characterization of the vast microbial

Table 3. Proportions of appropriate perioperative antimicrobial drug prophylaxis in Belgian hospitals after change in the reimbursement system*

	1986 (%)	1999 (%)
Correct timing	53	70
Correct indication	92	97
Duration >48 h	50	8
Choice of agent		
First-generation cephalosporin	28	66
Second-generation cephalosporin	17	29

*Source: Goossens et al (34).

diversity colonizing the human body (commensals and pathogens), which may lead to new probiotic strategies to prevent infections and reduce antimicrobial selection pressure.

Data sharing and increased international cooperation will lead to consistent control measures across different continents. Asian countries, users of large amounts of antimicrobial agents and important drivers of resistance until recently, will change paradigms and introduce modern infection control concepts and public health policies that will decrease overuse of antimicrobial agents.

Antimicrobial resistance among important pathogens will be slowly reversible. Trends in antimicrobial resistance follow an S-shaped curve with a quick ascent, a plateau and, sometimes, a slow decline. Antimicrobial resistance in high prevalence countries will be slowly reduced, especially for several gram-positive microorganisms (1).

Antimicrobial resistance will not have a major impact on life expectancy in the industrialized world. Deaths from panresistant infections without any treatment option will remain rare complications in high-income countries, since new antimicrobial agents and better use of currently available antimicrobial drugs will become standard policy.

The Dark Scenario

New resistance mechanisms will emerge and disseminate. Multiresistant group A streptococci will render penicillin and macrolides useless in the treatment of pharyngitis. *Salmonella* spp. infections can no longer be treated with advanced cephalosporins, fluorquinolones, or carbapenems.

We will observe raising resistance rates for most pathogens. Multiresistant *Acinetobacter* spp., enterococci, and staphylococci will cause substantial illness and increased treatment costs in those parts of the world that have not installed stringent control measures. Healthcare-associated infections due to vancomycin-resistant enterococci will become endemic in many countries.

Antibacterial-resistant S. aureus will become a massive public health problem. The scope of staphylococcal antimicrobial resistance will extend not only to new antimicrobial agents, but also to more settings. Although hospitals were once the sole province of methicillin-resistant *S. aureus* (MRSA), more and more community outbreaks of MRSA will occur in those persons who lack traditional risk factors for carriage of MRSA. The prevalence of MRSA in the US community will reach 25% within the next decade, with rates 3 times as high in hospitals (41).

Technological development will not fulfill its promise. No new antimicrobial classes or treatment strategies have been developed for gram-negative bacteria, and vaccines have not been widely effective. Serotype replacement in pneumococci allowed that organism to escape control. Fluoroquinolones are no longer effective against a wide array of infections and have not been replaced by any new class of orally available antimicrobial agents. New antimicrobial drugs with novel mechanisms of action (e.g., bacteriophages) have failed in large phase III trials.

Anthrax and pandemic influenza threats have led to mass prophylaxis, with disastrous consequences in terms of resistance. Several disasters and pandemics will increase the use of antimicrobial drugs on a global scale, leading to emergence and dissemination of resistance.

A continuing flood of consensus conference statements, position papers, and surveillance network reports will be issued about the problem of antimicrobial resistance, without any measurable and sustained effect on containment. Healthcare policy will not introduce stringent control measures because of a lack of precise estimates of the public health impact of antimicrobial resistance and the priority of other more pressing infectious disease problems such as HIV, tuberculosis, and malaria.

Conclusion

The high levels of uncertainty and complexity regarding antimicrobial resistance mandate that we build the capabilities to prepare not only for 1 specific future (following the pessimistic viewpoint of antimicrobial therapy: use it and lose it), but also across a range of alternative scenarios that may be less pessimistic.

Whether the current epidemic of antimicrobial resistance is sustainable or will succumb to current efforts to limit its spread will be decided by an interaction of factors related to microorganisms, host, use patterns of antimicrobial drugs, and the impact of infection control measures and technologic development (5). We hope that adding infection control and prudent use of antimicrobial agents to new drug development will avert the realization of pessimistic predictions about the future of antimicrobial resistance.

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Hidden Epidemic of Macrolide-resistant Pneumococci

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Community-acquired respiratory tract infections (RTIs) account for a substantial proportion of outpatient antimicrobial drug prescriptions worldwide. Concern over the emergence of multidrug resistance in pneumococci has largely been focused on penicillin-resistant *Streptococcus pneumoniae*. Macrolide antimicrobial drugs have been widely used to empirically treat community-acquired RTIs because of their efficacy in treating both common and atypical respiratory pathogens, including *S. pneumoniae*. However, increased macrolide use has been associated with a global increase in pneumococcal resistance, which is leading to concern over the continued clinical efficacy of the macrolides to treat community-acquired RTIs. We provide an overview of macrolide-resistant *S. pneumoniae* and assess the impact of this resistance on the empiric treatment of community-acquired RTIs.

Community-acquired respiratory tract infections (RTIs), including acute bacterial sinusitis, acute otitis media, acute exacerbations of chronic bronchitis, and community-acquired pneumonia, are among the most frequent infections treated by physicians and represent a major international health problem (1). Community-acquired pneumonia is one of the leading causes of hospitalization in the United States and the most common cause of death in patients with infectious diseases (2), while acute otitis media is the most frequent illness for which antimicrobial drugs are prescribed for children in the industrialized world. *Streptococcus pneumoniae* is the most common microbial pathogen identified in community acquired RTIs, and pneumococcal infections are among the leading causes of illness and death worldwide (3), particularly among children, the elderly, and persons with coexisting medical conditions.

In the past, β-lactam antimicrobial drugs (e.g., penicillin) were widely used to empirically treat community-acquired RTIs. Pneumococcal resistance to penicillin was first observed in the 1960s; since then, the emergence and spread of penicillin-resistant *S. pneumoniae* strains have been observed and tracked worldwide. With the β-lactams in widespread use, increasing levels of penicillin-resistant *S. pneumoniae* were thought to be of greater potential clinical importance than the emergence of macrolide-resistant *S. pneumoniae* strains. However, a number of studies and analyses of patients with pneumococcal pneumonia (4) have shown no association between penicillin resistance and patient death, although some studies have indicated that penicillin-resistant *S. pneumoniae* infection may be associated with an increased risk for suppurative complications, longer hospital stays, and higher treatment costs (5).

The growing concerns about the emergence and spread of drug-resistant pathogens (including penicillin-resistant *S. pneumoniae*) and an increased awareness of infection with atypical pathogens (e.g., *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*), led to the publication of community-acquired pneumonia treatment guidelines by the American Thoracic Society in 1993 (6). These guidelines included a recommendation that macrolide drugs be used as first-line empiric therapy for outpatients with community-acquired pneumonia. The macrolides have since been used extensively to treat community-acquired RTIs worldwide. However, increasing macrolide use has also been associated with an increase in pneumococcal resistance to these agents, and macrolide-resistant *S. pneumoniae* are now more common than penicillin-resistant *S. pneumoniae* in many parts of the world (7). We provide an overview of pneumococcal resistance to macrolides and assess the impact of macrolide-resistant *S. pneumoniae* on the empiric treatment of community-acquired RTIs.

Macrolide Resistance

Mechanisms of Resistance

Macrolides are microbiostatic agents that reversibly bind to the 23S ribosomal RNA in the 50S subunit of ribosomes and block protein synthesis (8). Two main macrolide resistance mechanisms have been identified in

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pneumococci: active efflux of the drug from the cell and target-site modification (8). Energy-dependent efflux of macrolides from target cells by a cell membrane transporter has been associated with the presence of *mef* genes. Recent work by Iannelli et al. (9) has implicated a second gene, *mat*(A), that encoded 2 ATP-binding domains, as a component of *mef*-mediated macrolide resistance in pneumococci. Irrespective of the identity of the gene responsible for macrolide efflux, *mef*(A)-positive *S. pneumoniae* strains displaying this macrolide efflux phenotype (termed the M phenotype) are resistant to 14- and 15-membered ring macrolides (but not lincosamides or streptogramins) and generally display a low level of in vitro resistance to these antimicrobial agents. However, data from surveillance studies suggest that erythromycin MICs for *mef*(A)-positive isolates may be increasing. MICs were 1–16 μg/mL for *mef*(A)-positive *S. pneumoniae* isolates collected from 1994 to 1995 (10), while results from a more recent study demonstrated an erythromycin MIC of 1 to >256 μg/mL (11).

The second major mechanism of macrolide resistance in streptococci, target-site modification, is predominantly encoded by the *erm*(B) gene, resulting in methylation of an adenine residue on the 23S rRNA by a methylase enzyme. This methylation blocks the binding of macrolide-lincosamide-streptogramin B antimicrobial drugs. Strains with the macrolide-lincosamide-streptogramin B phenotype generally show higher levels of in vitro resistance to macrolides compared to strains with the M phenotype (10).

Other target-site modifications occur rarely in clinical isolates of *S. pneumoniae*. These modifications include mutations that involve domain V of the 23S rRNA and genes encoding riboproteins L4 and L22 (12). Such mutations can confer resistance to macrolide-lincosamide-streptogramin B antimicrobial drugs and are associated with variable levels of in vitro resistance. Although the global prevalence of pneumococcal strains with macrolide resistance conferred by ribosomal gene mutations remains low (<2%), a study of macrolide resistance mechanisms among *S. pneumoniae* isolated in Canada from 1997 to 2003 indicated that the rate of resistance due to mechanisms other than efflux or ribosomal methylation increased from 1% in 1997 to 10% in 2003 (13).

Macrolide Resistance Trends

A number of industry-sponsored global surveillance studies, such as the Alexander Project (GlaxoSmithKline) and PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) (sanofi-aventis), have been designed to define and monitor the prevalence and distribution of antimicrobial resistance among respiratory pathogens, detect new patterns of resistance, provide early warning of emerging resistance, and evaluate the effects of interventions aimed at reducing antimicrobial resistance. Results from the Alexander Project indicate that in 1996 and 1997 the global rate of pneumococcal macrolide resistance was16.5%–21.9% (14); by 1998–2000, the resistance rate had increased to 24.6% (15). Data reported after completion of the first year of the PROTEKT study (1999–2000 respiratory season) confirmed this high global incidence (31.0%) of pneumococcal macrolide resistance (7), with similar overall levels of resistance among isolates collected as part of the PROTEKT US study (31.0% in 2000–2001 and 27.9% in 2001–2002) (16,17). The slight reduction in macrolide resistance among pneumococcal isolates collected as part of the PROTEKT US study from 2001 to 2002 may be a consequence of the February 2000 introduction of the 7-valent pneumococcal conjugate vaccine (18).

However, both macrolide resistance rates and resistance mechanisms may vary considerably depending on location. Macrolide resistance rates for isolates collected during the PROTEKT US study from 2001 to 2002 vary according to region; at a state level, the highest prevalence of pneumococcal macrolide resistance was recorded in Louisiana (48.2%) and the lowest in Vermont (15.2%) (11). Similarly, while *mef*(A) was the most prevalent pneumococcal macrolide resistance genotype identified in the United States overall (68.7% of genotyped isolates), the relative prevalence varied by state and ranged from 40% in Delaware to 85% in Georgia (11). While *erm*(B) was the second most prevalent genotype overall (16.8%), isolates possessing both the *erm*(B) and *mef*(A) genotype (12.2%) were more prevalent in 11 states than those harboring *erm*(B) alone. A recent analysis of PROTEKT US 2002–2003 data by Farrell et al. (19) indicates an increase in the prevalence of macrolide-resistant isolates containing both *erm*(B) and *mef*(A) from 9.7% in 2000–2001 to 16.4% in 2002–2003. Most (99.2%) of these *erm*(B) + *mef*(A)–positive isolates were resistant to ≥2 classes of antimicrobial drugs. Analysis of erythromycin MIC data for all macrolide-resistant isolates collected in 2000–2001 indicated that the MIC_{90} (MIC at which 90% of isolates were inhibited) varied according to resistance genotype (16 μg/mL for *mef*(A)-positive isolates vs. >256 μg/mL for *erm*(B)-positive isolates and those harboring both the *erm*(B) and *mef*(A) gene) (11).

Factors Contributing to Development and Spread of Macrolide Resistance

Inappropriate use of antimicrobial drugs is among the most important factors associated with the emergence and spread of pneumococcal macrolide resistance. Inappropriate use may include using antimicrobial drugs to treat nonmicrobial or self-limiting infections, using agents with a spectrum of activity that either does not cover the

appropriate causative pathogen(s) or which has too broad a spectrum of activity, and inappropriate dose or duration of treatment (20).

Other risk factors for carriage or infection with resistant pneumococcal strains include age (patients particularly at risk include those <2 or >65 years of age), history of macrolide use, and the presence of severe underlying disease (21). Analyses of data from national and international surveillance studies have suggested a link between increased use of macrolides and increased rates of pneumococcal resistance (22). In Portugal, the emergence of macrolide-resistant *S. pneumoniae* strains from 1994 to 2002 correlated with the use of azithromycin during the same period (23). Several studies have shown that macrolide administration is associated with increased nasopharyngeal carriage of resistant strains of *S. pneumoniae* in children (24); the clonal dissemination of macrolide-resistant pneumococcal strains in crowded environments (e.g., daycare centers, hospitals, jails, long-term care facilities) is also thought to be a major factor contributing to the spread of resistance.

Clinical Implications of Macrolide Resistance

Surveillance studies have shown that a substantial percentage of pneumococci are now macrolide resistant. Despite this rising rate of in vitro resistance, some researchers and clinicians have questioned whether resistance to macrolides is clinically relevant given the high concentrations achieved in respiratory tissues such as the epithelial lining fluid. Although macrolide levels in epithelial lining fluid have been reported to exceed the levels achieved in serum, the relevance of the fluid levels has been questioned (25), and sufficiently high macrolide blood levels remain essential to cure bacteremic pneumococcal pneumonia. Moreover, clinically achievable serum, epithelial lining fluid, and middle-ear fluid concentrations of azithromycin were insufficient to eradicate macrolide-resistant *S. pneumoniae*, irrespective of the resistance mechanism (26).

Prospective clinical studies have provided conflicting evidence for an association between discordant antimicrobial therapy (i.e., use of an agent to which the causative pathogen displayed in vitro resistance) and treatment outcome. For example, while results from 1 study of patients with community-acquired pneumonia and bacteremia due to bacteremic pneumococcal infection demonstrated an association between increased death rates and discordant antimicrobial drug therapy (27), no such association was observed in a different study (28) of patients with pneumococcal community-acquired pneumonia. However, the conclusions that can be drawn from such studies may be limited by factors such as small sample size, differences in patient inclusion or exclusion criteria (e.g., recent antimicrobial drug use), use of relatively insensitive measures of treatment outcome (e.g., death), and use of single or multiple antimicrobial drugs (many hospitalized patients receive combination antimicrobial therapy, thus limiting the opportunities to study the effects of discordant treatment on clinical outcomes); in the studies cited above, none of the cases of discordant therapy involved monotherapy with a macrolide.

In acute otitis media, tympanocentesis performed before and after drug therapy has been used in several studies to determine the clinical relevance of antimicrobial resistance. Using this method, Dagan et al. (29) showed that microbiologic failure (correlated with clinical failure) was associated with pneumococcal macrolide resistance among patients treated with azithromycin; treatment of 6 of 6 patients with high-level macrolide resistance failed microbiologically. Furthermore, analysis of data from a pediatric medical center in the United States (30) noted that the rising incidence of antimicrobial-resistant pneumococci corresponded to an increase in suppurative complications of acute otitis media and appeared to contribute to more aggressive infections that required surgical intervention.

In recent years, several reports have described clinical and microbiologic treatment failures that have occurred in hospitalized patients infected with macrolide-resistant pneumococci (31). Among these cases of treatment failure, 2 deaths occurred. In both cases, the previously healthy patients (a 28 year-old man and a 49-year-old woman) received monotherapy with intravenous azithromycin for pneumonia. The clinical status of both patients deteriorated while they were receiving azithromycin, and macrolide-resistant *S. pneumoniae* were isolated from blood and pleural fluid cultures taken while these patients were receiving medication.

A matched case-control study of hospitalized patients with bacteremia conducted by Lonks et al. (32) identified 86 patients with isolates of *S. pneumoniae* that were fully or intermediately resistant to macrolides and 141 controls who had macrolide-susceptible pneumococcal infection. When patients with meningitis were excluded from the analysis, 18 (24%) of 76 patients were taking a macrolide at the time of bacteremia compared to none of the controls ($p<0.0001$). Moreover, 5 (24%) of the 21 bacteremic patients infected with pneumococci expressing the M phenotype were taking a macrolide (compared with none of the 40 matched control patients; $p<0.0016$). These data show that breakthrough bacteremia and treatment failure occurred only in those patients infected with a macrolide-resistant pneumococcus; no incidences of breakthrough bacteremia were seen in those infected with a macrolide-susceptible pneumococcus. Similarly, a study of all pneumococcal bacteremias from a hospital in Belgium (33)

showed that 4 (12%) of 33 patients with a macrolide-resistant pneumococcus were taking a macrolide when blood cultures were obtained, i.e., they had breakthrough bacteremia; in contrast, none of the 103 patients with macrolide-susceptible pneumococci was taking a macrolide.

The overall incidence of treatment failure caused by macrolide-resistant pneumococci cannot be estimated from the case reports and observational studies published to date. These reports of treatment failure likely only represent the tip of the iceberg, as most case studies published to date have only captured treatment failures that resulted in breakthrough bacteremia. These published studies underreport the magnitude of treatment failures because nonbacteremic pneumococcal pneumonia is 3–5 times more common than bacteremic pneumonia. In addition, these treatment failures resulted in hospitalization, which is more expensive than outpatient therapy. Most macrolides are prescribed as part of empiric treatment regimens for ambulatory patients in the outpatient setting; microbiologic cultures are not usually obtained from these patients, and antimicrobial susceptibility testing is rarely performed (even if treatment failure occurs).

Macrolide Resistance and Treatment Guidelines

In the United States, guidelines for the treatment of community-acquired RTIs have been established by a number of groups, including the American Thoracic Society, the Infectious Diseases Society of America, the Centers for Disease Control and Prevention (CDC), and the Sinus and Allergy Health Partnership. The clinical relevance of macrolide-resistant *S. pneumoniae* has been addressed in updates to these groups' guidelines for the treatment of community-acquired pneumonia (34,35) and in a report published by the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group convened by CDC (36). The consensus among these guidelines is that empiric therapy should be stratified based on likely cause, treatment setting (inpatient versus outpatient), and the risk for pneumococcal antimicrobial resistance. In general, all 3 guidelines recommend that monotherapy with macrolides should be restricted to specific patient subgroups (i.e., those with no coexisting cardiopulmonary disease and no risk factors for infection with drug-resistant *S. pneumoniae* [e.g., recent antimicrobial drug use]). For outpatients with risk factors for drug-resistant *S. pneumoniae*, current recommended treatment options include combination therapy with a β-lactam (such as high-dose amoxicillin or high-dose amoxicillin-clavulanate) plus a macrolide or an antipneumococcal fluoroquinolone (34,35). The increased use of fluoroquinolones has been associated with the emergence and spread of resistance to these agents (37), and local clonal dissemination of *S. pneumoniae* strains with very high-level resistance to penicillin has been reported in the United States (38). Although the prevalence of these resistant isolates remains low, such findings emphasize the necessity for local resistance patterns to be considered when prescribing empiric antimicrobial drug therapy for patients with community-acquired RTIs.

The Sinus and Allergy Health Partnership guidelines for the treatment of acute microbial rhinosinusitis also highlight the need to consider the increasing prevalence of pneumococcal resistance when making treatment choices, with patients divided into categories dependent on their recent exposure to antimicrobial drugs (39). Similarly, a recent American Thoracic Society statement on the management of acute microbial exacerbations of chronic obstructive pulmonary disease emphasizes the need to consider local resistance patterns when prescribing antimicrobial drugs (40).

Conclusions

National and international surveillance studies demonstrate a high global prevalence of in vitro macrolide resistance among pneumococcal isolates obtained from patients with community-acquired RTIs. In recent years, a number of studies have clearly linked in vitro macrolide resistance to microbiologic and clinical treatment failure, indicating that macrolide resistance is an emerging problem. As pneumococcal community-acquired RTIs (particularly community-acquired pneumonia) are a leading cause of illness and death worldwide, appropriate empiric antimicrobial therapy should be used to treat these infections. Recent updates to a number of treatment guidelines have reflected this changing situation by emphasizing the need for clinicians to consider local antimicrobial resistance patterns and risk factors for infection with drug-resistant pathogens when prescribing empiric antimicrobial therapy.

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Community-associated Methicillin-resistant *Staphylococcus aureus* in Hospital Nursery and Maternity Units

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Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has rarely been reported in the hospital setting. We report an outbreak of 7 cases of skin and soft tissue infections due to a strain of CA-MRSA. All patients were admitted to the labor and delivery, nursery, or maternity units during a 3-week period. Genetic fingerprinting showed that the outbreak strain was closely related to the USA 400 strain that includes the midwestern strain MW2. All isolates contained the staphylococcal chromosome cassette *mec* type IV. Genes for Panton-Valentine leukocidin and staphylococcal enterotoxin K were detected in all isolates, and most contained other enterotoxin genes. Testing of nearly 2,000 MRSA isolates collected during citywide surveillance studies from 1999 to 2003 showed that ≈1% were genetically related to MW2. CA-MRSA strain MW2 has been present in this region at least since 1999. This study documents the spread of this strain among healthy newborns at 1 hospital.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an established pathogen in most healthcare facilities. Recently, infections due to MRSA have been documented in children and adults who lack traditional risk factors (1–4). Most infections caused by these community-associated (CA) MRSA appear to involve the skin. However, these strains may occasionally cause pneumonia or death in previously healthy patients (5,6). In one of the initial reports of CA-MRSA, 4 deaths were reported in children infected with a prototypical strain designated MW2 (5).

Several lines of evidence suggest that the emerging CA-MRSA isolates are distinct from typical nosocomial strains (7–9). First, CA-MRSA isolates are generally susceptible to non-β-lactam antimicrobial agents and genetic fingerprinting suggests that they are unrelated to hospital-associated strains (7–9). CA-MRSA isolates possess a small (21- to 24-kb) and mobile staphylococcal chromosome cassette *mec* type IV (SCC*mec*IV)–encoding penicillin-binding protein (8). This gene cassette has been rarely found in contemporary healthcare-associated MRSA strains. Finally, most of these strains have genes that encode for multiple virulence factors, including Panton-Valentine leukocidin (PVL) and superantigens (5,10).

Strains of CA-MRSA have recently caused infections in hospitalized neonates in the nonoutbreak setting (11). They have rarely been linked to nosocomial outbreaks. One report involving postpartum women documented hospital transmission of the strain MW2 (12). We describe an outbreak in a nursery and maternity unit involving the MW2 strain of CA-MRSA. The prevalence of strains resembling MW2 in Brooklyn, New York, is also reported.

Materials and Methods

Outbreak Investigation at Hospital A

From October to November 2002, a cluster of skin and soft tissue infections due to MRSA involving pediatric and maternity patients occurred at a New York City hospital. The hospital has a labor and delivery unit and 2 units that house both healthy newborns and maternity patients. Healthcare workers on these units typically care for patients on all the units. After the outbreak was recognized, the following interventions were implemented: 1) nursing and medical personnel from the involved areas were informed of the outbreak and potential modes of transmission of staphylococci, 2) contact precautions were empha-

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sized for all patients with suspected or proven skin infections, 3) alcohol-based hand sanitizers were placed in involved areas, 4) healthcare workers from involved units were screened for nasal MRSA colonization, and 5) environmental surfaces (including cord clamps, antitheft transponders, and temperature sensors of baby warmers) were tested for MRSA contamination. Healthcare workers colonized with MRSA were treated with intranasal mupirocin and furloughed until repeat cultures were negative. To identify any other potential case-patients, letters concerning the outbreak were sent to pediatricians who cared for newborns discharged from the affected units during the outbreak period. Cases were defined as MRSA infections in patients who stayed on the labor and delivery, nursery, or maternity units at any time from October 2002 to December 2002. The medical records of the patients were reviewed for information regarding prior healthcare exposures, receipt of antimicrobial agents, underlying medical conditions, treatment, and clinical outcome.

Cultures related to the outbreak were grown on tryptic soy agar plates supplemented with 3% sheep blood; colonies consistent with *S. aureus* were identified according to standard techniques. All isolates underwent susceptibility testing with the Etest method (AB Biodisk, Solna, Sweden). Ribotyping was performed with the Riboprinter Microbial Characterization System (Qualicon, Wilmington, DE, USA), as previously noted (13). In addition, isolates of MRSA collected during the outbreak were fingerprinted by pulsed-field gel electrophoresis (PFGE), as previously described (13). PFGE results were interpreted according to known criteria (14).

SCC*mec* typing was performed by using multiplex polymerase chain reaction (PCR), under conditions described by Oliveira et al. (15). Primers to detect the *mecA* gene were included as an internal positive control (15). Multilocus sequence typing (MLST) was performed on selected isolates as described by Enright et al. (16). Bidirectional DNA sequencing of 7 amplified housekeeping genes was performed with an automated fluorescent dye-terminator sequencing system (Applied Biosystems, Foster City, CA, USA). Allelic types were assigned by using the MLST database (available from www.mlst.net).

The presence of genetic sequences encoding several staphylococcal toxins was also investigated for the outbreak isolates. Based on the previously reported distribution of enterotoxins in CA-MRSA from the United States (7), the following toxins were selected for investigation: staphylococcal enterotoxin A (SEA), B (SEB), C (SEC), H (SEH), and K (SEK). In addition, strains were screened for PVL and toxic shock syndrome toxin-1 (TSST-1). Previously published primers and conditions were used to detect sequences encoding for SEA, SEB, SEC, SEH, PVL, and TSST-1 (17–19). Genes encoding for SEK were detected with the following primers: SEK forward: 5′-TGGATCAATGGAAATCACAAAA-3′ and reverse: 5′-TTTGGTAGCCCATCATCTCC-3′ (predicted product size 287 bp). The specificity of amplification was verified by bidirectional sequencing of the product.

Surveillance Study

The identification of MW2 in the outbreak of the neonatal-maternity unit prompted a retrospective investigation to determine the regional prevalence of MRSA resembling this strain. In 1999, 2001, and 2003, surveillance studies were performed in Brooklyn, New York. Each surveillance study involved collecting all single-patient isolates of *S. aureus* from clinical microbiology laboratories during a 3-month interval. Each study included 11–15 hospitals. Susceptibility testing was performed in the central research laboratory by using the agar dilution method according to NCCLS methodology (20). All MRSA isolates were then screened for a phenotype of susceptibility to clindamycin and ciprofloxacin (typical for MW2). Isolates possessing this susceptibility pattern underwent ribotyping and SCC*mec* typing. The study was approved by the Institutional Review Board at the State University of New York (SUNY) Health Science Center and Maimonides Medical Center.

Results

Outbreak Investigation at Hospital A

From October 18 to November 28, 2002, a total of 8 patients with skin and soft tissue infections due to MRSA were identified. During this period, 3.5 cases of MRSA infection occurred each month in the nursery and maternity units. In contrast, no MRSA infections had been reported from the involved units in the 10 months before the outbreak. Two patients were mothers, and 6 were neonates; in no instance were both the mother and her child infected. All had been hospitalized on an involved unit at some point from October 16 to November 6, 2002. Review of medical records showed that none of the patients had prior hospital exposure, underlying chronic medical conditions, or recent antibiotic therapy.

Clinical manifestations of the infections are included in Table 1. None of the patients had evidence of infection upon admission to the hospital. The timing of hospitalization and onset of clinical symptoms are shown in Figure 1. Patients stayed on the unit for an average of 5 days (range 2–12 days). Clinical infection developed in 4 of the newborns and 1 mother while in the hospital. Symptoms developed in 2 newborns and 1 mother 2, 10, and 24 days, respectively, after discharge. β-Lactam antimicrobial agents were initially administered for 6 patients. Definitive therapy generally consisted of topical or systemic antimicrobial agents active

Table 1. Clinical information for patients with methicillin-resistant *Staphylococcus aureus* infection during the outbreak period

Patient	Age at onset	Sex	Strain	Infection type	Initial therapy	Definitive therapy
P1, newborn	8 d	F	USA 400	Preseptal cellulitis	Nafcillin, cefotaxime	Topical gentamicin
P2, newborn	13 d	F	USA 400	Omphalitis, otitis externa	Ampicillin, cefotaxime	Topical mupirocin
P3, mother	33 y	F	USA 400	Breast abscess	Cefazolin	Surgical drainage, vancomycin, topical mupirocin
P4, newborn	2 d	M	USA 400	Omphalitis, pustulosis	Nafcillin Gentamicin	Gentamicin, topical mupirocin
P5, newborn	4 d	M	USA 400	Pustulosis	Cephalexin	Topical bacitracin
P6, newborn	2 d	M	USA 400	Pustulosis	None	Local wound care
P7, newborn	1 d	F	USA 400	Pustulosis, mastitis	Topical mupirocin	Vancomycin
P8, mother	24 y	F	Unique	Peripheral IV catheter site	Cefazolin	Trimethoprim-sulfamethoxazole, catheter removal

against MRSA; 1 patient required surgical drainage. All patients had clinical resolution of infection.

Two additional suspected cases were reported by pediatricians to the Infection Control Department. The first was in an infant, born in November 2002, who was seen as an outpatient for pustulosis; however, the site was not cultured. The second case involved another infant, also born in November 2002, who was readmitted to the hospital 4 days later for treatment of omphalitis. Multiple cultures yielded no growth. No additional cases were reported from December10, 2002, to December 31, 2003.

Susceptibility testing showed that all 8 isolates were susceptible to clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, rifampin, doxycycline, linezolid, and vancomycin. Of the 8 clinical isolates, 7 (isolates P1-P7) belonged to 1 ribotype that was identical to the prototypical MW2 strain (Figure 2). PFGE confirmed that the 7 isolates were identical and closely related to MW2 (Figure 2). All 7 contained SCC*mec* type IV. Since the 7 isolates appeared identical, MLST was performed on one of the isolates and showed sequence type 1. The PFGE and MLST pattern are the same as CA-MRSA clone USA 400, which also includes MW2 (21). Among these 7 isolates, all contained SEK and PVL, 6 contained SEC and SEH, and 5 contained SEA. None was found to have genes encoding SEB or TSST-1. The eighth clinical isolate, from a catheter-site infection, was distinct from the outbreak strain by ribotyping and PFGE (Figure 2). For this isolate, SCC*mec* was nontypable, and MLST typing confirmed a distinct allelic profile. None of the genes encoding toxins was detected.

A total of 189 healthcare workers worked on the involved units during the outbreak period. Screening cultures of the anterior nares were performed in 176 of the workers in November 2002. Three of the cultures were positive for MRSA, including 2 from the nursing staff and 1 from a pediatrician. The 3 MRSA strains possessed a susceptibility pattern typical for the multidrug-resistant hospital strains, with resistance to clindamycin and ciprofloxacin. They belonged to ribotypes distinct from the outbreak clone, and PFGE confirmed these isolates were unrelated to MW2 (Figure 2). For the 3 isolates, SCC*mec* was nontypable with the multiplex PCR method. None of the 27 environmental samples collected in November 2002 yielded positive cultures for MRSA.

Surveillance Study

A total of 4,345 isolates of *S. aureus* were collected in the 3 surveillance studies conducted in 1999, 2001, and 2003; susceptibility data for these isolates are given in Table 2. A total of 1,913 (44%) isolates were methicillin-resistant. Of the 1,913 MRSA isolates, 118 (6%) possessed the screened phenotype (susceptible to both clindamycin and fluoroquinolones). Among the 118 isolates, 40 different ribotypes were identified. A total of 11 isolates possessed the same ribotype pattern as the out-

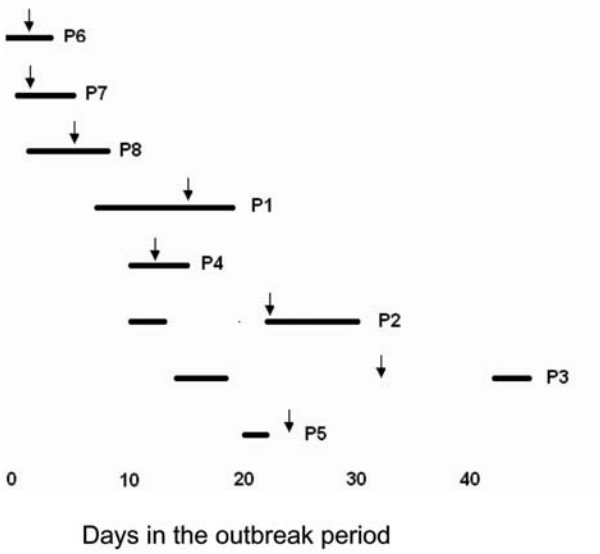

Figure 1. Time course of hospitalizations and onset of methicillin-resistant *Stpahylococcus aureus* illness during the outbreak at Hospital A. Solid bars represent period of hospitalization; arrows represent onset of clinical infection.

Table 2. Susceptibility data on *Staphylococcus aureus* isolates collected from 11 to 15 hospitals in 1999, 2001, and 2003*

	1999 (N = 567)	2001 (N = 772)	2003 (N = 588)
% MRSA	36	46	52
Antimicrobial agent (% susceptible)			
Azithromycin	14	5	5
Clindamycin	18	15	20
Vancomycin	100	100	100
Daptomycin	ND	100	100
Tigecycline	ND	ND	100
Minocycline	98	ND	98
Linezolid	100	ND	100
Rifampin	88	92	95
Imipenem	37	49	56
Ciprofloxacin	10	8	7
Trimethoprim-sulfamethoxazole	75	80	89

*MRSA, methicillin-resistant *Staphylococcus aureus*; ND, not done.

break clone, MW2. Of the 11 isolates, 4 were known to come from children. One HIV-infected adult died of overwhelming sepsis within 24 hours of hospitalization. Sources of the cultures included skin and soft-tissue in 7 patients, blood/sterile body fluid in 3 patients, and the genital tract in 1 patient. Nine of the 11 isolates had SCC*mec*IV. The number of isolates resembling MW2 remained relatively constant during the 3 surveillances (4 in 1999, 3 in 2001, and 4 in 2003).

Discussion

This report characterizes the nosocomial transmission of the CA-MRSA strain MW2 among healthy newborns and, possibly, a postpartum woman. Symptoms developed in 3 patients 2–24 days after hospitalization; 2 may have acquired the bacteria in the hospital or the community. An eighth patient, a mother with catheter-site infection, had an unrelated strain with a pattern suggestive of a hospital-associated strain. The source of the outbreak and mechanism of transmission were not evident, as no cultures of staff members or the environment yielded this particular strain of MRSA. Transmission may have occurred after MW2 was introduced into the hospital by transient colonization of healthcare workers or by contamination of shared medical equipment. The infection control measures enacted in response to the initial cases may have had a role in controlling the outbreak. Widespread screening of healthcare workers for MRSA did not detect the outbreak strain in this and another report (12). While a potential role for this practice cannot be excluded, current evidence does not support routinely implementing widespread screening for CA-MRSA.

In the pediatric population, risk factors associated with MRSA infections include premature birth or low birth weight, chronic underlying diseases, prolonged hospitalization, invasive or surgical procedures, indwelling catheters, and prolonged use of antimicrobial agents (22–25). Outbreaks of *S. aureus* have been especially challenging in neonatal nursery units. Prior outbreaks involving the pandemic strain phage type 80/81 were characterized by high colonization rates among infants discharged from nurseries and subsequent transmission to family members (26). In this report, infection developed in the outpatient setting for 2 patients (following an admission on the involved unit), which suggests carriage of MW2 from the hospital back into the community. Unrecognized CA-MRSA colonization during hospitalization could become an additional method of its dissemination in the community.

Increased prevalence of CA-MRSA has been reported in Chicago, Los Angeles, Texas, and Minnesota (2,3,27,28). In New York City, CA-MRSA appears less common; 1 investigation reported MRSA carriage in 0.26% of children and their guardians (29). In our present report, a retrospective analysis of isolates collected from citywide surveillance studies conducted from 1999 to 2003 suggests that ≈1% of all MRSA isolates in Brooklyn are genotypically related to the prototypical North American

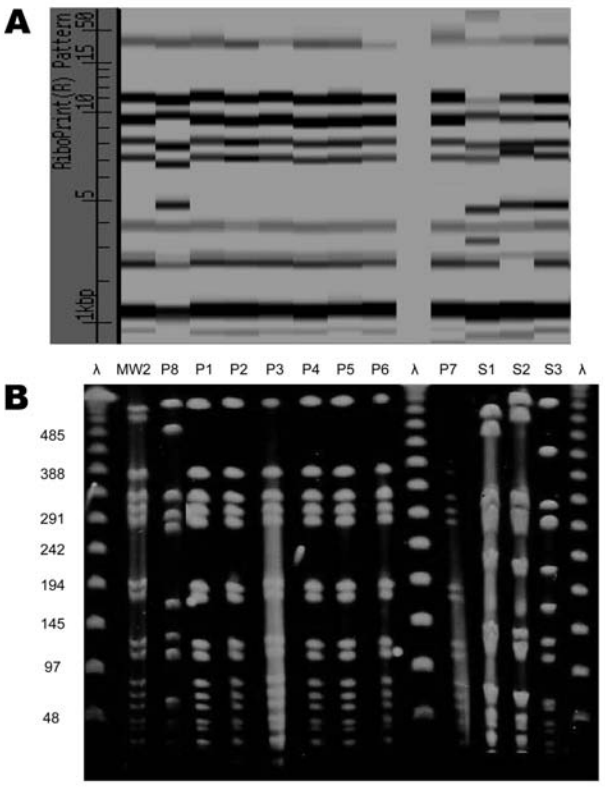

Figure 2. A) Ribotype and B) pulsed-field gel electrophoresis patterns of 8 clinical isolates of methicillin-resistant *Staphylococcus aureus*. Outbreak-related isolates P1–P7 are closely related to MW2. Clinical isolate P8 and the 3 isolates from healthcare workers (S1–S3) are unrelated to the outbreak strain.

CA-MRSA, MW2. Since only MRSA isolates that were susceptible to both clindamycin and ciprofloxacin were analyzed, this analysis probably underestimates the true prevalence. Other strains of CA-MRSA (e.g., USA 300) and USA 400 strains that acquired resistance to these antimicrobial agents would have been missed by our screening methods.

The introduction of CA-MRSA strains into neonatal units represents an especially serious challenge. Many of the infections caused by these strains, including some in our report, can be unusually severe and life-threatening (11). Careful vigilance involving surveillance, identification of these dangerous strains, and implementation of infection control measures, should be helpful in preventing further transmission both within and outside of the hospital.

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Dr. Bratu is a senior research fellow in the Division of Infectious Diseases at SUNY Downstate. Her research interests include virulence factors in *S. aureus* and mechanisms of antimicrobial resistance.

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Relative Fitness of Fluoroquinolone-resistant *Streptococcus pneumoniae*

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Fluoroquinolone resistance in *Streptococcus pneumoniae* is primarily mediated by point mutations in the quinolone resistance–determining regions of *gyrA* and *parC*. Antimicrobial resistance mutations in housekeeping genes often decrease fitness of microorganisms. To investigate the fitness of quinolone-resistant *S. pneumoniae* (QRSP), the relative growth efficiencies of 2 isogenic QRSP double mutants were compared with that of their fluoroquinolone-susceptible parent, EF3030, by using murine nasopharyngeal colonization and pneumonia models. Strains containing the GyrA: Ser81Phe, ParC: Ser79Phe double mutations, which are frequently seen in clinical QRSP, competed poorly with EF3030 in competitive colonization or competitive lung infections. However, they efficiently produced lung infection even in the absence of EF3030. The strain containing the GyrA: Ser81Phe, ParC: Ser79Tyr double mutations, which is seen more frequently in laboratory-derived QRSP than in clinical QRSP, demonstrated reduced nasal colonization in competitive or noncompetitive lung infections. However, the strain was equally able to cause competitive or noncompetitive lung infections as well as EF3030.

Streptococcus pneumoniae causes otitis media, bacteremia, and meningitis and is a leading cause of community-acquired bacterial pneumonia worldwide. Pneumococcal infections are commonly treated with β-lactams, macrolides, and, increasingly, fluoroquinolones. Pneumococcal resistance to each of these drug classes has increased in recent years (1,2). Initially, antimicrobial resistance in a pathogen may come at a cost: modifications that allow survival in the presence of antimicrobial drugs may render the pathogen less efficient at host infection, even in the absence of the antimicrobial agent (3). Little is known about the fitness of antimicrobial-resistant *S. pneumoniae* (4–8). The emergence of quinolone-resistant *S. pneumoniae* (QRSP) appears to be more dependent on fluoroquinolone selection of de novo spontaneous point mutations in the quinolone resistance–determining regions (QRDRs) of the topoisomerase genes *gyrA* and *parC* than on clonal dissemination (9–13). However, some studies reported occurrences of clonal relatedness among QRSP (11,14–16).

To investigate the relative fitness of QRSP, we conducted a competition study of a fluoroquinolone-susceptible clinical strain of *S. pneumoniae* (EF3030) with 2 of its fluoroquinolone-resistant isogenic mutants that had 2 common QRDR point mutation combinations. These 3 strains were analyzed by using an in vitro growth model, an in vivo nasopharyngeal colonization model, and an in vivo pneumonia model. We also carried out the nasopharyngeal colonization and pneumonia infections in the absence of competition to assess the ability of the mutants to colonize and to produce pneumonia in the absence of competition from the susceptible parent. To our knowledge, this is the first extensive investigation into the relative fitness of QRSP using in vitro models in combination with nasopharyngeal colonization and lung infection models.

Materials and Methods

Generation of Fluoroquinolone-resistant Mutants

For this study, naturally occurring fluoroquinolone resistance mutations were placed in the serotype 19F strain EF3030 by using established techniques (17). Briefly, 1,325-bp fragments of *gyrA* and 778 bp of *parC* were amplified by polymerase chain reaction from 2 previously characterized (18) clinical isolates of QRSP (CT01147 and UAB169; gemifloxacin MIC = 1 μg/mL) by using the primers shown in the Table. Phenotypic expression was carried out for 2 to 24 h. First-step trans-

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Table. Primers used in the study

Name	Sequence	Product size (bp)
gyrA-F	5′-TTTAGGTGAAGTGAAGGCAAGGG-3′	1,325
gyrA-R	5′-GAATAACATTGGCTGAGGCGTC-3′	
parC-F	5′-TTTGAAAGGAGTTGAACACGCC-3′	778
parC-R	5′-TCCGTCCATAGAACCGTTATTACC-3′	

formants were generated by the introduction of a *parC* or *gyrA* fragment, and these were selected on 0.06 μg/mL gemifloxacin (SmithKline Beecham Pharmaceuticals, Collegeville, PA, USA). Second-step transformants were generated by the introduction of the second fragment (*gyrA* or *parC*) into first-step transformants, and these were selected on 0.5 μg/mL gemifloxacin, a concentration that effectively inhibited the growth of first-step mutants and permitted the growth of second-step mutants. Two of the isogenic *gyrA, parC* double mutants, Phe/Phe and Phe/Tyr, were chosen for fitness studies. In addition, levofloxacin MICs were determined for these 2 mutants by using broth microdilution.

Competitive Growth of QRSP Mutants In Vitro

Phe/Phe contained a GyrA: Ser81Phe mutation and a ParC: Ser79Phe mutation. Phe/Tyr contained a GyrA: Ser81Phe mutation and a ParC: Ser79Tyr mutation. In vitro competition experiments were carried out between EF3030 and Phe/Phe (N = 7) and between EF3030 and Phe/Tyr (N = 12) by coincubating them in Todd-Hewitt broth containing yeast extract (Difco, Detroit, MI, USA). The number of generations of each strain was calculated as previously described (4) by using the formula g = (logB – logA)/(log2), where relative fitness (RF) = g_{res}/g_{sus}, g is the number of generations, res is gemifloxacin-resistant transformants (Phe/Phe or Phe/Tyr), sus is the gemifloxacin-susceptible parent EF3030, B is the CFU/mL at time 1 (6 h), and A is the CFU/mL at time 0.

Murine Pneumonia Models

For both models of pneumococcal infection, 6-week-old, female CBA/CaHN-*Btk*^{xid}/J (CBA/N) mice (Jackson Laboratories, Bar Harbor, ME, USA) were used. Infection leading to pneumonia and colonization were induced over a period of 7 days, and samples were obtained from nasopharynges, lungs, and blood of mice as previously described (19,20). The pneumonia model entailed anesthetizing the mouse by inhalation of isoflurane before delivery of bacteria in 40 μL of lactated Ringer solution to ensure delivery to the lungs. In the colonization model, nonanesthetized mice were infected intranasally with bacteria in 10μL of lactated Ringer solution to ensure colonization of the nasopharynx, as previously described (19,20). All mouse experiments were carried out under the approval of the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Competitive Growth of QRSP Mutants In Vivo

To determine relative nasopharyngeal growth during colonization, 10 μL of a 1:1 mixture containing 10^6 CFUs each of EF3030 and the fluoroquinolone-resistant mutant (Phe/Phe or Phe/Tyr) were instilled into the nares as described for the colonization model (20). Ten mice received the EF3030 and Phe/Phe mixture, and 10 mice received the EF3030 and Phe/Tyr mixture.

To determine relative growth in the lungs, 40 μL of a 1:1 mixture containing EF3030 and the fluoroquinolone-resistant mutant (Phe/Phe or Phe/Tyr) were instilled into the nares as previously described for the pneumonia model (19). For the EF3030 and Phe/Phe competitive infections, 9 mice received 10^4 CFUs of each strain, and 14 mice received 10^6 CFUs of each strain. For the EF3030 and Phe/Tyr competitive infections, 9 mice received 10^4 CFUs of each strain, and 14 mice received 10^6 CFUs of each strain. Initially, the lower dose was used because of concerns for mouse mortality. When this turned out not to be an issue, the infectious dose was raised to 10^6 CFU to increase lung infection levels, yield more countable colonies, and allow the effects of a range of infectious doses to be examined.

For recovery of EF3030 and mutants from mice in the pneumonia and colonization models, mice were killed 7 days postinfection, samples were collected, and CFUs were counted in nasal washes, lungs, and blood as described previously (19,20). Serial dilutions of specimens were cultured with gentamicin (which allowed growth of EF3030 and both mutants but reduced growth of oral commensal organisms) and with gemifloxacin (which allowed growth of only Phe/Phe and Phe/Tyr). Samples were incubated on blood agar plates containing 5 μg/mL gentamicin with or without 0.08 μg/mL gemifloxacin at 37°C for 16 h in a candle jar.

Percentage recovery units (PRUs) were determined for bacteria recovered from mice co-colonized or coinfected with both strains. PRUs were calculated by multiplying the recovery ratio (CFUs recovered from nasal wash or lung homogenate divided by CFUs used to infect mice intranasally) by 10^6 (to simplify statistical comparisons and facilitate visual comparisons).

Noncompetitive Growth of QRSP Mutants in Vivo

To establish noncompetitive pneumococcal infections with EF3030, Phe/Phe, and Phe/Tyr, 10^6 CFUs were used for colonizations, and 10^7 CFUs were used for lung infections, as described above. EF3030 was used to infect 39 mice (10 for colonization and 29 for pneumonia), Phe/Phe was used to infect 25 mice (5 for colonization and 20 for

pneumonia), and Phe/Tyr was used to infect 24 mice (5 for colonization and 19 for pneumonia). Mice were killed after 7 days, and samples were collected and analyzed as described above.

Statistical Analysis

The Wilcoxon matched-pairs signed-rank test was used to compare the numbers of generations for each competing pair in in vitro competitive growth experiments and to compare the PRUs in in vivo competitive infections. For noncompetitive infections, PRUs of EF3030, Phe/Phe, and Phe/Tyr were compared by using the Mann-Whitney unpaired 2-tailed test. Statistical tests were conducted with the InStat program (GraphPad Software, Inc., San Diego, CA, USA). A p value <0.05 was considered statistically significant.

Results

QRDR Mutations

The QRDR mutations in *gyrA* and *parC* of the clinical QRSP (donor strains CT01147 and UAB169), the mutants (Phe/Phe and Phe/Tyr), and parent strain (EF3030) were sequenced to confirm the presence of QRDR mutations and because genetic transformation has been associated with increased mutation frequency (21,22). The transformation fragment for *gyrA* consisted of 1,325 bp, of which 660 inclusive of the QRDR were sequenced. Likewise, the transformation fragment for *parC* consisted of 778 bp, of which 446 were sequenced. The *gyrA* and *parC* QRDR mutations in the mutants (Phe/Phe and Phe/Tyr) matched those of the corresponding donor strains (CT01147 and UAB169). Phe/Phe also contained 2 additional synonymous, nonquinolone resistance–conferring mutations in *gyrA* (data not shown). The levofloxacin MICs for the Phe/Phe and Phe/Tyr mutants were both 16 μg/mL, verifying the degree of resistance to the fluoroquinolone class of antimicrobial agents.

Colonization Model

Overall, EF3030 underwent more generations per 6-hour in vitro growth period than either Phe/Phe (p<0.016) (Figure 1A) or Phe/Tyr (p<0.007) (Figure 1B). Of 10 mice intranasally infected with approximately equal amounts (10^6 CFUs) of EF3030 and Phe/Phe, 8 were colonized. Among these 8 mice, EF3030 outcompeted Phe/Phe (p<0.023) (Figure 2A). Of 10 mice intranasally infected with approximately equal amounts of EF3030 and Phe/Tyr, 8 were colonized. Among these 8 mice, EF3030 outcompeted Phe/Tyr (p<0.008) (Figure 2B).

When mice were infected intranasally with 10^6 CFUs of EF3030, Phe/Phe, or Phe/Tyr, Phe/Phe and EF3030 were recovered in similar numbers (p = 1.069), but Phe/Tyr was recovered in much lower numbers than EF3030 (p<0.004) (Figure 2C). Thus, although Phe/Phe was less efficient at nasopharyngeal colonization when competing with EF3030, it colonized as well as EF3030 when tested alone. Phe/Tyr was less efficient than EF3030 at colonizing, whether or not it was in direct competition with EF3030.

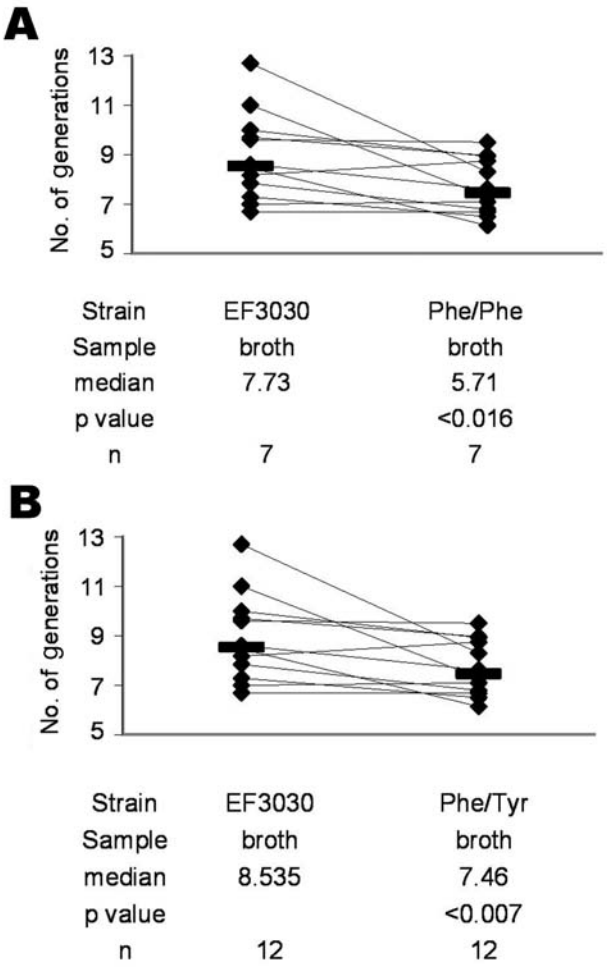

Figure 1. In vitro competition between *Streptococcus pneumoniae* EF3030 and the Phe/Phe mutant (A) and between EF3030 and the Phe/Tyr mutant (B) in liquid medium (broth). Bars indicate medians. Lines connect strains competing in the same broth. p values were calculated by the Wilcoxon matched-pairs signed-rank test.

Pneumonia Model

Of the 23 mice infected with approximately equal amounts (10^4 CFUs of each strain or 10^6 CFUs of each strain) of EF3030 and Phe/Phe, all 23 were colonized nasopharyngeally, and lung infection developed in 13 of 23. EF3030 outcompeted Phe/Phe in both the nasopharynx (p<0.001) and the lungs (p<0.001) (Figure 3A).

Of the 23 mice infected with approximately equal amounts (10^4 CFUs or 10^6 CFUs of each) of EF3030 and

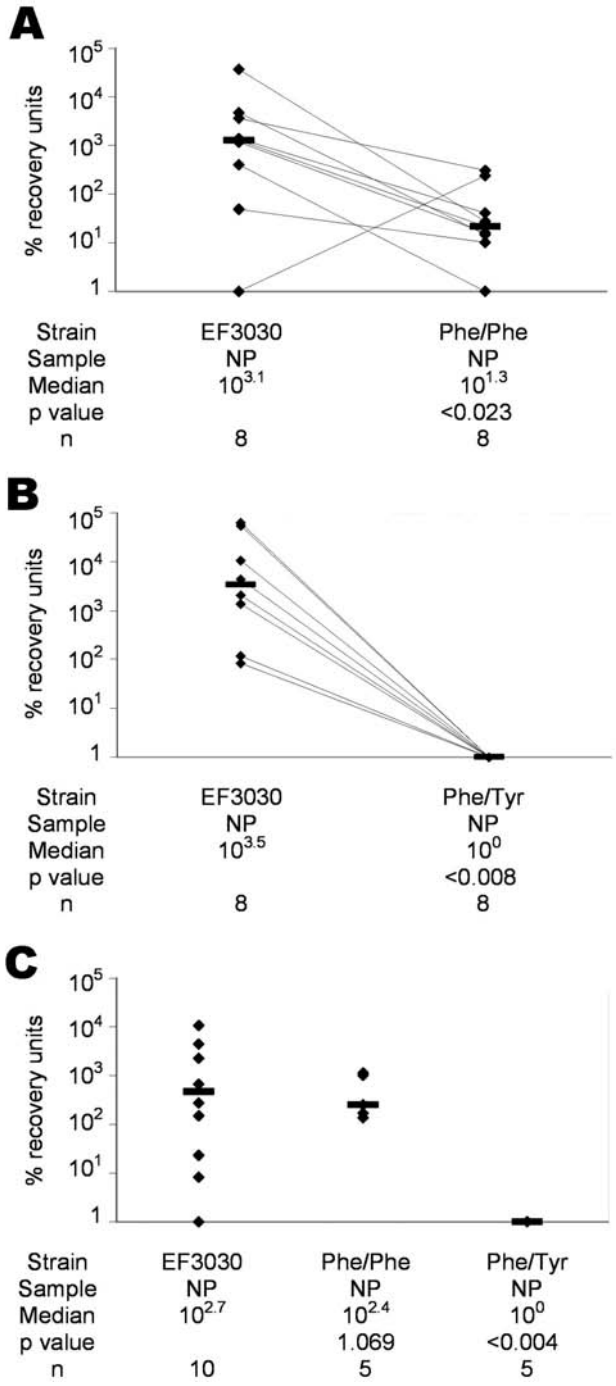

Figure 2. Percentage recovery units (PRUs: CFUs recovered from nasal wash or lung homogenate divided by CFUs originally used to infect mice and multiplied by 106) for competitive colonization between *Streptococcus pneumoniae* EF3030 and the Phe/Phe mutant (A), EF3030 and the Phe/Tyr mutant (B), and noncompetitive colonization of EF3030, Phe/Phe, and Phe/Tyr (C). Lines connect data from the same mouse. Bars indicate median PRUs. NP, nasopharynx. p values were calculated by the Wilcoxon matched-pairs signed-rank test (A and B) and the Mann-Whitney unpaired 2-tailed test (C).

Phe/Tyr, all 23 were colonized nasopharyngeally, and lung infections developed in 12 of 23. We observed no significant difference in PRUs with the 2 different inocula. EF3030 outcompeted Phe/Tyr in the nasopharynx ($p<0.008$) but not in the lungs ($p<0.176$) (Figure 3B). Thus, when anesthetized mice were infected with both EF3030 and a mutant (Phe/Phe or Phe/Tyr), EF3030 outcompeted each mutant in the nasopharynx, but EF3030 outcompeted only Phe/Phe in the lungs.

Of the 29 mice monoinfected with 10^7 CFUs of EF3030, 5 died of infection and 24 were colonized nasopharyngeally. Lung infections developed in 19 of these 24 (Figure 3C). Of the 20 mice monoinfected with 10^7 CFUs of Phe/Phe, 4 died of infection and 16 were colonized nasopharyngeally; lung infections developed in all 16. Of the 19 mice monoinfected with 10^7 CFUs of Phe/Tyr, 5 died of presumed pneumonia, and 14 were colonized nasopharyngeally; lung infections developed in all 14.

Among these monoinfections, EF3030 was recovered from the nasopharynx in quantities significantly different from those of Phe/Phe ($p<0.001$) and Phe/Tyr ($p<0.001$) (Figure 3C). In the lungs, however, EF3030 was not recovered in numbers significantly different those of from either Phe/Phe ($p = 0.453$) or Phe/Tyr ($p = 0.152$). Thus, even in the absence of competition, EF3030 was recovered in higher numbers than those of both mutants in the nasopharynx, but was not recovered in higher numbers than those of either mutant in the lungs.

Discussion

Although fluoroquinolone resistance in *S. pneumoniae* remains very low in North America, it has begun to increase in recent years (15,23) and is especially high in some Asian countries that already have high β-lactam and macrolide resistance rates (24). Pneumococcal resistance to fluoroquinolones is largely mediated by de novo point mutations in the *gyrA* and *parC* genes encoding DNA gyrase and topoisomerase, respectively, in the QRDRs (25). A specific single-point mutation in either of these genes confers low-level resistance, with high-level resistance generally requiring a point mutation in both *gyrA* and *parC* QRDRs. QRSP are generally clonally unrelated, although there have been some reports of clonal dissemination, and fluoroquinolone resistance has now been reported in several international clones (10–13,26).

The fitness of pathogenic bacteria to cause disease relies on several factors, including colonization of the host, evasion of host defenses, propagation on or inside the host, and transmission to a new host. Antimicrobial resistance can be associated with a decrease in bacterial fitness (3,27). A measure of fitness of antimicrobial-resistant pathogens could aid in the prediction of the future rates of

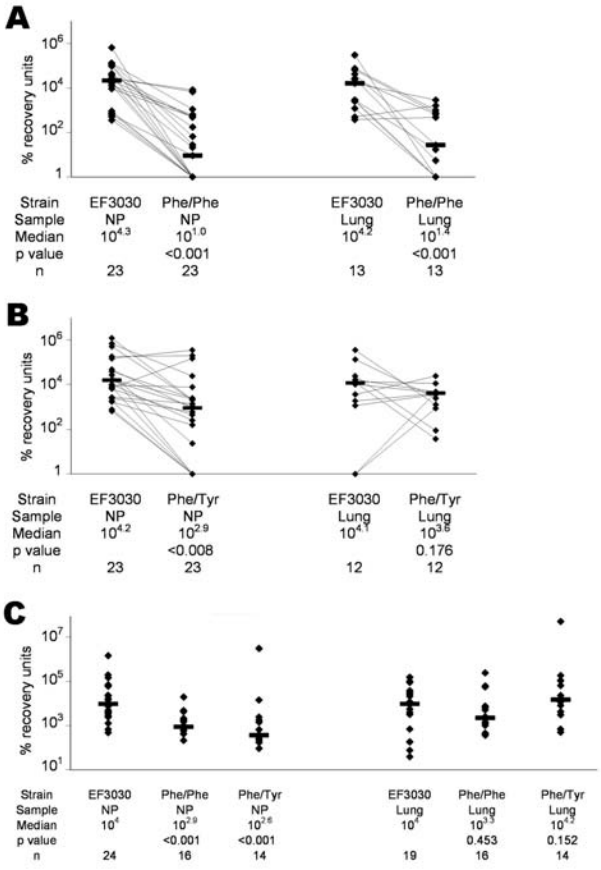

Figure 3. Percentage recovery units (PRUs: CFUs recovered from nasal wash or lung homogenate divided by CFUs originally used to infect mice and multiplied by 106) for competitive pneumonia infection with *Streptococcus pneumoniae* EF3030 and the Phe/Phe mutant (A), EF3030 and the Phe/Tyr mutant (B), and noncompetitive pneumonia with EF3030, Phe/Phe, and Phe/Tyr (C). Lines connect data from the same mouse. Bars indicate median PRUs. NP, nasopharynx. p values were calculated by the Wilcoxon matched-pairs signed-rank test (A and B) and the Mann-Whitney unpaired 2-tailed test (C).

disease caused by these bacteria, guide recommendations for empiric therapy for some bacterial infections, and direct the development of new antimicrobial drugs. Although other studies have investigated the fitness of antimicrobial-resistant pathogens including *S. pneumoniae* (6,7), the focus has frequently been on resistance to β-lactam antimicrobial drugs, and only a few have investigated the relative fitness of QRSP (4,5).

In our current study, we sought to investigate the fitness of QRSP mutants. We postulated that QRSP may have reduced fitness because fluoroquinolone resistance rates remain very low, and naturally occurring QRSP isolates are generally clonally unrelated. When in competition with EF3030, the Phe/Phe mutant, which contains the GyrA: Ser81Phe and ParC: Ser79Phe mutation combination often found in clinical QRSP (11,18,24,28–30), was inferior in all 3 models tested. However, in the absence of competition with EF3030, Phe/Phe was only inferior in nasopharyngeal colonization but was as able as EF3030 to produce lung infection. Conversely, the Phe/Tyr mutant, which contains the GyrA: Ser81Phe and ParC: Ser79Tyr mutation combination found more often in laboratory-selected mutants than in clinical QRSP (18,24,28–30), was inferior in vitro and in nasopharyngeal colonization but was as able as EF3030 to produce lung infection, regardless of competition from EF3030. Though counterintuitive, this probably occurred because of the nature of the lung infection model, in which bacteria are intranasally instilled into anesthetized mice without the prerequisite for nasopharyngeal colonization. In fact, nasopharyngeal colonization resulting from the lung infection model is more the result of retrograde movement of bacteria from the lungs to the nasopharynx than of initial collection of bacteria in the nasopharynx when first infected. Why QRDR mutations tended to confer more fitness costs in the nasopharyngeal mucosa than in the lungs is not clear, but it is possible that commensal bacteria may have provided more competition in the nasopharynx than in the lungs, and therefore the mutants displayed greater fitness reductions when in competition with both wild-type *S. pneumoniae* and commensal bacteria. Alternatively, phase variation in pneumococcal opacity may play a role in the difference in fitness of the mutants in the lung versus the nasopharynx, since the opaque phase tends to predominate in invasion, and the transparent phase predominates in colonization (31). These 2 phases express very different complements of virulence factors, which suggest that the processes involved in bacterial survival in these 2 niches can be very different.

In mice that had been colonized and in those with lung infections, fewer organisms were recovered than were infected, i.e., no bacterial growth was detectable in the animals. It may be postulated that these models are simply measuring the relative death rates of the Phe/Phe and Phe/Tyr mutants, as compared to EF3030, and not actual survival and growth. In a study by Balachandran et al. (32), evidence was presented indicating that pneumococci multiply during colonization. To our knowledge, no studies have investigated pneumococcal turnover in the lung, but since the lungs of CBA/N mice contain many neutrophils (33), the bacteria would likely have to multiply to compensate for being killed, based on the number of bacteria recovered from the lungs.

Although several studies have investigated the fitness of antimicrobial-resistant pathogens (8,34,35) and antimicrobial-resistant *S. pneumoniae* (6,7,36,37), few have investigated the fitness of fluoroquinolone-resistant bacteria (4,5). Our results are in contrast to those of Gillespie

et al. (4), who found a significant decrease in the relative fitness of Tyr/Tyr, but not of Phe/Tyr, compared to wild-type, in in vitro growth experiments with *S. pneumoniae*. Conversely, our results are supported by Giraud et al. (8), who reported a decrease in relative fitness in high-level fluoroquinolone-resistant *Salmonella enterica* serovar Typhimurium in in vitro growth and chicken gut colonization experiments, and by Azoulay-Dupuis et al. (5), who demonstrated that clinical strains of QRSP were less virulent in outbred mice than a quinolone-susceptible laboratory strain and its quinolone-resistant isogenic mutant.

If QRSP are less efficient than fluoroquinolone-susceptible *S. pneumoniae* at colonizing humans, the result could explain the few reports of clonal lineages of QRSP. Nasopharyngeal colonization precedes pneumonia and is the reservoir from which person-to-person transmission occurs (38). Therefore, a pneumococcus that is inefficient in colonizing the nasopharynx would be less efficient in producing lung infection, no matter how efficiently the organism infects the lungs; likewise, this organism would be less likely to disseminate clonally in the community. However, the fact that lung infection is not attenuated by fluoroquinolone resistance indicates that the resistant strains selected in patients by antimicrobial treatment may still cause severe disease, and possibly death, as has been reported (39,40).

We have attempted to measure the relative fitness of the 2 most commonly occurring QRDR mutation combinations. While different mutations may have different affects on fitness, we found that strains containing these common QRDR mutations appeared to have reduced fitness in the absence of antimicrobial drugs both in vitro and in vivo. Thus, QRSP may have reduced ability to initiate infections in the absence of fluoroquinolone selection and may be inefficient at displacing resident susceptible strains and therefore causing disease. This suggests that the judicious use of antimicrobial drugs may keep the prevalence of QRDR clones low because of their relatively low fitness.

The few reports of clonal spread of QRSP and of fluoroquinolone resistance in multidrug-resistant isolates raise the possibility that these isolates may have already acquired compensatory mutations. Continued surveillance is very important in understanding the epidemiology of QRSP. Overall, fluoroquinolone resistance rates remain very low, most resistance arises in genetically diverse strains, and clonal dissemination is likely still not a major contributor to the appearance of QRSP.

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Global Spread of Vancomycin-resistant *Enterococcus faecium* from Distinct Nosocomial Genetic Complex

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Vancomycin-resistant enterococci (VRE) have caused hospital outbreaks worldwide, and the vancomycin-resistance gene (*vanA*) has crossed genus boundaries to methicillin-resistant *Staphylococcus aureus*. Spread of VRE, therefore, represents an immediate threat for patient care and creates a reservoir of mobile resistance genes for other, more virulent pathogens. Evolutionary genetics, population structure, and geographic distribution of 411 VRE and vancomycin-susceptible *Enterococcus faecium* isolates, recovered from human and nonhuman sources and community and hospital reservoirs in 5 continents, identified a genetic lineage of *E. faecium* (complex-17) that has spread globally. This lineage is characterized by 1) ampicillin resistance, 2) a pathogenicity island, and 3) an association with hospital outbreaks. Complex-17 is an example of cumulative evolutionary processes that improved the relative fitness of bacteria in hospital environments. Preventing further spread of this epidemic *E. faecium* subpopulation is critical, and efforts should focus on the early disclosure of ampicillin-resistant complex-17 strains.

The emergence of vancomycin-resistant enterococci (VRE) followed a worst-case scenario for nosocomial pathogens: the first VRE isolates that harbored the *vanA* transposon were identified in 1987 in Europe (1,2), and within 10 years VRE represented >25% of enterococci associated with bloodstream infections in hospitalized patients in the United States (3).

Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals. Two species cause most enterococcal infections, *Enterococcus faecalis* and *E. faecium*. The relative importance of *E. faecium* as a pathogen has increased with the occurrence of high-level resistance to multiple antimicrobial drugs, such as ampicillin and vancomycin (4). The rapid increase of vancomycin resistance compromises physicians' ability to treat infections caused by many of these strains because often no other antimicrobial drugs are available. The epidemiology of VRE infection differs between Europe and the United States. In Europe, VRE are frequently isolated from farm animals, which have been associated with the abundant use of avoparcin as a growth promoter in the agricultural industry, until it was banned in 1997 (5). The reported prevalence of VRE in hospitals has been low, but increasing rates (>10%) in stool and clinical samples were reported recently (6–9). In the United States, avoparcin was never approved for use in agriculture, and neither were any other glycopeptides; consequently, VRE have not been found in animals or healthy persons. However, nosocomial VRE infection and transmission have occurred much more frequently in the United States. Recent reports have documented, in hospitalized patients, horizontal transfer of the *vanA* gene from vancomycin-resistant *E. faecalis* to methicillin-resistant *Staphylococcus aureus* (MRSA), creating MRSA with high-level resistance to vancomycin (10–13). Nosocomial spread of VRE may therefore create a reservoir of mobile resistance genes for other, more virulent, nosocomial pathogens. Without extensive control measures, large-scale emergence of vancomycin-resistant *S. aureus* (VRSA) may be the next stage in the global crisis of antimicrobial resistance.

The existence of VRE in different ecologic niches complicates the understanding of its epidemiology. Although previous molecular epidemiologic studies on limited numbers of strains suggested host specificity and overrepresentation of certain clones in hospital outbreaks (14,15), these studies did not elucidate the patterns of evolutionary

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descent among VRE. We determined the population structure of 411 VRE and vancomycin-susceptible *E. faecium* (VSE) isolates by using multilocus sequence typing (MLST), explored the evolutionary origin of epidemic isolates associated with documented hospital outbreaks and other isolates, and assessed the association with ampicillin resistance and the presence of a recently discovered putative pathogenicity island (PAI) in *E. faecium* (16).

Materials and Methods

The strain collection included 5 categories of VRE and VSE: 1) 96 animal surveillance (bison, calves, cats, dogs, ostriches, poultry, pigs, rodents) isolates (43 VRE, 53 VSE) from 7 countries in Africa and Europe; 2) 57 epidemiologically unrelated community surveillance isolates (20 VRE, 37 VSE) from nonhospitalized persons from 7 countries in Australia and Europe; 3) 64 epidemiologically unrelated surveillance (fecal) isolates (45 VRE, 19 VSE) from hospitalized patients not linked to hospital outbreaks from 9 countries in Australia, Europe, and North and South America; 4) 162 epidemiologically unrelated hospital isolates (43 VRE, 118 VSE, 1 not determined) from clinical specimens (blood, pus, and urine) from 17 countries in Africa, Australia, Europe, and North and South America; and 5) 1 strain from each of 32 different documented hospital outbreaks (28 VRE, 4 VSE) in 10 countries in Australia, Europe, and North and South America (W. Grubb and D. Jonas, pers. comm.; 15,17–23).

We determined vancomycin susceptibilities for 410 isolates and ampicillin susceptibilities for 381 isolates by using standard agar dilution methods according to NCCLS guidelines. Isolates with MIC ≥16 μg/mL for ampicillin and ≥8 μg/mL for vancomycin were considered to be resistant. In total, 394 strains were screened for the *esp* gene with primer sets and amplification conditions described previously (24). Independent and combined effects of virulence and resistance markers on the abundance of complex-17 were estimated by using multiple logistic regression analysis (Stata 7.0, StataCorp LP, College Station, TX, USA).

MLST was carried out with a standard set of primers that amplify the 7 genes included in the *E. faecium* MLST scheme (14). Information on these loci, the latest set of primers, amplification conditions, and details of all isolates are available on the MLST Web site (http://efaecium.mlst.net).

The eBURST program was used to assess the genetic relationships of genotypes, to assign isolates to genetic complexes, and to study patterns of evolutionary descent of isolates within a complex (25). Complexes were identified by using the stringent (6/7 shared alleles) group definition with 1,000 bootstrap replicates. The BLAND program was used to examine the relationship between pairwise allelic differences and nucleotide sequence differences (26). If genetic diversity in *E. faecium* is mainly the result of accumulated point mutations, then recently diverged strains will have a high level of similarity in both their allelic profiles as well as in the nucleotide sequence of the nonidentical alleles, which results in a positive correlation between the number of nucleotide differences in nonidentical alleles and the number of allelic differences. However, such a trend will be absent when recombination plays an important role in generating the genetic diversity, since nonidentical alleles of closely related isolates can differ at multiple nucleotide sites.

To assess the effect of recombination on the population structure of *E. faecium* in more detail, the topologies of the 7 MLST gene trees were compared by using the Shimodaira-Hasegawa test (27). Briefly, maximum likelihood trees for each MLST gene were obtained under a general time-reversible model, with a proportion of invariant sites and rate heterogeneity among sites assuming a discrete gamma distribution with 8 categories (GTR+I+Γ model). PAUP* 4.0b10 was used to obtain the maximum likelihood trees by using a neighbor-joining starting tree followed by tree-bisection reconnection branch swapping (28). For a given gene, the Shimodaira-Hasegawa test compares the difference in log likelihoods of competing tree topologies. A null distribution of differences in log likelihoods was obtained by 1,000 replicates of nonparametric bootstrapping of reestimated log likelihoods. We conducted 107 Shimodaira-Hasegawa tests for each MLST gene by comparing the 7 MLST gene trees and 100 random trees separately generated for each of the MLST genes. In a clonal population, the different MLST housekeeping genes have similar tree topologies, but with recombination, the different genes may have different tree topologies that may fit random trees better.

Associations between ampicillin resistance, presence of a novel putative *E. faecium* PAI, and genetic clustering in complex-17 were described by linear logistic regression models: log odds = $b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \ldots + b_ix_i$. Log odds denotes the natural logarithm of the proportion of samples from an epidemiologic group belonging to complex-17, b_i denotes the parameter estimated by maximum likelihood methods and x_i the level of exposure, e.g., 0 and 1 for ampicillin resistance, vancomycin resistance, and the presence of PAI and 0–4 for the epidemiologic source of isolates: animal surveillance, community surveillance, hospital surveillance, clinical sample, and hospital outbreak, respectively.

Results

Identification of Clonal Lineages

MLST of 411 *E. faecium* isolates resulted in 175

different sequence types (ST). Clustering these types with the eBURST algorithm (25) showed 1 large complex of genetically related types. ST-22 was the primary founder; 3 minor complexes had ST-1, -69, and -94 as primary founders; 6 complexes had only 2 or 3 STs; and 57 singletons were not linked to the aforementioned complexes (Figure 1). Within complex-22, ST-17 represents an important secondary founder of a distinct branch designated complex-17.

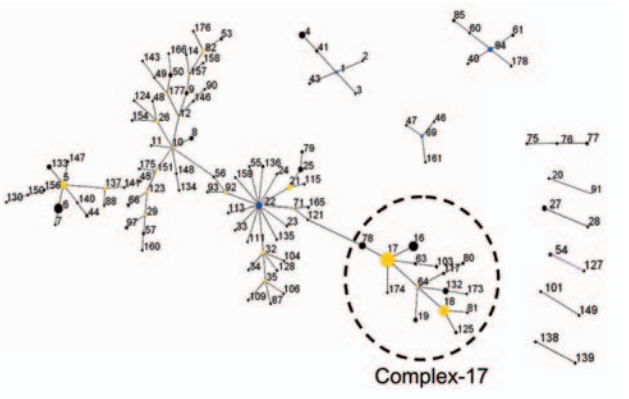

Figure 1. Clustering of 175 sequence types representing 411 isolates with eBURST (25). This algorithm identifies the founder of a complex or genogroup of related sequence types (ST) and subsequent patterns of evolutionary descent. The primary founder, indicated in blue, of a complex is defined as the ST with the largest number of single locus variants (SLVs). Larger complexes may contain secondary founders of additional lineages that have a number of SLVs of their own. These secondary founders are indicated in yellow. Numbers correspond to ST numbers. The area of each circle corresponds to the number of isolates of the ST. All complexes (major and minor) are shown. In addition, 57 STs did not group into any of the complexes and were considered singletons (STs 13, 15, 30, 31, 36, 37, 38, 39, 42, 51, 52, 58, 59, 62, 65, 67, 68, 70, 72, 73, 74, 83, 84, 86, 89, 95, 96, 98, 99, 100, 102, 105, 107, 108, 110, 112, 114, 116, 118, 126, 129, 131, 142, 144, 145, 152, 153, 155, 162, 163, 164, 167, 168, 169, 170, 171, 172). The "epidemic" genetic complex-17 derived from secondary founder ST-17 is indicated. A measurement of statistical confidence in each of the assigned primary founders is made by a bootstrap resampling procedure (25).The predicted primary founders of the complexes 22, 94, 1, and 69 have a bootstrap value of 73%, 84%, 85%, and 59%, respectively.

Selective Advantage of the Successful Hospital-adapted Complex-17

In all, 142 of 411 isolates belonged to complex-17, with a gradual increase in proportion among animal isolates (1/96), human community isolates (3/57), human hospital surveillance isolates (15/64), and human clinical isolates (95/162), to hospital-outbreak isolates (28/32) (Table 1). Ampicillin resistance, presence of the *E. faecium* PAI (16), and genetic clustering in complex-17 were strongly associated (Table 2).

When controlling for individual and combined effects of ampicillin resistance, presence of PAI, and vancomycin resistance, we can show that 1) the loglinear assumption holds for all effect parameters, and linear models describe the observed frequencies without substantial loss of goodness of fit; 2) individual genetic markers exert an independent and multiplicative effect; and 3) all genetic markers combined explain ≈48% of the category-specific abundance of complex-17 (Figure 2). The effect of vancomycin resistance did not increase the explanatory value of the model, owing to the fact that determinants for vancomycin resistance could be found in equal proportions within and outside of complex-17, likely a result of

Table 1. Frequency of ampicillin and glycopeptide resistance, the presence of the pathogenicity island (PAI), and log odds of all complex-17 and non–complex-17

Epidemiologic source	Genetic and phenotypic features*												Complex-17‡	Other†‡	Log odds§
	Complex-17		Other†		Complex-17		Other†		Complex-17		Other†				
	AmR	AmS	AmR	AmS	PAI+	PAI–	PAI+	PAI–	VanR	VanS	VanR	VanS			
Animal surveillance (n = 96), %	1	0	2	93	0	1	0	94	0	1	43	52	1 [1]	95 [99]	–4.55
Community surveillance (n = 57), %	0	0	1	46	3	0	0	47	3	0	17	37	3 [5]	54 [95]	–2.89
Hospital surveillance (n = 64), %	14	0	7	40	7	8	0	49	13	2	32	17	15 [23]	49 [77]	–1.18
Clinical (n = 162), %	85	2	13	47	47	47	4	57	21	73	22	45	95 [59]	67 [51	0.35
Hospital outbreak (n = 32), %	26	0	3	1	20	6	3	1	24	4	4	0	28 [88]	4 [12]	1.95

*Ampicillin resistant (AmR) or susceptible (AmS) not determined in 30 isolates, PAI present (PAI+) or absent (PA–) not determined in 17 isolates, vancomycin resistant (VanR) or susceptible (VanS) not determined in 1 isolate.
†Not belonging to complex-17.
‡Numbers in brackets refer to the percentage of isolates that belong to the complex.
§The natural logarithm of the proportion of samples from an epidemiologic source belonging to complex-17.

Table 2. Parameter estimates by using a logistic regression model*

Regression lines	Parameter estimates					p value
	b_0	b_{amp}	b_{PAI}	b_{gly}	b_{epi}	
Complex-17 crude	–4.44	0	0	0	1.6	0.000
Corrected for amp	–6.61	5.38	0	0	1	0.000
Corrected for PAI	–6.29	5.08	1.06	0	0.84	0.038
Corrected for gly	–6.07	5.12	1.01	–0.45	0.83	0.316

*Amp, ampicillin resistance; PAI, presence of the *Enterococcus faecium* pathogenicity island; gly, glycopeptide resistance; epi, epidemiologic source (animal surveillance, community surveillance, hospital surveillance, clinical sample, hospital outbreak).

widespread horizontal transfer of *vanA* (Table 1). This finding suggests that the epidemiologic success of descendants of ST-17 that results in clinical infections and hospital epidemics was at least partly related to antimicrobial resistance and the presence of putative virulence genes. The fact that 126 of 128 isolates of complex-17 were resistant to ampicillin and only 77 of 139 isolates of complex-17 contain PAI (Table 1) suggests that *E. faecium* acquired ampicillin resistance first, which resulted in a selective advantage in hospitals, followed by the acquisition of PAI, which further facilitated transmission.

Estimates of Recombination

To assess the effect of recombination on the population structure of *E. faecium*, we estimated whether single locus variants (SLVs) from the presumed founders of complex-1, -17, -22, -69, and -94 have arisen by point mutations or by recombination (Table 3). Of all allelic differences between ancestor-SLV pairs (n = 30), 22 (4 in complex-17) included >1 nucleotide, were found in multiple clonal complexes, and thus were most likely a result of recombination. Eight allelic differences (2 in complex-17) included only a single nucleotide change, were unique within the dataset, and thus were most likely a result of mutation. Therefore, most alleles of SLVs in complex-17 and in the other complexes have arisen by recombination in the initial stages of diversification rather than by de novo point mutation. An important role for recombination in genetic diversification in *E. faecium* was confirmed by the lack of a positive trend between the number of nucleotide differences in nonidentical alleles and the number of allelic differences (Figure 3) (26). The finding of high average numbers (≥4) of nucleotide differences in the nonidentical alleles of SLVs in the total *E. faecium* population as well as in complex-17 also points towards frequent recombination.

The degree of phylogenetic congruence between the 7 MLST genes was examined in a set of 24 diverse STs. These 24 STs were separated from each other by a linkage distance of >0.4 on a UPGMA (unweighted pair-group method with arithmetic mean) tree constructed from the pairwise comparisons of their allelic profiles (data not shown) and included the primary founders of CC1 (ST1), CC22 (ST22), CC69 (ST69), and CC94 (ST94); secondary founders of important subgroups complex-5 (ST5) and complex-17 (ST17); 1 ST (ST76) belonging to a small complex of 3 STs; and 17 singletons (STs 15, 38, 39, 54, 67, 74, 83, 84, 89, 96, 98, 99, 101, 107, 118, 142, 163). The results of the congruence analysis presented in Table 4 show that 25 (60%) of 42 of the pairwise comparisons of the 7 MLST loci were incongruent. Of the 7 genes, *atpA* is the most incongruent. This analysis confirms that recombination played a substantial role in the evolution of *E. faecium*.

Discussion

Nosocomial VRE, which rapidly emerged in the United States in the 1990s after their initial discovery in Europe, are found in increasing rates in hospitals in Europe, Asia, and South America (5–7,9,23,29,30). The data presented in

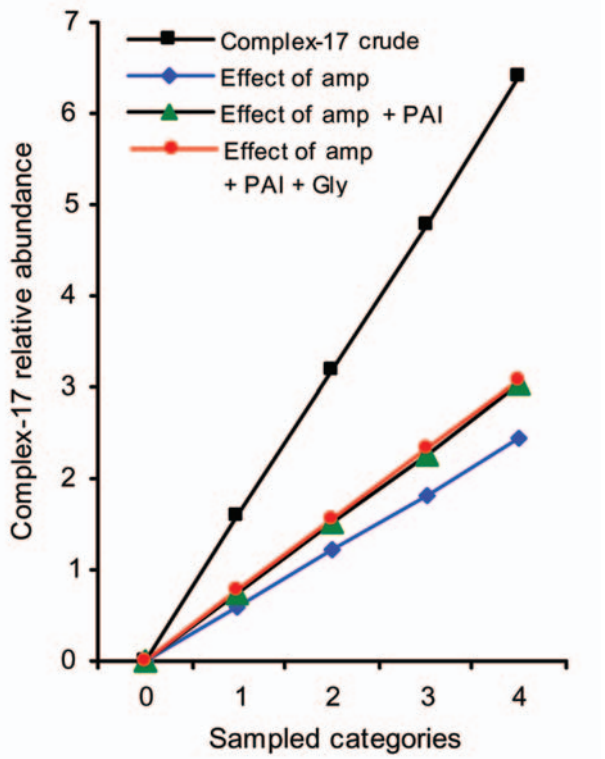

Figure 2. Relative abundance of complex-17 in various sampled categories and proportion increase explained by combined effect of 3 parameters. 0, animal surveillance samples; 1, human community surveillance samples; 2, human hospitalized patient samples; 3, human clinical samples; 4, hospital outbreak samples; amp, ampicillin resistance; PAI, pathogenicity island; gly, glycopeptide resistance.

Table 3. Variant alleles of single locus variants (SLVs) within 5 genetic complexes*

ST of ancestor	ST of SLV	Variant locus	Ancestral allele	SLV allele	No. nucleotide differences (amino acid change)
17	64	*atpA*	1	7	4
17	117	*atpA*	1	9	20
17	78	*atpA*	1	15	22
17	16	*ddl*	1	2	1
17	63	*purK*	1	21	1 (C-Y)†
17	174	*purK*	1	29	1†
22	32	*atpA*	2	3	2
22	21	*atpA*	2	9	18
22	92	*atpA*	2	5	19
22	71	*atpA*	2	15	20
22	135	*atpA*	2	27	18
22	159	*atpA*	2	30	1†
22	113	*atpA*	2	26	19
22	55	*ddl*	3	1	6
22	111	*gdh*	1	6	1
22	24	*purK*	2	7	3
22	136	*purK*	2	26	1 (H-Y)†
22	33	*pstS*	1	5	1 (L-V)†
22	23	*adk*	1	7	1†
1	43	*atpA*	8	3	16
1	41	*purK*	7	3	4
1	2	*gyd*	1	9	1 (Y-H)†
1	3	*pstS*	1	12	1 (Y-N)†
94	40	*atpA*	13	10	3
94	60	*gyd*	6	11	1
94	61	*pstS*	10	17	4
94	178	*pstS*	10	27	2
69	46	*atpA*	9	5	1
69	161	*atpA*	9	3	16
69	47	*adk*	6	5	4

*Genetic complexes 1, 17, 22, 69, and 94 were included in this analysis. ST, sequence type; C, cysteine; Y, tyrosine; H, histidine; L, leucine; V, valine; N, asparagine.
†Single nucleotide changes that are unique in the dataset and thus are due to mutation.

this study show that most of these hospital-derived VRE are part of a single clonal lineage. This lineage, designated complex-17 after its presumed founder ST-17, represents most hospital outbreak and clinical isolates, apparently because it successfully adapted to hospital environments.

The >400 strains analyzed in this study were selected from a large representative collection of 2,000 *E. faecium* isolates. A wide variety of sources were used as selection criteria: hospital-associated outbreaks; clinical samples and stool samples from hospitalized patients, healthy persons, and animals; and a wide geographic distribution (21 countries on 5 continents).

Complex-17 probably evolved from the primary *E. faecium* ancestor ST-22 through a combination of mutation and recombination. The following observations suggest that recombination has been especially important in the genetic diversification of the *E. faecium* population: 1) within clonal complexes, most SLVs (73%) have arisen by recombination rather than point mutations; 2) no positive correlation exists between the degree of allelic diversity and the number of nucleotide differences in nonidentical alleles; and 3) most (60%) of the comparisons of MLST gene tree topologies were incongruent.

Exploitation of a novel ecologic niche as hospital settings by *E. faecium* ST-17 often starts with adaptive changes (31). On the basis of our findings, we postulate that ST-17 acquired ampicillin resistance and a novel putative PAI. This amplifying selective process in which variants with a selective advantage can more easily acquire additional adaptive mechanisms has been called "genetic capitalism" (32). After successfully exploiting the hospital environment, ST-17 increased in frequency to become the dominant clone. Genetic diversification over time finally resulted in a meroclone, complex-17, of highly related genotypes, fully adapted as a nosocomial pathogen, that has spread globally (Figure 4). In addition, *E. faecium* STs, predominantly ST-78, belonging to complex-17, have recently also been found in the Republic of South Korea (K.S. Ko and J.-H. Song, pers. comm.). Considering the short period in which multiresistant *E. faecium* emerged as a nosocomial pathogen (33), complex-17 represents the first globally dispersed nosocomial-adapted clonal lineage.

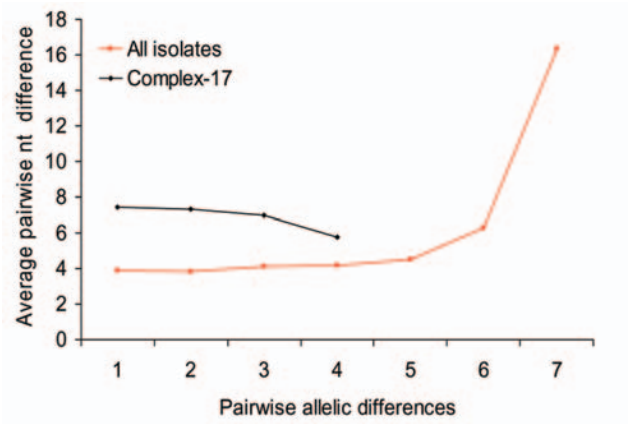

Figure 3. Sequence diversity versus allelic diversity. The average number of nucleotide (nt) differences in nonidentical alleles for all pairwise comparisons of the 178 *Enterococcus faecium* sequence types (STs), and the 15 STs belonging to complex-17 was calculated separately for allelic profiles that differ in 1–7 alleles. This computation shows no positive correlation between the number of nucleotide differences and allelic differences, which suggests that recombination has played an important role in the genetic diversification in *E. faecium*, including the STs that constitute complex-17.

Table 4. Summary of gene congruence analysis

Gene	No. incongruence genes by SH test*	Random trees†
adk	1 (*atpA*)	8 (*atpA*)
atpA	6 (*adk*, *ddl*, *gdh*, *gyd*, *pstS*, *purK*)	76 (*adk*)
ddl	6 (*adk*, *atpA*, *gdh*, *gyd*, *pstS*, *purK*)	8 (*atpA*)
gdh	1 (*atpA*)	1 (*atpA*)
gyd	2 (*adk*, *atpA*)	0 (*atpA*)
pstS	6 (*adk*, *atpA*, *ddl*, *gdh*, *gyd*, *purK*)	3 (*atpA*)
purK	3 (*atpA*, *adk*, *gyd*)	1 (*atpA*)

*Number of incongruent genes at the p<0.05 level based on a Shimodaira-Hasegawa (SH) test of tree topologies. The incongruent genes are in parentheses.
†Number of random tree topologies out of 100 random trees that are better fit to the gene tree from the most incongruent multilocus sequence typing (MLST) gene. The most incongruent MLST gene is given in parentheses.

Despite the frequency of recombination events, clonal complex-17 is still detectable within the *E. faecium* population, which suggests that the emergence of this complex is relatively recent.

The existence of epidemic clones, even in recombining populations, is also seen in other bacterial species (34,35). However, the evolution of a single epidemic and clinically relevant genetic complex, as seen with *E. faecium*, differs from the evolution of other gram-positive pathogens like *Streptococcus pneumoniae* and *Staphylococcus aureus*. In *S. pneumoniae*, pandemic clones such as ST81, ST90, and ST156 represent major invasive and multidrug-resistant isolates that have spread globally (36). The allelic profiles of these clones, however, are highly diverse, which suggests that they are genetically unrelated and do not constitute a single genetic lineage, as does *E. faecium*. Furthermore, the serotype of *S. pneumoniae* seems a more important marker of invasiveness than the overall genotype (37). In *S. aureus* isolates, major pandemic MRSA clones that are responsible for most hospital-acquired infections are found in multiple genetically unrelated lineages, though most previously identified pandemic clones are found in clonal complex 8 (38,39). Therefore, the genetic diversity of major epidemic clones as seen in *S. pneumoniae* or *S. aureus* may not have yet emerged in *E. faecium* epidemic populations.

Stress-inducing conditions in hospitals, such as antimicrobial drug use, may have favored the selection of an enterococcal subpopulation, complex-17, with enhanced antibacterial resistance, virulence, and ability to spread. Whether reducing antimicrobial selection pressure in hospitals will reestablish a susceptible and less transmissible enterococcal population is unknown and will at least partly depend on the relative fitness costs of sustaining antimicrobial resistance and virulence determinants in *E. faecium*. Furthermore, the hospital-adapted complex-17 has rapidly spread globally during the last 2 decades. Subsequent acquisition of *vanA*- or *vanB*- containing transposons by horizontal gene transfer resulted in VRE with pandemic potential. Rapid diagnosis of complex-17 strains based on multiple locus variable number of tandem repeat analysis (MLVA) may help control its spread (40). Whether this effort will be successful depends on the level of complex-17 endemicity in the hospital. In many European countries, a relatively large community reservoir of VRE exists, a result of the massive use of the antimicrobial drug avoparcin as a growth promoter, while in general the prevalence of hospital-adapted (complex-17) VRE is much lower. In such a setting, hospital transmission of isolates

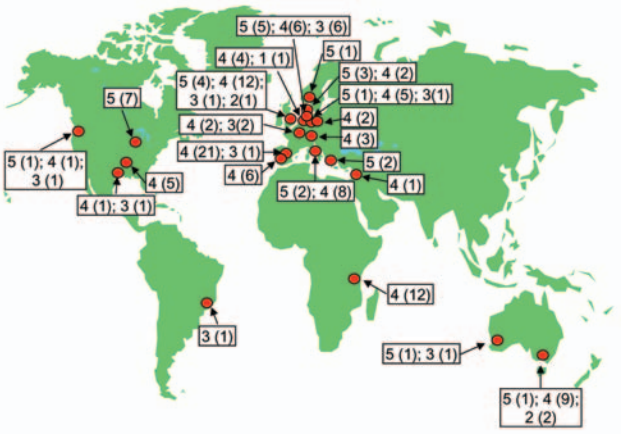

Figure 4. Global distribution of complex-17 isolates. Red circles indicate cities where complex-17 isolates were recovered. Numbers indicate epidemiologic sources: 1, animal isolates; 2, human community surveillance isolates; 3, surveillance (feces) isolates from hospitalized patients; 4, human clinical isolates; 5, isolates from documented hospital outbreaks. Numbers of isolates are indicated in parentheses.

belonging to complex-17 can be halted by using a fast genotyping scheme like MLVA to discriminate between hospital-adapted (complex-17) and community *E. faecium* strains followed by strict infection control measures. The combination of infection control measures plus genotyping controlled an outbreak of VRE in a Dutch hospital (41).

Establishing nosocomial co-endemicity of VRE and MRSA will facilitate the horizontal transfer of *vanA*- or *vanB*- containing transposons, transforming MRSA into VRSA, with implications for patient care. Until now, 3 sporadic cases of *vanA*-induced VRSA have been reported in the United States in 2002 and 2004. Spread of multidrug-resistant *E. faecium* strains and their resistance genes will have serious implications for health care, and control efforts should focus on early detection of *E. faecium* isolates belonging to complex-17.

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Dr. Willems began this research at the National Institute for Public Health and the Environment and continued at the University Medical Center Utrecht, where he is currently working. His research interests are the molecular epidemiology, population structure, and genetic evolution of multidrug-resistant nosocomial pathogens.

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Community Prescribing and Resistant *Streptococcus pneumoniae*

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We investigated the association between prescribing antimicrobial agents and antimicrobial resistance of *Streptococcus pneumoniae* among children with acute otitis media in southern Israel. During a 6-year period, all prescriptions of a sample of ≈20% of Jewish and Bedouin children <5 years of age were recorded and all pneumococcal isolates from middle ear fluid were collected. Although antimicrobial drug use was significantly higher in Bedouin children, the proportion of *S. pneumoniae* isolates with penicillin MIC ≥1.0 mg/mL was significantly higher in Jewish children. In both populations, antimicrobial prescriptions were markedly reduced over time, especially for penicillins and erythromycin. In contrast, azithromycin prescriptions increased from 1998 to 2001 with a parallel increase in macrolide and multidrug resistance. Penicillin resistance was associated with macrolide resistance. These findings strongly suggest that azithromycin affects increased antimicrobial resistance, including multidrug resistance, in *S. pneumoniae*.

Community-acquired respiratory infections are the main reason for prescribing antimicrobial agents in young children (1). Antimicrobial drug use is a major contributor to the emergence of resistance in respiratory pathogens (2–5). Rates of antimicrobial drug use vary between countries, leading to different rates of antimicrobial-resistant pathogens (6,7). Selection for resistant organisms also depends on the class of antimicrobial agent and pharmacokinetic and pharmacodynamic characteristics of the drug (2,3,7,8).

Acute otitis media is the most common microbial respiratory tract infection in early childhood and a leading reason for antimicrobial drug use in children in most industrialized countries (9). *Streptococcus pneumoniae* and *Haemophilus influenzae* are the 2 most common pathogens isolated from patients with acute otitis media worldwide, comprising >75% of all microbial episodes (10). During the last decade, an alarming increase in resistance of *S. pneumoniae* to many antimicrobial agents has been observed (11–14). In contrast, the prevalence of β-lactamase production in *H. influenzae* is relatively stable.

The dynamics of the relationship between antimicrobial drug use and prevalence of resistance in *S. pneumoniae* has not been systemically studied in children. We attempted to determine the association between prescribing antimicrobial agents and resistance patterns of *S. pneumoniae* recovered from middle ear fluid of children with acute otitis media in southern Israel over a 6-year period. We hypothesized that 1) the dynamics of antimicrobial resistance patterns of *S. pneumoniae* causing acute otitis media are associated with that of prescribing antimicrobial agents, and 2) not all antimicrobial classes contribute equally to resistance in this pathogen.

Materials and Methods

Setting

In southern Israel (the Negev), Jewish and Bedouin populations live side by side; the socioeconomic conditions and the lifestyles of the 2 groups differ, but both have access to the same medical services (both clinic and hospitalization services). The Jewish population is mainly urban, whereas the Bedouin population, formerly composed of desert nomads, is in transition to a western lifestyle (15). The 2 pediatric populations also differ in disease patterns and rates. Hospitalization rates for respiratory and other infectious diseases are higher among Bedouin children (16,17). The only medical center providing hospital services to the Negev is the Soroka University Medical Center, in which all children in the area are born and treated.

Medical insurance in Israel is universal and provided free of charge. Approximately 60% of Jewish children and 85%–90% of Bedouin children in the Negev are insured in the largest health plan in Israel, the General Health Insurance Plan. Antimicrobial treatment policies are identical in the 2 populations since drug formulations, prices,

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and availability of antimicrobial agents are the same at all General Health Insurance Plan clinics. Criteria for referral to a hospital are also similar, and no change in policy occurred during the study period regarding referral to a pediatric emergency room with a recommendation to perform tympanocentesis.

The 7-valent pneumococcal conjugate vaccine (Prevenar, Wyeth-Lederle Vaccines, Pearl River, NY, USA) has been licensed in Israel since 2004, but has not yet been introduced into the country because of a supply shortage. Thus, none of the children were vaccinated during the study.

Measuring Antimicrobial Drug Prescriptions

In the Negev region, 53 primary urban General Health Insurance Plan clinics each care for a minimum of 3,000 members. Data were gathered from the 7 largest pediatric primary care clinics where all prescriptions were computerized during all study years. Five clinics, caring for a yearly average population of 6,163 children <5 years age, were located in urban Jewish centers; 2 clinics, caring for a yearly average population of 6,636 children <5 years of age, were located in Bedouin towns. This accounts for ≈20% of the region's children in this age group. Twenty-five to 30 physicians treated children at these clinics. All computerized data regarding prescriptions were generated from the economic department of the General Health Insurance Plan in southern Israel. All antimicrobial drug prescriptions for outpatient children <5 years of age were recorded from 1998 through 2003 and grouped as follows: penicillins (amoxicillin, amoxicillin-clavulanate, and phenoxymethyl penicillin); cephalosporins (cefaclor, cephalexin monohydrate, and cefuroxime-axetil); erythromycin (stearate or ethyl-succinate); and azithromycin. Internal data analysis showed no differences in prescribing antimicrobial agents between the different clinics within each ethnic group.

Microbial Isolates

All middle ear fluid specimens from patients treated at the Soroka University Medical Center pediatric emergency room and patients hospitalized with acute otitis media, and >90% of the middle ear fluid specimens obtained by otolaryngologists in the community, are sent for culture to the Clinical Microbiology Laboratory of this medical center, the only microbiology laboratory in the Negev. Two thirds of the aspirates were obtained at this medical center (40% in the emergency room and another 26% in pediatric wards). Patients at this center with acute otitis media and bulging tympanic membrane undergo tympanocentesis, which is done by an otolaryngologist. In the outpatient clinics (approximately one third of the patients), tympanocentesis is typically carried out in patients with recurrent or nonresponsive acute otitis media. A small proportion of middle ear fluid aspirates were obtained as part of double tympanocentesis studies. All *S. pneumoniae* isolates obtained from the middle ear fluid of children with episodes of acute otitis media from 1999 through 2003 that were processed at Soroka University Medical Center were included.

An episode was defined as a pathogen-free interval of ≥30 days between isolations for the same serotype or by any interval for different serotypes. Only 1 isolate was counted per episode; if the same strain was isolated from both ears, 1 of the 2 was randomly selected.

Microbiologic Analysis

Swabs were placed in MW173 Amies transport medium (Transwab, Medical Wire and Equipment, Potley, UK), plated immediately on trypticase agar media containing 5% sheep blood and 5 μg/mL of gentamicin, and incubated aerobically at 35°C for 48 h. Presumptive identification of *S. pneumoniae* was based on the presence of α-hemolysis and inhibition by optochin and was confirmed by slide agglutination (Phadebact, Pharmacia Diagnostics, Uppsala, Sweden). Serotyping was conducted by using the quellung reaction with reagents from Statens Serum Institut (Copenhagen, Denmark) (18). Susceptibility of *S. pneumoniae* to sulfamethoxazole, tetracycline, erythromycin, clindamycin, and chloramphenicol was determined by using the Kirby-Bauer disk diffusion method and interpreted according to the National Committee for Clinical Laboratory Standards (19). Macrolide resistance was reported as erythromycin resistance. Isolates exhibiting inhibition zones ≤19 mm with a 1-μg oxacillin disk were further tested by Etest (PDM Epsilometer, AB Biodisk, Solna, Sweden) for penicillin (20). Isolates with a penicillin MIC ≥0.1 μg/mL were considered nonsusceptible to penicillin. Isolates with a penicillin MIC ≥1.0 μg/mL were analyzed separately. Isolates resistant to ≥3 antimicrobial classes were considered multidrug resistant.

Statistical Analysis

Statistical analysis was done with SPSS (Chicago, IL, USA) 12.0 software for Windows. The chi-square test was used to compare the distribution of categoric data. Linear regression analysis with the Pearson correlation coefficient was done to test the linear increase or decrease of antimicrobial prescription rates. A similar procedure was performed for exponential increases or decreases in prescription rates, but variables were tested after logarithmic transformation. Comparisons of mean yearly differences in antimicrobial prescription rates between Bedouin and Jewish children were done using the paired samples *t* test. A p value <0.05 was considered significant.

Results

Prescribing of Antimicrobial Agents

A total of 236,466 prescriptions (149,589 for Bedouin and 86,877 for Jewish children) were recorded in the 7 clinics, which represented 12,799 child-years in children <5 years of age (Table). Overall prescribing of antimicrobial agents to Bedouin children was higher (mean ± SD 3.79 ± 0.4 prescriptions per child-year) than to Jewish children (2.37 ± 0.3 prescriptions per child-year). This represents a mean difference of 1.41 prescriptions per child-year ($p<0.001$).

As a group, penicillins were the most frequently prescribed agents and accounted for 80.6% (120,514/149,589) of prescriptions for Bedouin and 80.4% (69,872/86,877) for Jewish children (Table and Figure 1). The cephalosporin group ranked second in Bedouin children (12.5% of all prescriptions), and azithromycin ranked second in Jewish children (10.4% of all prescriptions). Significantly more amoxicillin (1.53-fold), amoxicillin-clavulanate (2.10-fold), cephalosporins (3.04-fold), and erythromycin (1.44-fold) were prescribed for Bedouin than for Jewish children. Jewish children received more prescriptions for phenoxymethyl penicillin (1.20-fold) and azithromycin (1.41-fold) than Bedouin children. All differences between prescription rates of Bedouin and Jewish children were significant (Table).

A significant decrease in total antimicrobial prescriptions was noted with time (Figure 1). In Bedouin children, total antimicrobial prescription rate was reduced 29% from 4,389 per 1,000 children in 1998 to 3,106 per 1,000 children in 2003 ($p = 0.038$, $r = –0.835$ for exponential decrease). In Jewish children, total prescription rate was reduced 30% from 2,949 per 1,000 children in 1998 to 2,079 per 1,000 children in 2003 ($p = 0.014$, $r = –0.902$ for exponential decrease). The decrease in total antimicrobial prescription rate in both populations was caused mostly by a reduction in the prescription rate of penicillins. The erythromycin prescription rate decreased sharply in both populations. In Bedouin children, the rate was reduced 76% from 181 prescriptions per 1,000 children in 1998 to 44 per 1,000 children in 2003 ($p<0.001$, $r = –0.984$ for exponential decrease); in Jewish children, the rate was reduced 81% from 149 prescriptions per 1,000 children in 1998 to 29 per 1,000 children in 2003 ($p = 0.002$; $r = –0.963$ for exponential decrease).

Azithromycin was introduced in Israel in 1998. The prescription rate of this drug increased dramatically to 310 prescriptions per 1,000 Bedouin children ($p = 0.04$, $r = 0.996$ for linear increase) and 357 prescriptions per 1,000 Jewish children ($p = 0.011$, $r = 0.989$ for linear increase) in 2001. Thereafter, a sharp decrease in azithromycin prescription rate (a 51% decrease to 152/1,000) was noted in Bedouin children ($p = 0.024$, $r = –0.999$ for linear decrease from 2001 to 2003). In Jewish children, the azithromycin prescription rate decreased 31%, but this did not reach statistical significance (p = not significant for linear decrease from 2001 to 2003). No significant change in the cephalosporin prescription rate was observed in these populations.

Acute Otitis Media Episodes

A total of 11,311 episodes of acute otitis media requiring tympanocentesis were recorded in the region during the study period. At least 1 pathogen was isolated in 59% (6,678/11,311) of the episodes.

S. pneumoniae Acute Otitis Media Episodes

A total of 3,651 *S. pneumoniae* acute otitis media episodes were recorded: 39% (1,425/3,651) of the isolates were obtained from Jewish children and 61% (2,223/3,651) from Bedouin children. The ethnicity of the child was not recorded for 3 isolates. In 89% (3,258/3,651) of the children, a complete history regarding previous episodes of acute otitis media and antimicrobial treatment was available. Twenty-nine percent (978/3,396) of the children had had 1–3 episodes of acute otitis media and 36% (1,225/3,396) had >3 episodes of acute otitis media in

Table. Antimicrobial drug prescriptions among Bedouin and Jewish children <5 years of age, southern Israel, 1998–2003

	Bedouin children		Jewish children			
Antimicrobial agent	Total prescriptions	Mean ± SD yearly prescription rate/1,000 children	Total prescriptions	Mean ± SD yearly prescription rate/1,000 children	Mean ± SD yearly difference (Bedouin children vs. Jewish children)/prescription/ 1,000 children	p value
Amoxicillin	89,598	2,269 ± 266	54,090	1,480 ± 264	789 ± 175	<0.001
Amoxicillin-clavulanate	27,729	703 ± 91	12,173	335 ± 88	368 ± 51	<0.001
Phenoxymethyl penicillin	3,187	84 ± 47	3,609	101 ± 47	–16 ± 10	0.012
Cephalosporins*	18,625	471 ± 69	5,751	155 ± 34	316 ± 66	<0.001
Erythromycin	3,492	92 ± 51	2,256	64 ± 45	28 ± 66	0.002
Azithromycin	6,958	167 ± 108	8,998	236 ± 124	–70 ± 49	0.018
Total	149,589	3,787 ± 442	86,877	2,371 ± 315	1,416 ± 224	<0.001

*Cefaclor, cephalexin monohydrate, and cefuroxime-axetil.

the preceding year. Twenty-five percent (823/3,258) of the episodes occurred in children who had already undergone tympanocentesis. A total of 24% (809/3,373) of the children were receiving antimicrobial agents at the time of tympanocentesis, and 36% (1,231/3,373) had received antimicrobial agents in the preceding month. No major differences in the proportions of these characteristics were seen within each ethnic group.

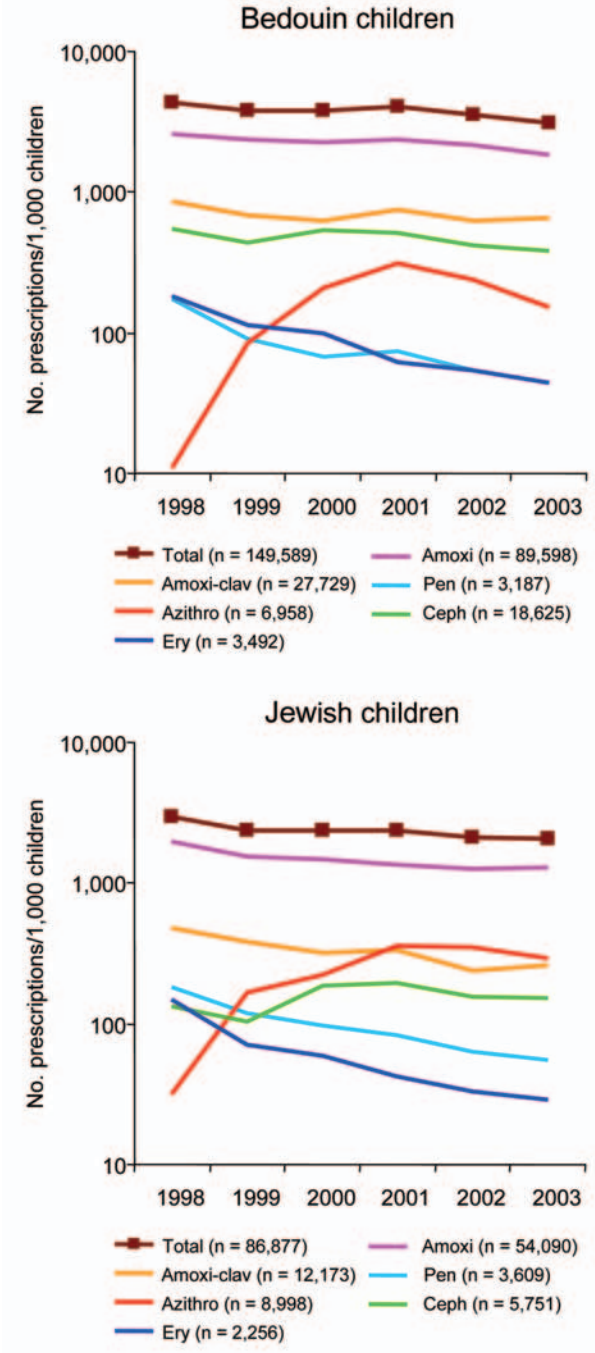

Figure 1. Antimicrobial drug prescription rates for Bedouin and Jewish children <5 years of age in southern Israel from 1998 through 2003. Amoxi, amoxicillin; Amoxi-clav, amoxicillin-clavulanate; Pen, phenoxymethyl penicillin; Azithro, azithromycin; Ceph, cephalosporins (cefazolin, cefaclor, cephalexin monohydrate, and cefuroxime-axetil); Ery, erythromycin.

S. pneumoniae Antimicrobial Resistance Patterns

A total of 98% (3,600/3,651) of *S. pneumoniae* isolates were available for antimicrobial susceptibility testing. Penicillin-nonsusceptible isolates constituted most of the isolates (62% [869/1,399] and 64% [1,418/2,201] of all isolates in Jewish and Bedouin children, respectively) without a significant change in time or in the relative proportions between the 2 populations (Figure 2). However, resistance patterns differed significantly. Among Jewish children, 52%–67% of all penicillin-nonsusceptible isolates had a penicillin MIC ≥1.0 μg/mL each year (532 of all 1,399 isolates, 38%). Among Bedouin children, only 30%–40% of the nonsusceptible isolates (528 of all 2,201 isolates, 24%) had a penicillin MIC ≥1.0 μg/mL each year ($p<0.001$). Thus, despite the reduction in the prescription rates of penicillins in both populations, no parallel decrease in penicillin nonsusceptiblility was observed. Furthermore, the highest penicillin MIC values were continuously observed in Jewish children, for whom the lowest penicillin prescription rates were recorded.

Erythromycin resistance was more common in Jewish (25%, 356/1,425) than in Bedouin children (16%, 355/2,221) ($p<0.001$). In Bedouin children, rates of erythromycin resistance increased significantly from 1999 through 2002, from 9% (41/456) to 21% (93/440), respectively ($p<0.001$). In Jewish children, erythromycin resistance decreased from 23% (61/261) in 1999 to 18% (48/270) in 2000 ($p = 0.01$). Thereafter, the proportion of resistant isolates increased as well, reaching 29% (84/291) in 2002 ($p = 0.02$). Erythromycin resistance did not increase in either population during 2003. In 2003, 17% (63/375) and 27% (90/336) of isolates in Bedouin and Jewish children, respectively, were erythromycin-resistant (Figure 3). The multidrug-resistance pattern paralleled that of erythromycin resistance in each population. Thus, both erythromycin and multidrug resistance increased in parallel with the increase in the azithromycin prescription rate, and resistance tended to decrease with the change in this rate. Furthermore, the population with the highest azithromycin prescription rate also had the highest rates of erythromycin resistance.

Penicillin MIC values were not equally distributed among erythromycin-susceptible and erythromycin-resistant *S. pneumoniae* isolates (Figure 4). In both Jewish and Bedouin children, the proportion of penicillin-susceptible *S. pneumoniae* was greater in erythromycin-susceptible isolates, and the proportion of isolates with a penicillin MIC ≥1.0 μg/mL was greater in erythromycin-resistant *S. pneumoniae* ($p<0.001$ for each comparison).

Discussion

We attempted to determine whether antimicrobial prescription patterns were associated with resistance patterns among *S. pneumoniae* isolates from young children with acute otitis media in 2 populations treated by the same medical system, but living in separate communities with different lifestyles. We used prescription rates of ≈20% of the children <5 years of age in each population and a large collection of middle ear fluid *S. pneumoniae* isolates obtained from these 2 populations during the same time period. This unique opportunity enabled us to not only relate resistance dynamics to prescriptions dynamics for each population, but also to observe whether differences in antimicrobial prescription rates between populations could explain some of the differences in antimicrobial resistance patterns.

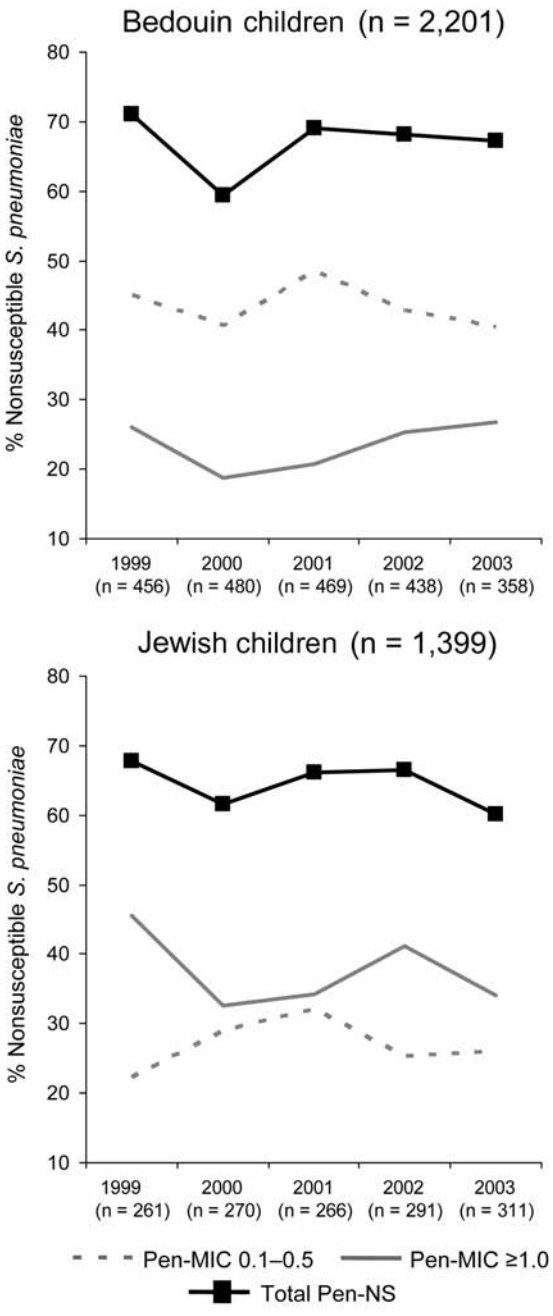

Figure 2. Proportions of penicillin-resistant *Streptococcus pneumoniae* isolated during episodes of acute otitis media in Bedouin and Jewish children <5 years of age in southern Israel from 1999 through 2003. Pen-MIC, penicillin MIC (μg/mL); Pen-NS, penicillin-nonsusceptible.

The association of azithromycin prescriptions with antimicrobial resistance patterns among *S. pneumoniae* isolated from middle ear fluid is noteworthy for several reasons. First, the prescription rate for azithromycin was the only one that was higher among Jewish children than among Bedouin children. Second, the azithromycin prescription rate pattern closely paralleled both macrolide and multidrug resistance in each population. Third, higher penicillin MIC values were associated with macrolide resistance, which explained, at least in part, the higher rates of *S. pneumoniae* isolates from middle ear fluid with a penicillin MIC ≥1.0 μg/mL among Jewish children who received relatively fewer amoxicillin (with or without clavulanate) prescriptions than Bedouin children who received more amoxicillin prescriptions (with or without clavulanate).

The distinct pattern of reducing total antimicrobial drug prescriptions that resulted from reducing prescribed penicillins, although azithromycin use increased, was reported in other regions, including the United States (21,22) and western Europe (23). The pattern of reduced antimicrobial drug use could be the result of campaigns such as those conducted in the United States following the initiative by the Centers for Disease Control and Prevention for the judicious use of antimicrobial drugs, which recommended the first-line use of amoxicillin to treat acute otitis media (21) (http://www.cdc.gov/drugresistance/community/files/ads/Otitispa.pdf). However, the increase in azithromycin prescriptions, along with a reduction in penicillin prescriptions, could be partly the result of commercial promotion campaigns for the use of azithromycin, which were launched in parallel with campaigns to reduce the overall use of antimicrobial agents.

The increase in the azithromycin prescription rate in our study, as in Europe and North America, is partly attributable to the properties that make this drug an attractive agent for children. The long half-life of azithromycin (≤72 h) (24) makes a convenient dose regimen of once a day for ≤5 days. However, it is eliminated very slowly and remains at subinhibitory concentrations in tissues of persons with pneumococcal infections. Subinhibitory concentrations of antimicrobial agents favor the selection of resistant mutants. This has been shown in vitro for *S. pneumoniae* exposed to subinhibitory macrolide concentrations

(25). In a clinical trial, children treated with azithromycin harbored significantly more resistant strains in their oral flora than those who randomly received other macrolides. After 6 weeks, 85% still had macrolide-resistant organisms (26). In European countries, the increase in prescriptions of long-acting macrolides resulted in selection for macrolide resistance in *S. pneumoniae* (27,28).

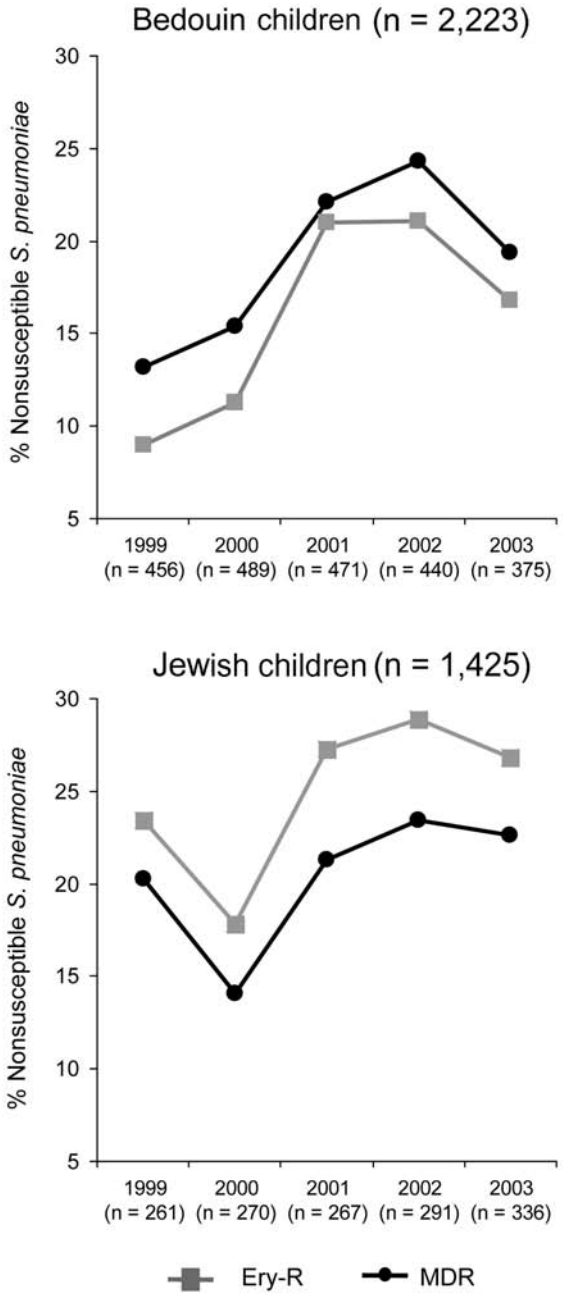

Figure 3. Proportions of erythromycin-resistant and multidrug-resistant *Streptococcus pneumoniae* isolated during episodes of acute otitis media in Bedouin and Jewish children <5 years of age in southern Israel from 1999 through 2003. Ery-R, erythromycin resistance; MDR, multidrug resistance (resistance to ≥3 antimicrobial classes).

Our finding that azithromycin prescriptions were associated with *S. pneumoniae* multidrug resistance was noteworthy. The ability of certain antimicrobial agents to promote resistance to other drug classes has been previously reported. Several studies showed that use of long-acting macrolides was an important factor in increasing penicillin resistance in a given community (29,30). This could explain the higher rates of resistant *S. pneumoniae* with MIC ≥1.0 μg/mL in Jewish than in Bedouin children, despite significantly lower prescription rates for penicillins, but significantly higher prescription rates for azithromycin. This pattern of increasing penicillin and macrolide resistance in association with increased prescribing of azithromycin was also observed in the United States, where it was predicted that in the absence of pneumococcal conjugate vaccine, by 2004 ≈40% of all *S. pneumoniae* isolates would be resistant to both penicillin and macrolides and that the increased rate would be exponential (31).

The differential effect of azithromycin versus amoxicillin on nasopharyngeal carriage of antimicrobial-resistant *S. pneumoniae* in patients was demonstrated in a study conducted in southern Israel. In this study, carriage of both macrolide- and multidrug-resistant *S. pneumoniae* markedly decreased in children with acute otitis media receiving amoxicillin-clavulanate, but increased markedly in those receiving azithromycin (32). This differential effect lasted more than 1 month.

Our study has 3 limitations. First, the factors contributing to differences in antimicrobial drug use between the 2 populations could not be controlled. Antimicrobial drug prescriptions could not be matched with individual use. In addition, potential confounders such as family structure and daycare exposure could not be assessed. The higher prescription rate for antimicrobial agents in Bedouin children could be explained by differences in accessibility to healthcare facilities. However, since there is no financial burden for healthcare in Israel and all clinics belong to the same health plan, acute otitis media is unlikely to be treated differently in either population. The similar reduction in antimicrobial drug prescriptions in both populations suggests that no difference in prescribing policies existed between the 2 populations. Lower socioeconomic status and overcrowding in the Bedouin population, which led to a higher rate of respiratory illness in this group (16,17), may explain the difference in rates of antimicrobial drug prescriptions.

Second, *S. pneumoniae* were obtained only from children with acute otitis media. However, *S. pneumoniae* is part of the normal nasopharyngeal flora and is exposed to antimicrobial agents regardless of the diagnosis for which the agent was prescribed. Therefore, we believe that the effect of prescribing antimicrobial drugs in the community on resistance patterns of *S. pneumoniae* isolated from

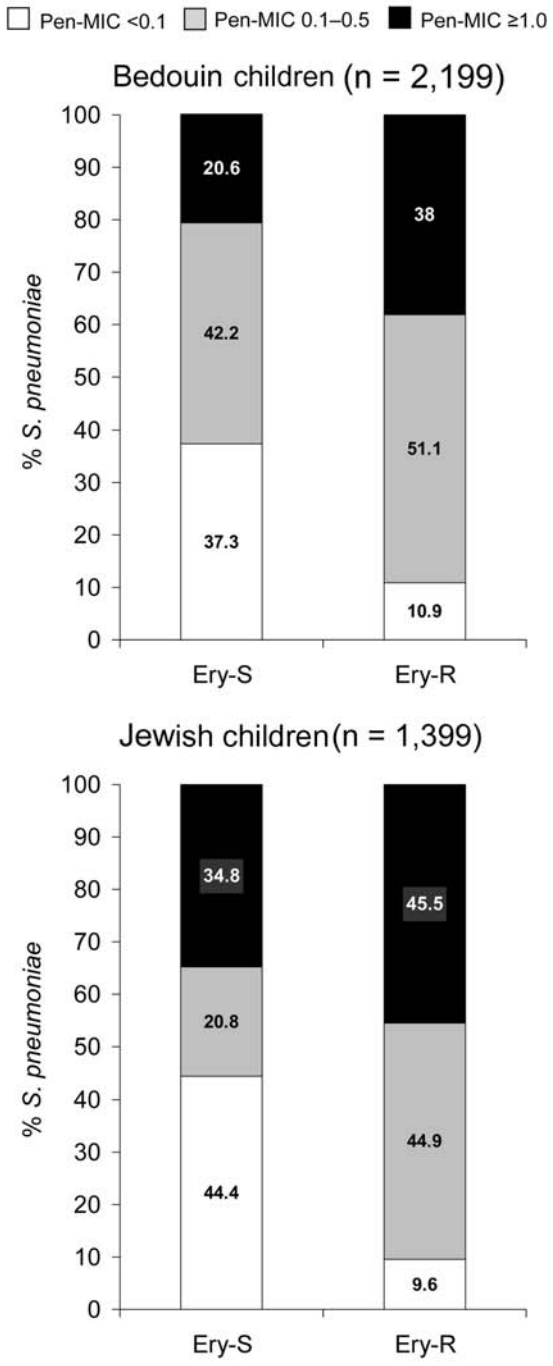

Figure 4. Distribution of penicillin MICs (μg/mL) in erythromycin-susceptible and erythromycin-resistant *Streptococcus pneumoniae* isolated during episodes of acute otitis media in Bedouin and Jewish children <5 years of age in southern Israel from 1999 through 2003. P was calculated for the difference in overall distribution of penicillin MICs between erythromycin-susceptible and erythromycin-resistant isolates, as well as for difference in relative contribution of isolates with MICs ≥1.0 μg/mL to both groups (p<0.001, Ery-S versus Ery-R in both Bedouin and Jewish children). Ery-S, erythromycin susceptible; Ery-R, erythromycin resistant; Pen-MIC, penicillin MIC.

middle ear fluid represents the effect on the entire spectrum of *S. pneumoniae* disease.

Third, this was not an intervention study; therefore, we could not demonstrate unequivocally the causative effect of azithromycin use on macrolide and multidrug resistance in *S. pneumoniae*. However, the association demonstrated in this study, together with published data, strongly suggest such a causative effect.

The introduction of the 7-valent pneumococcal conjugate vaccine to infant and toddler immunization programs in the United States was associated with a reduction in invasive diseases (33,34) and acute otitis media (35,36) caused by antibiotic-resistant *S. pneumoniae*. However, persistence of antimicrobial resistance within vaccine and nonvaccine serotypes (37–40) suggests that vaccine alone may not reduce antimicrobial resistance, and that if the use of antimicrobial drugs is not controlled, the ability of the pneumococcal conjugate vaccine to reduce antimicrobial-resistant S. *pneumoniae* may be only transient.

During the last 2 years of this study, prescription rates were reduced in both populations. This reduction could be partly explained by efforts of pediatric infectious diseases specialists to educate pediatricians and family physicians in the study area to reduce use of antimicrobial drugs, especially oral use of cephalosporins and macrolides-azalides. However, this effect may not be the main reason for this decrease. A decrease in macrolide and multidrug resistance of *S. pneumoniae* observed in the last year of this study may indicate that the effect of azithromycin use on antimicrobial resistance is reversible. Continuous monitoring of antimicrobial prescriptions and resistance in respiratory pathogens should help determine if a further decrease in azithromycin prescriptions would be followed by a further decrease in antimicrobial resistance of *S. pneumoniae*.

In conclusion, azithromycin prescriptions were associated with macrolide, penicillin, and multidrug resistance among *S. pneumoniae* isolated from the middle ear fluid of children with acute otitis media. Such an association was not found with amoxicillin (with or without clavulanate) prescriptions. When promoting judicious use of antimicrobial drugs, selective reduction in prescribing specific antimicrobial drugs such as azithromycin should be emphasized, in addition to total reduction in antimicrobial use.

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pVir and Bloody Diarrhea in *Campylobacter jejuni* Enteritis

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The plasmid pVir may play a role in the virulence of *Campylobacter jejuni*, a leading cause of bacterial gastroenteritis. The pVir plasmid was identified in 17% of 104 *C. jejuni* clinical isolates studied and was significantly associated with the occurrence of blood in patient stool, a marker of invasive infection. The pVir plasmid was not associated with greater occurrence of diarrhea, fever, pain, vomiting, or need for patient hospitalization. Isolates containing pVir were also associated with the presence of a tetracycline-resistance plasmid, but pVir did not transfer with tetracycline-resistance plasmids to recipient strains of *C. jejuni*. The association of pVir and bloody stool suggests that pVir may be clinically relevant in *C. jejuni* infections.

Campylobacter jejuni is a major foodborne pathogen and a leading cause of bacterial gastroenteritis (1,2). Infection with *C. jejuni* can result in a wide array of clinical symptoms, including diarrhea, fever, abdominal pain, and vomiting, as well as bloody stool with severe invasive infection (3). Various virulence factors, which allow for adherence, colonization, and invasion of the intestinal epithelium, have been proposed to contribute to the pathogenesis of *C. jejuni* (4). Potential virulence components include flagella (5,6), invasion proteins (7), and toxins (8,9). Although the genome of *C. jejuni* has been sequenced (10), its mechanisms of pathogenicity remain poorly understood (11).

A number of bacterial enteric pathogens contain plasmids that contribute to pathogenesis, including *Shigella* sp. (12), *Salmonella* sp. (13), and enteropathogenic *Escherichia coli* (14). No evidence was seen for the involvement of plasmids in the virulence of *C. jejuni* until Bacon et al. (15) identified plasmid pVir in strain 81-176. pVir is an ≈37.5-kb plasmid that contains components of a type IV secretion system (T4SS) (15,16) known to be important for the virulence of a number of major bacterial pathogens (17). Bacon et al. (15) suggested that the pVir plasmid is important in vitro for both adherence and invasion of intestinal epithelial cells in culture. In a survey of fresh clinical isolates of *C. jejuni* from Thailand, 10% (n = 58) contained pVir (15).

C. jejuni gastroenteritis is primarily self-limiting and is usually treated by supportive therapy (fluid and electrolyte replacement) (3). Erythromycin is the drug of choice for treating severe clinical infections with *C. jejuni* (18), and fortunately the prevalence of erythromycin resistance has remained low (19). Worldwide, tetracycline resistance in *C. jejuni* is high: 56% in Canada (20) and up to 95% in Thailand (21). In Alberta, Canada, only 8.6% of human clinical isolates were tetracycline-resistant in 1981 (22). In *C. jejuni*, tetracycline resistance is primarily mediated by plasmids that carry the *tet(O)* gene (23,24). The Tet(O) protein binds to the bacterial ribosome and displaces tetracycline (25,26). The aim of this study was to investigate the prevalence of the pVir plasmid in *C. jejuni* isolated from clinical specimens in Alberta and the relationship of pVir to the clinical expression of the disease in gastroenteritis.

Materials and Methods

Source of *C. jejuni* Isolates

We obtained a random sample of 104 human isolates of *C. jejuni* (fresh and frozen at –70°C in double-strength skim milk) cultured from stool samples from 1999 to 2002 at the University of Alberta Hospital and Provincial Laboratory for Public Health (Microbiology) in Edmonton, Alberta, Canada, for this study. All stool specimens were routinely cultured for enteric pathogens tested according to standard laboratory protocols. Most *C. jejuni* isolates were from northern Alberta and represented ≈10%

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of *C. jejuni* infections in Alberta reported annually to Health Canada. The Health Research Ethics Board (Biomedical Panel) of the University of Alberta approved the protocol for access to human isolates and collection of patient information for this study.

Collection of Clinical Data

Clinical data were collected by letter or phone from family physicians' offices and emergency medical records and included information on age, sex, place of residence, travel within the last month, coexisting conditions, symptoms (diarrhea, bloody diarrhea, pain, fever, vomiting) and their duration (interval from symptom onset to initial evaluation), hospitalization (if needed), antimicrobial therapy, and complications. Fever was defined as a complaint of fever stated by the patient or a documented temperature of >38°C. Blood in the stool was observed by the patient and reported to the attending physician. The investigators conducting the chart review and laboratory analyses (pVir screening, antimicrobial susceptibility testing) were blinded to each other's data.

Culture of *C. jejuni* Isolates

Isolates of *C. jejuni* were spread on brain heart infusion agar (Difco-Becton-Dickinson, Sparks, MD, USA), supplemented with 0.4% yeast extract (Difco), and incubated at 37°C in microaerobic conditions (5% CO_2, 10% H_2, 85% N_2) for 48 h. Isolates were not passaged more than once. All isolates were stored frozen in brain heart infusion broth (Difco) with 20% glycerol at –80°C.

Antimicrobial Susceptibility and Plasmid Isolation

C. jejuni susceptibility was tested by using the disk diffusion method; the following antimicrobial disks (Oxoid, Nepean, Ontario, Canada) were included: tetracycline (30 μg), kanamycin (30 μg), erythromycin (15 μg), chloramphenicol (30 μg), and nalidixic acid (30 μg). All nalidixic acid-resistant isolates were further tested for resistance to ciprofloxacin (1 μg). Susceptibility testing to all antimicrobial agents was carried out on Mueller-Hinton agar plates that were spread with a 0.5 McFarland standard suspension of *C. jejuni* in phosphate-buffered saline (Sigma, St. Louis, MO, USA) and incubated for 48 h at 37°C under microaerobic conditions. Zones of inhibition were measured as described by Gaudreau and Gilbert (27). Plasmid isolations were performed by using a Qiagen Midi or Mini Plasmid Kit (Qiagen, Mississauga, Ontario, Canada), by alkaline lysis (28) or by a method used for *Helicobacter pylori* (29). Tetracycline resistance detected by disk diffusion was confirmed by Etest (Oxoid) and conventional agar dilution.

DNA-DNA Hybridizations for pVir

Purified plasmid DNA from *C. jejuni* clinical isolates was applied to nitrocellulose paper (Osmonics, Westborough, MA, USA) and air dried. Plasmid pMS11EH was used as a negative control, and plasmid DNA isolated from *C. jejuni* 81-176 was used as a positive control. Plasmid DNA was denatured in 0.5 mol/L NaOH, 0.15 mol/L NaCl for 5 min, neutralized twice in 10 mmol/L Tris-HCl, 0.15 mol/L NaCl, pH 7.5 for 5 min each time, soaked in 2× SSPE solution (20× SSPE consists of 3.0 mol/L NaCl, 0.2 mol/L NaH_2PO_4, and 0.02 mol/L EDTA) for 5 min, air dried, and baked overnight at 65°C.

The *cjp5* (*virB11*) DNA probe was prepared with primers *virB11* Fwd (5′ GAACAGGAAGTGGAAAAACTAGC 3′) and *virB11* Rev (5′ TTCCGCATTGGGCTATATG 3′) (15) that were used to amplify by polymerase chain reaction (PCR) a 708-bp product from within the *cjp5* gene on pVir. Conditions for the PCR were as follows: initial denaturation at 95°C for 1 min, followed by 30 cycles of denaturation (1 min, 95°C), annealing of primers (1 min, 50°C) and primer extension (1 min, 72°C). PCR was performed in a BioRad Gene Cycler (BioRad, Mississauga, Ontario, Canada). The *cjp5* PCR product was purified with a PCR purification kit (Qiagen) and eluted with TE (Tris-EDTA) buffer. DNA was denatured after boiling for 5 min, and the tube was then placed directly on ice. A random primers DNA labeling system (Gibco, Burlington, ON) was used to prepare the ^{32}P-labeled *cjp5* DNA probe. The ^{32}P-labeled *cjp5* DNA probe was used immediately in DNA-DNA hybridizations to screen isolated *C. jejuni* plasmids for the presence of pVir, under the same stringency conditions defined by Bacon and coworkers (15). Hybridizations were performed in a solution consisting of 6× SSC (20× consists of 3.0 mol/L NaCl and 0.3 mol/L sodium acetate), 5× Denhart solution (50× Denhardt solution consists of 1% wt/vol Ficoll 400 (Sigma), 1% wt/vol polyvinylpyrrolidone (Sigma), and 1% wt/vol bovine serum albumin (Sigma, Fraction V), 0.1% sodium dodecyl sulfate (SDS, BioRad) and 100 μg/mL herring sperm DNA (Invitrogen, Burlington, Ontario, Canada). The ^{32}P-labeled *cjp5* DNA probe was added directly to the hybridization solution in the tube, mixed, and incubated overnight (18 h) at 50°C. The probed nitrocellulose paper was washed in 0.5× SSC 4 times, dried, and then exposed to BioMax MS Film (Eastman Kodak Company, Rochester, NY, USA) overnight at –70°C.

Identifying Tetracycline-Resistance Plasmids

Tetracycline-resistance plasmids were identified by a PCR screen for the *tet(O)* gene on purified plasmid preparations. Primers *tet(O)* Fwd (5′ GGCGTTTTGTTTATGTGCG 3′) and *tet(O)* Rev (5′ ATGGACAACCCGACAGA

AGC 3′) were used to amplify a 559-bp fragment of the *tet(O)* gene from isolated *C. jejuni* plasmid DNA (15,30). PCR conditions were the same as for the *cjp5* PCR described above. Gel electrophoresis was used to confirm the presence of an ≈40-kb plasmid.

Transfer of Plasmids between *C. jejuni* Strains

The transfer of plasmids from *C. jejuni* clinical isolate 23-51 (containing both a tetracycline-resistance plasmid and pVir) to *C. jejuni* UA 543 (a nalidixic acid–resistant, tetracycline-susceptible recipient strain with no plasmids) was carried out (23). Suspensions of donor strain 23-51 and UA 543 recipient strains were mixed in ratios of 1:4 and 1:6, centrifuged, and resuspended in 100 μL of Lennox broth (Difco). Mating suspensions were inoculated onto a 0.22-μm Millipore filter (Millipore Corporation, Nepean, Ontario, Canada), placed on brain heart infusion agar, and incubated for 24 h. Cultures were resuspended in 1 mL of phosphate-buffered saline, diluted, and plated onto Mueller-Hinton agar containing 25 μg/mL tetracycline and 50 μg/mL of nalidixic acid. Plasmids were isolated from the transconjugants by a Qiagen Mini Kit. The *cjp5* DNA probe was used to screen for pVir as described above.

Statistical Analysis

The Fisher exact test was used to test the significance ($p<0.05$) of the association between pVir and clinical symptoms, association of pVir and tetracycline-resistance plasmids, and the frequency of tetracycline resistance from this study and a previous study (22).

Results

Clinical Data

The patients were 7 months to 87 years of age (average 35 years); 47% were female. Ninety-five percent of the patients resided in Alberta, and the remaining 5% were from other Canadian provinces. Only 10 patients reported a history of travel outside Canada before becoming ill, namely to Mexico (3 patients), Alaska, Nicaragua, Ecuador, England, India, Lebanon, and Africa (1 patient each). The most commonly reported clinical symptom was diarrhea (99%); the exception was in an 86-year-old woman, who had an ileus. Other commonly reported symptoms were abdominal pain (83%) and fever (77%). Bloody stool was reported in 27% of patients and vomiting in 30% of patients; 18% of patients were hospitalized for treatment of severe dehydration. Fifty-eight patients received antimicrobial therapy: 34 ciprofloxacin (1 was switched to erythromycin after culture results, 2 received ciprofloxacin in combination with metronidazole), 20 macrolides (13 erythromycin, 5 clarithromycin, 3 azithromycin), 2 metronidazole (1 in combination with cephalexin), 1 amoxicillin, and 1 cotrimoxazole. Sixteen patients had other associated conditions: 4 cardiac disorders (2 arrhythmias, 2 ischemic heart disease), 3 hypertension (1 also had chronic obstructive pulmonary disease), 3 diabetes, 2 neoplasms (1 leukemia, 1 Wilm tumor), 2 gastrointestinal disorders (1 ulcerative colitis, 1 irritable bowel), 1 hepatitis C, and 1 seizure. Campylobacteremia with febrile seizures developed in a previously healthy patient.

Antimicrobial Susceptibility

The antimicrobial susceptibility of 104 human isolates of *C. jejuni* is shown in Table 1. Tetracycline resistance was identified in 63 isolates (60%); of these, 4 isolates were also resistant to nalidixic acid, 2 isolates were also resistant to kanamycin, and 1 isolate was also resistant to both nalidixic acid and kanamycin. Three of the 4 nalidixic acid–resistant isolates were also resistant to ciprofloxacin. In the tetracycline-sensitive isolates, no resistance to other antimicrobial agents was detected.

Plasmid Content

Tetracycline-resistance plasmids were found in 50 (79%) of 63 tetracycline-resistant isolates. DNA-DNA hybridizations for the *cjp5* gene on pVir determined that 18 (17%) of 104 *C. jejuni* isolates contained the pVir plasmid. Stocked frozen clinical isolates had a slightly higher frequency of pVir than fresh clinical isolates (18% vs. 13%, respectively), most likely because of sampling error. Tetracycline-resistance plasmids were found in 17 (94%) of 18 pVir-positive *C. jejuni* isolates compared with 33 (38%) of 86 pVir-negative isolates. The presence of pVir plasmids was associated with the presence of tetracycline-resistance plasmids ($p<0.00001$). Alternatively, 33 (66%) of the 50 isolates that contained tetracycline-resistance plasmids did not contain pVir, demonstrating that plasmid-mediated tetracycline resistance does not occur exclusively with pVir.

Relationship of Signs and Symptoms to Plasmid Content

Pain, diarrhea, vomiting, and fever were equally likely to occur in all *C. jejuni* infections, regardless of the pres-

Table 1. Antimicrobial resistance frequencies in human clinical isolates of *Campylobacter jejuni* (n = 104) as determined by antimicrobial disk diffusion, 1999–2002

Antimicrobial agent	Resistance frequency (%)
Tetracycline	60
Nalidixic acid	4
Ciprofloxacin	3
Kanamycin	3
Erythromycin	0
Chloramphenicol	0

Table 2. Association of pVir plasmid with clinical symptoms in patients with *Campylobacter jejuni* gastroenteritis, Alberta, Canada, 1999–2002

	Clinical symptom (%)						
pVir	Pain	Diarrhea	Vomiting	Blood in stool	Fever	Hospitalization	Duration <7 days (%)
Present (n = 18)*	83 (n = 18)	100 (n = 18)	28 (n = 15)	53† (n = 17)	59 (n = 17)	22 (n = 18)	77 (n = 14)
Absent (n = 86)	80 (n = 72)	99 (n = 85)	30 (n = 73)	21 (n = 76)	64 (n = 64)	17 (n = 86)	82 (n = 68)

*Samples sizes (n) for each clinical symptom differ as symptoms were not known for each patient.
†p<0.05, presence vs. absence of pVir.

ence of pVir (Table 2). However, 53% of patients infected with pVir-positive *C. jejuni* strains had bloody stool, as opposed to 21% of patients infected with pVir-negative *C. jejuni* strains (p = 0.011). pVir was not associated with age, sex, antimicrobial therapy, coexisting conditions, or travel. The patient with campylobacteremia and febrile seizures was infected with a pVir-negative *C. jejuni*. None of the patients infected with pVir-positive *C. jejuni* strains had a history of travel outside of Alberta in the week preceding the illness, and all of the patients who traveled outside of Alberta were infected with pVir-negative *C. jejuni* strains.

Transfer of Plasmids between *C. jejuni* Strains

Tetracycline-resistant transconjugants contained ≈40-kb plasmids that carried *tet(O)* but were negative for pVir in DNA-DNA hybridizations with the *cjp5* probe (data not shown). This finding confirmed the finding of Bacon et al. (15) that pVir could not be transferred with tetracycline-resistance plasmids in conjugal mating between *C. jejuni* strains.

Discussion

The role of pVir in human *C. jejuni* infections has not been investigated previously. Bacon et al. (15,16) presented evidence for the role of pVir in the virulence of *C. jejuni* based on in vitro cell-culture experiments and limited animal data with the ferret model (15). In our study, symptoms associated with *C. jejuni* infection were correlated with the presence or absence of pVir plasmids. Symptoms varied from mild discomfort to severe cases with bloody diarrhea. Travel and coexisting medical conditions were not predictors of severity of *C. jejuni* infections and were not associated with a higher frequency of blood in the stool or pVir-positive *C. jejuni* infections. Although the pVir virulence plasmid did not correlate with most clinical symptoms, patients infected with a pVir-positive *C. jejuni* strain were more likely to produce bloody stool than those infected with a pVir-negative strain. The time course of the disease may have affected whether or not blood was observed in the stool sample. The clinical data collected reflect both the observations of the patient as reported to the physician and the physician's records. Accordingly, these conclusions are based upon the necessary limitations of this retrospective study of patients from different unconnected study sites within a region, with perhaps some differences in diagnostic definitions.

Bloody stool in *C. jejuni* gastroenteritis indicates the progression of the infection into the tissues of the colon and rectum (3). This invasion of the intestinal epithelium is responsible for the mucosal damage and inflammatory lesions seen in *C. jejuni* infections and is a major component of pathogenesis, although the mechanism is currently unknown (11). pVir was previously found to be important for the in vitro invasion of intestinal epithelial cell lines (15,16). The association of pVir in *C. jejuni* with bloody diarrhea in a clinical setting supports the role of pVir for in vivo epithelial cell invasion and stresses its potential as a marker for the risk of developing a more severe clinical infection.

The lack of association of pVir with bloody stool in a small proportion of patients suggests that other virulence determinants are likely to be involved in severe *C. jejuni* infections, as are several other host factors that determine the clinical expression of the disease (3). Further studies are necessary to clarify these aspects of clinical *C. jejuni* infection.

Large variations in invasion frequency have been observed among strains of *C. jejuni* (4). Fearnely et al. (31) found that *C. jejuni* strains can either invade cell cultures at high frequencies (hyperinvasive) or have low invasive potential. Subsets of the *C. jejuni* strains may exist that use different mechanisms to produce disease (15,32). Whether or not the pVir virulence plasmid is the defining feature of a hyperinvasive subset of *C. jejuni* strains remains to be determined.

The finding that the pVir virulence plasmid is present in *C. jejuni* isolates containing a tetracycline-resistance plasmid is of considerable interest. One potential explanation for this association is the impact on the invasive ability of the strain. Variation in the invasion frequencies of *C. jejuni* 81-176 pVir gene mutants has been observed (33) and may be due to the functional redundancy of T4SS genes found on pVir and a tetracycline-resistance plasmid (16). This finding may, in part, explain the dependence of pVir on the tetracycline-resistance plasmid observed in this study.

The prevalence of tetracycline resistance in *C. jejuni* in our study (60%) represents a significant increase from 8.6% in 1981 (p<0.0001) (22), but the frequency of resist-

ance to erythromycin or ciprofloxacin has remained low. These results confirm those of other Canadian studies that have identified increasing levels of tetracycline resistance and low levels of erythromycin resistance (20,34,35). Considering that erythromycin is the drug of choice for treating *C. jejuni* gastroenteritis, that its efficacy has not been compromised by the emergence of resistance is surprising. In our study, a low level of resistance to ciprofloxacin, commonly prescribed to prevent travelers' diarrhea, is also a surprising finding. A temporal link between veterinary use of fluoroquinolones and the emergence of fluoroquinolone resistance in human isolates of *C. jejuni* coincided with the licensing of fluoroquinolones, such as enrofloxacin, in the late 1980s and early 1990s in the Netherlands, Spain, United Kingdom, the United States, and Canada (36). In the eastern Canadian province of Quebec, resistance to ciprofloxacin increased from 13% in 1995 to 47% in 2001 (20,37). Approval for fluoroquinolone as a therapeutic agent for use in agriculture in Canada was withdrawn in 1997 (38) and may explain the low prevalence of ciprofloxacin-resistant *C. jejuni* isolated from human specimens in the western Canadian province of Alberta.

Serotyping was not conducted in our study since on its own it is not a useful discriminating marker for the ability of *C. jejuni* isolates to cause severe gastroenteritis. Molecular subtyping studies in the United States reported virtually identical isolates of *C. jejuni* in locally purchased retail poultry products and human infections (39). Future studies will continue to investigate the association of pVir-positive *C. jejuni* infections with the presence of blood in stool and employ a variety of genotypic tools (molecular typing, DNA microarrays) and phenotypic assays (i.e., invasion) to identify the characteristics of *C. jejuni* isolates from animal and human sources that allow them to be effective pathogens in humans.

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Community-associated Methicillin-resistant *Staphylococcus aureus*, Canada

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A total of 184 methicillin-resistant *Staphylococcus aureus* (MRSA) strains were collected from patients who sought treatment primarily for skin and soft tissue infections from January 1, 1999, to March 31, 2002, in east-central Saskatchewan, Canada. Molecular subtyping analysis using pulsed-field gel electrophoresis showed 2 major clusters. Cluster A (n = 55) was composed of a multidrug-resistant MRSA strain associated with a long-term care facility and was similar to the previously reported nosocomial Canadian epidemic strain labeled CMRSA-2. Cluster B (n = 125) was associated with cases identified at community health centers and was indistinguishable from a community-associated (CA)-MRSA strain identified previously in the United States (USA400). Cluster B remained susceptible to a number of classes of antimicrobial agents and harbored the *lukF-PV* and *lukS-PV* toxin genes. Over 50% of both clonal groups displayed high-level resistance to mupirocin. This is the first report of the USA400 strain harboring the *lukF-PV* and *lukS-PV* toxin genes in Canada.

The first report of a highly virulent community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strain occurred in 1993 in Australia (1), and since that time CA-MRSA has been reported in many countries. CA-MRSA strains are different genetically and epidemiologically from strains commonly associated with nosocomial infections. Common nosocomial risk factors generally do not apply to CA-MRSA, although previous antimicrobial drug use has been identified as a potential risk factor for CA-MRSA (2–4). In addition, reports have documented CA-MRSA as having caused serious, and sometimes fatal, disease, especially in otherwise healthy children (5–7). Most CA-MRSA strains remain susceptible to a number of classes of antimicrobial agents such as aminoglycosides, tetracyclines, and fluoroquinolones. Many reports of CA-MRSA have described strains harboring the Panton-Valentine leukocidin determinant, a virulence factor for primary skin infection and pneumonia (8,9).

Over the past decade, MRSA has been observed sporadically as a community-acquired pathogen in Canada (10,11). On the Canadian prairies a disproportionate number of aboriginal persons admitted to acute care facilities are infected or colonized with MRSA, compared to persons of nonaboriginal origin (10,12).

This report describes the emergence of 2 different strains of MRSA in east-central Saskatchewan, Canada. The first was associated with a long-term care facility and the second was a clone of MRSA harboring the *lukF-PV* and *lukS-PV* toxin genes and generated an indistinguishable fingerprint from the previously described USA400 strain. This is the first report describing the emergence in Canada of the strain of USA400 that contains *lukF-PV* and *lukS-PV*.

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Materials and Methods

Setting

The investigation focused on an area in east-central Saskatchewan. This region consisted of a city of ≈7,127 persons that was serviced by a hospital with a central laboratory in which the MRSA strains were identified. This locale also contained a number of community health centers and a long-term care facility housing ≈100 persons. The region also included a number of smaller communities that consisted of small rural First Nations and Metis communities (aboriginal populations) and small towns.

Case Definitions

Surveillance for MRSA was laboratory-based and involved nonrepeat MRSA cases. Case-patients were residents of east-central Saskatchewan with laboratory-confirmed MRSA infections identified from January 1, 1999, to March 31, 2002, by the local hospital or provincial laboratory. All MRSA isolates identified by the hospital laboratory were subsequently confirmed at the provincial laboratory by using standard protocols. Residential status was determined by the location of the patient's treatment facility because case-patient address information was unavailable. General patient demographic information was collected (date of birth, sex, date of sample collection, and invasiveness) for all case-patients. Invasive infections were defined according to the guidelines from the Centers for Disease Control and Prevention Active Bacterial Core Surveillance Program and included obtaining isolates from a normally sterile site, such as blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, surgical aspirate, bone, joint fluid, or internal body site (www.cdc.gov/ncidod/dbmd/abcs/meth-case.htm). Antimicrobial susceptibility testing by microbroth dilution was performed according to NCCLS recommendations (13). Breakpoints used for mupirocin were as follows: susceptible, MIC <4 mg/L; low-level resistance, MIC ≥4 and <256 mg/L; high-level resistance, ≥256 mg/L (14).

Molecular Characterization of MRSA Strains

mecA and *nuc* genes from MRSA isolates were coamplified with a multiplex real-time polymerase chain reaction (PCR) assay. Nucleic acid was isolated from 4–5 colony picks by boiling in a 2% (wt/vol) homogeneous suspension of Chelex 100 resin (Bio-Rad Laboratories Ltd, Mississauga, Ontario, Canada). Primer and probe sequences with their reaction concentrations are shown in Table 1. Master mix was composed of the Applied Biosystems TaqMan PCR Core kit (Applied Biosystems, Foster City, CA, USA) that uses reaction concentrations of: 1x PCR buffer A, 4.0 mmol/L $MgCl_2$, 200 μmol/L dATP, 200 μmol/L dCTP, 200 μmol/L dGTP, 400 μmol/L dUTP, 1.25 U AmpliTaq Gold, and 0.5 U uracil-DNA-N-glycosylase (UNG) for carryover prevention. Thermal cycling and data collection were performed on an ABI Prism 7700 Sequence Detector using the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 55 cycles of 95°C for 15 s and 60°C for 1 min. Amplification was confirmed for each target by the generation of a sigmoid amplification plot.

lukF-PV and *lukS-PV* detection was carried out by using PCR with primers and protocols previously described (15). PCR was used to detect the exfoliative toxin genes *eta* and *etb* as previously described (16).

Isolates were subtyped by using pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I following the Canadian Standardized Protocol as described previously (17). PFGE-generated DNA fingerprints were digitized and analyzed with BioNumerics Ver. 3.5 (Applied Maths, Sint-Martens-Latem, Belgium) by using a position tolerance of 1.0 and an optimization of 1.0. Relatedness was determined following established criteria with major strain clusters (designated by a letter) grouped with banding patterns of <7 band changes (18). Fingerprints were compared to those in the national MRSA fingerprint database, which comprised of >600 unique MRSA fingerprint types. Isolates with specific DNA profiles were grouped into 1 of 6 Canadian epidemic strains of MRSA (CMRSA-1, CMRSA-2, CMRSA-3, etc.) as previously described (19,20). Multilocus sequence typing (MLST) was conducted on a representative isolate from each of the unique PFGE types as previously described (21). Staphylococcal chromosome cassette (SCC) *mec* typing was conducted as previously described (22).

Results

Epidemiologic Analysis of MRSA

Before 1999, MRSA was rarely isolated in east-central

Table 1. DNA oligonucleotides used in this study

Primer/Probe	Oligonucleotide sequence	Final concentration (μmol)
*mec*A forward	5′ GGC AAT ATT ACC GCA CCT CA 3′	0.30
*mec*A reverse	5′ GTC TGC CAC TTT CTC CTT GT 3′	0.30
*mec*A probe	5′ FAM - AGA TCT TAT GCA AAC TTA ATT GGC AAA TCC - TAMRA 3′	0.10
nuc forward	5′ CAA AGC ATC AAA AAG GTG TAG AGA 3′	0.05
nuc reverse	5′ TTC AAT TTT CTT TGC ATT TTC TAC CA 3′	0.05
nuc probe	5′ VIC - TTT TCG TAA ATG CAC TTG CTT CAG GAC CA - TAMRA 3′	0.05

Saskatchewan. It was identified only 2 other times in persons from this region since 1996. However, in April 1999, a resident of a long-term care facility in the region under study tested positive for MRSA. This patient had recently been hospitalized in Saskatoon, Saskatchewan, and tested positive for MRSA shortly after being transferred back to the long-term care facility. Following this case, an additional 183 nonrepeat MRSA were isolated from infected persons from this region between January 1999 and April 2002. The annual rates of MRSA-related infections in the region under study were 3.1/1,000 persons in 1999, 4.8/1,000 persons in 2000, and 14.6/1,000 persons in 2001. In 1 specific community within the study area with a population of approximately 1,400 persons, MRSA rates were 0/1,000 in 1999, 7.1/1,000 in 2000, and 46/1,000 in 2001. Only a single isolate was considered invasive, having causes a blood infection. Approximately 18% (n = 33) of the cases were identified from a single long-term care facility and made up of >50% of cases identified between April 1999 and June 2000 (Figure 1A). After June 2000, a larger cluster of cases emerged, which peaked in October 2001 and were primarily identified from patients presenting at local health clinics or nursing stations from surrounding communities (Figure 1A).

Molecular Characterization of MRSA

All of the 184 strains were PCR positive for the *mec*A and *nuc* genes, respectively. DNA fingerprinting of all strains using PFGE resulted in the identification of 5 major fingerprint patterns labeled pattern A to E (Figure 2). Clonal group A (n = 55) comprised 8 subtypes with subtype A1 representing 86% (n = 47, Canadian Diseases Network (CDN) type 417) of the total, with other A subtypes as follows: A2 (n = 1, CDN type 697); A3 (n = 1, CDN type 695); A4 (n = 2, CDN type 691); A5 (n = 1, CDN type 696); A6 (n = 1, CDN type 550); A7 (n = 1, CDN type 726); A8 (n = 1, CDN type 725). Group B was the most predominant molecular subtype with 68% (n = 126) of the strains in this cluster. This group was comprised of 3 PFGE subtypes labeled B1 (n = 123, CDN type 142), B2 (n = 1, CDN type 378), and B3 (n = 2, CDN type 418). Three additional unique PFGE fingerprint types were labeled C1 (n = 1, CDN type 494), D1 (n = 1, CDN type 334), and E1 (n = 1, CDN type 147).

Characterization of Clonal Group A Isolates

Clonal group A isolates were first identified on April 12, 1999, from a patient in a long-term care facility who had been transferred from a hospital in Saskatoon, Saskatchewan. A rapid increase in cases was related to this clonal group from persons from the long-term care facility (n = 33, 60%) as well as community health centers (n = 22, 40%) during the next few months (Figure 1B). Cases related to this group peaked June 1999; however, isolates continued to be identified over the course of this study from both patients in long-term care facilities and community health centers (Figure 1). Clonal group A strains were found to cause a large number of noninvasive infections. Only a single isolate was identified as causing an invasive blood infection. A slightly higher number of female

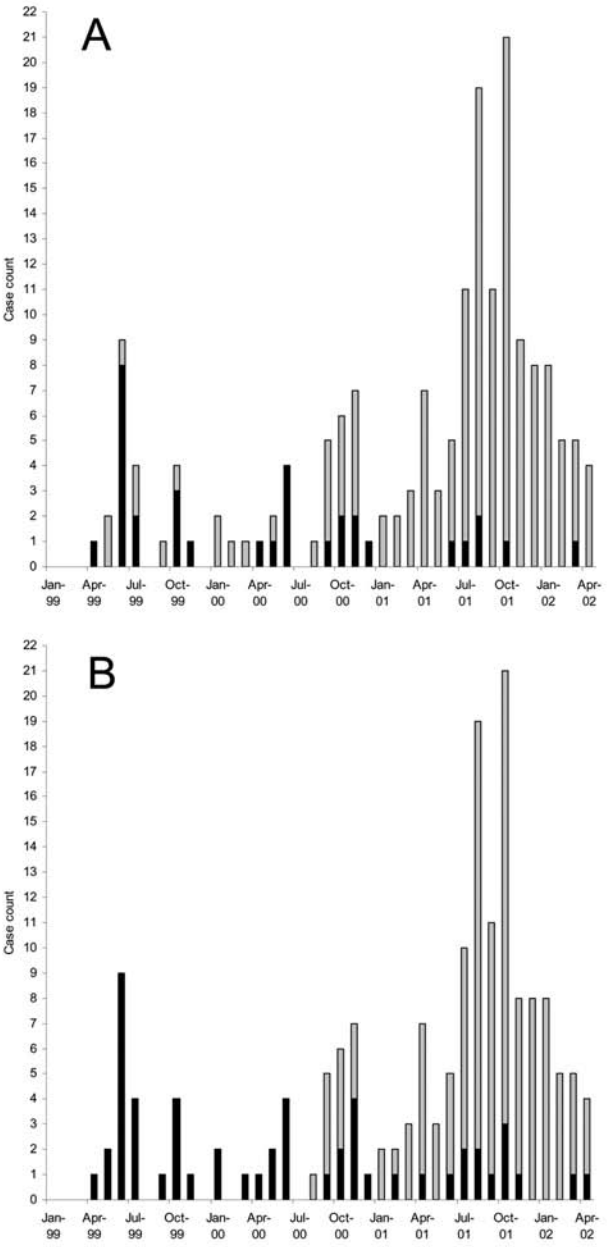

Figure 1. Epidemiologic curve showing the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in central-eastern Saskatchewan. A) Number of nonrepeat cases over the length of study; solid bars, cases identified in a long-term care facility; gray bars, cases identified in community health centers. B) Same data as (A), with solid bars representing isolates of clone A and gray bars showing isolates of clone B.

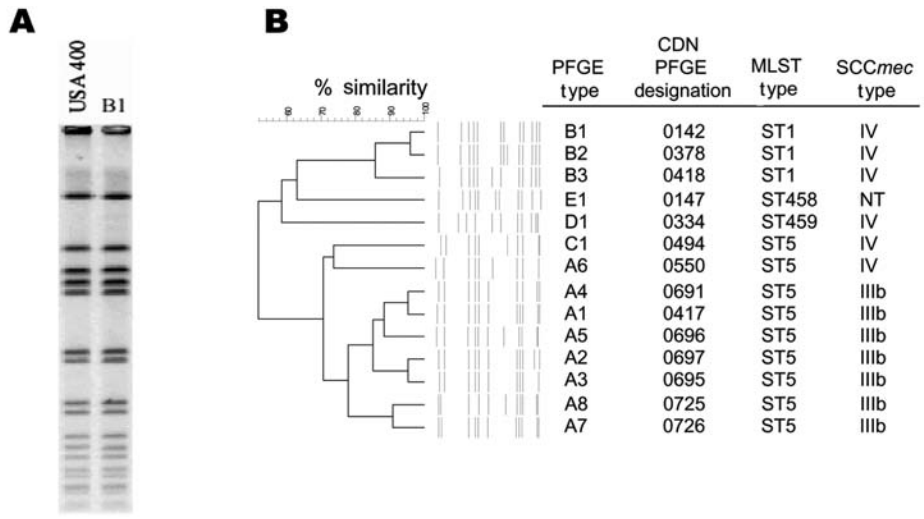

Figure 2. A) Pulsed-field gel electrophoresis (PFGE) fingerprint of USA400 and PFGE pattern B1. B) Dendrogram showing relationship of the unique fingerprints, along with the PFGE type designation (11) and other molecular characteristics of each subtype. CDN, Canadian Diseases Network; MLST, multilocus sequence typing; SCC, staphylococcal chromosome cassette.

patients 58% (n = 32) were identified with clonal group A–related infections. Approximately 64% (n = 35) of these strains were identified from persons >59 years of age, although 29% (n = 16) of cases were identified in persons <10 years of age (Figure 3). Of the 22 cases reported from the community health centers, 73% (n = 16) were <18 years of age. Comparison of the representative PFGE clonal group A1 fingerprint pattern to the Canadian National Fingerprint Database showed that these strains were similar to the previously identified epidemic MRSA-labeled CMRSA-2 (19,20). A representative strain from each of the unique fingerprint patterns from the A clonal group was typed by using MLST, and all were found to be of the sequence type ST5. All of the unique A subtypes as well as a 25% (n = 12) sample of strains that were of the A1 subtype did not harbor the *lukF-PV*, *lukS-PV*, *eta*, and *etb* toxin genes. SCC*mec* typing of 4 A1 subtypes and subtypes A2, A3, A4, A5 showed all contained SCC*mec* IIIb. Most clonal group A isolates were resistant to ciprofloxacin, erythromycin, gentamicin, tetracycline, mupirocin, and fusidic acid (Table 2).

Characterization of Clonal Group B Isolates

The first reported case of infection due to clonal group B occurred 16 months after the description of first reported MRSA infection on August 31, 2000, and the cases continued to increase with a peak of occurring in October 2001 (Figure 1B). Clonal group B correlated with a large number of noninvasive infections reported from community health centers, and only 1 report was made of a long-term care patient with MRSA type 0142 infection. The distribution by sex was similar; 49% of patients were female (n = 62) and 51% were male (n = 64). More than 67% (n = 85) of these strains were identified from persons <20 years of age, although ≈17% (n = 21) of cases were identified in persons >40 years (Figure 3).

The PFGE B1 fingerprint pattern (CDN type 142) was indistinguishable from the USA CA-MRSA strain labeled USA400 (Figure 2) (21). MLST was conducted on 1 representative strain from each clonal group B subtype, and all were the same sequence type (ST1). In addition, these MRSA strains were shown to contain SCC*mec* type IV. A selection of ≈25% of the PFGE type 142 strains (n = 33), including all of the unique fingerprint patterns B2 and B3, was shown by PCR to harbor the *lukF-PV* and *lukS-PV* genes, and none of the strains tested harbored the *eta* and *etb* toxin genes. Clonal group B strains were susceptible to most antimicrobial agents tested; however, 40% and 55% of the isolates displayed resistance to erythromycin and high-level resistance to mupirocin, respectively (Table 2).

Characterization of Other Molecular Subtypes

Three other unique PFGE subtype groups were identified; these were composed of single isolates labeled C1, D1, and E1, respectively (Figure 2). None of these isolates carried the *lukF-PV* and *lukS-PV* toxin genes or the

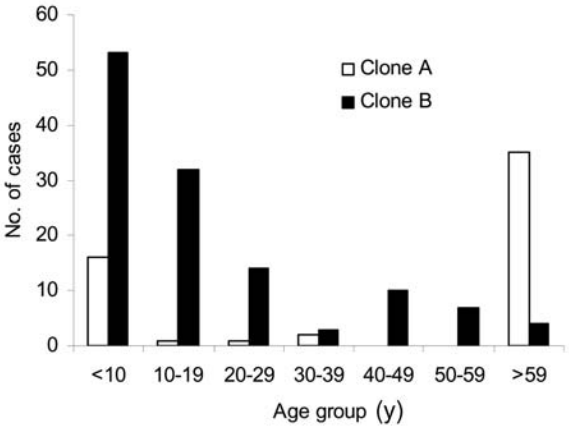

Figure 3. Age distribution of patients with cases caused by each clonal type A (white bars) or type B (black bars).

Table 2. Antimicrobial susceptibilities of the different clonal groups identified using pulsed-field gel electrophoresis

	Clonal group A (n = 55)				Clonal group B (n = 126)			
Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	% resistant	Range	MIC_{50} (mg/L)	MIC_{90} (mg/L)	% resistant	Range
Oxacillin	16	32	100	4–64	16	32	100	4–64
Cefazolin	32	32	75	4–32	16	32	31	2–32
Ciprofloxacin	32	≥32	86	0.25–≥32	0.25	0.25	0	0.25–0.5
Clindamycin	<0.25	≥8	11	<0.25–≥8	<0.25	<0.25	1.6	<0.25–≥8
Erythromycin	>8	≥8	87	<0.25–≥8	<0.25	≥8	40	<0.25–≥8
Gentamicin	>16	≥16	64	<0.5–≥16	<0.5	<0.5	0	<0.5
Rifampin	<0.25	<0.25	3.6	<0.25–≥4	<0.25	<0.25	0	<0.25
Tetracycline	16	≥16	55	<2–≥16	<2	<2	0	<2
Trimethoprim/sulfamethoxazole	<0.25	0.5	5.5	<0.25	<0.25	<0.25	0	<0.25
Vancomycin	1.0	1.0	0	1.0	1.0	1.0	0	0.5-1.0
Linezolid	4.0	4.0	0	4.0	2.0	4.0	0	1.0-4.0
Mupirocin	≥128	≥128	58	≥128	≥128	≥128	55.6	<0.25–≥128
Fusidic acid	≥8	≥8	100	≥8.0	0.12	0.25	0	0.12–0.25

exfoliative toxins. Similar to the PFGE B clonal group, these strains remained susceptible to most non–β-lactam drugs tested (data not shown).

Discussion

A dramatic increase of MRSA in the east-central region of Saskatchewan has been documented in this study. Molecular typing analysis has shown that 2 major unrelated strain clusters were in circulation in the communities under study. The emergence of clonal group A was linked to a patient in a long-term care facility who had recently been transferred from a hospital in Saskatoon, and this strain began to spread within the facility. Comparisons to the Canadian National Fingerprint Database showed the A1 pattern is related to the CMRSA-2 epidemic strain cluster observed in Canadian tertiary care hospitals (19,20). Forty percent of infections caused by the A clonal group were identified from community health centers, which suggests that this strain may have spread from the long-term care facility into the community. In fact, CMRSA-2 has been previously shown to be more likely to be associated with community isolations than the other epidemic CMRSA clusters (19). Unlike previously described CA-MRSA strains, this strain does not harbor the PVL or exfoliative toxin genes, and it displays a multidrug resistance phenotype. However, analysis of the community health center cases showed that most (73%) were from persons <18 years of age. Current studies that use comparative genomics are under way in our laboratory to determine whether any genetic differences exist between community, nosocomial, and sporadic MRSA that may explain the association with community isolation (23).

The more predominant clone (cluster B) in this study was, with 1 exception, identified from cases reported from community health centers. Comparison of the B1 fingerprint pattern showed that it was indistinguishable to the USA 400 strain, which is responsible for CA-MRSA infections in the midwestern United States (24,25). Although the source of the USA 400 strain into this community cannot be determined, a community-based cluster of MRSA was reported in a rural community in southwestern Manitoba in 1997, and this strain had been identified previously from persons in northwestern Manitoba since 1995 (26). We have demonstrated that this strain is also indistinguishable from the USA400 fingerprint pattern (data not shown). This strain may have spread from northwestern Manitoba to the community in this current study because they are geographically close (J. Wylie, pers. comm.).

When all of the typing information is compared, some observations warrant mention. The single isolate with the molecular subtype designated A6 contains SCC*mec* IV, which is different from the other A-subtypes that contain SCC*mec* IIIb. Notably, all A-subtypes are clustered into the MLST group ST5. Although the SCC*mec* IV region is smaller than that of the SCC*mec* IIIb, it could not have arisen from a simple deletion event because sequence analysis of these 2 cassettes has shown l significant difference that cannot be explained by a simple deletion event (27). One possible explanation is that a recombinational event occurred, which led to the replacement of SCC*mec* IIIb with the SCC*mec* IV. Instability in these regions has been reported, although the events are likely rare (28,29). Since clone B harboring the SCC*mec* IV is also circulating in this community, a recombination event may have occurred. Furthermore, the MRSA strains with macrorestriction patterns C1 and D1 also contained the SCCmec IV region, which suggests that horizontal transfer of this cassette to additional *S. aureus* strains may have occurred. Alternatively, the strain with the A6 pattern may have emerged independently from the other strains within the A cluster.

This study documents the finding of high-level mupirocin resistance in >50% of all study strains (clones A and B). Mupirocin can be used to treat superficial skin infections and has been used to decolonize patients. High-level mupirocin resistance in MRSA was first described in 1996 among patients in a burn unit in Kuwait (30). Mupirocin resistance is mediated by the *mupA* gene, which is generally plasmid encoded, although recently the gene has been identified on the chromosome of *S. aureus* (30,31). Since high-level resistance has been documented on plasmids that vary in size and restriction patterns (1,32), examining the plasmid restriction fragment length polymorphisms may be useful in monitoring monitor plasmid dissemination between and within the clonal types. In a similar manner, we previously described extended-spectrum β-lactamase–containing plasmids in gram-negative organisms (33). Information from this exercise could determine if horizontal transfer of the gene coding for mupirocin resistance occurred between the two clonal groups A and B.

We retrospectively reviewed a sample of charts from patients with skin and soft tissue infections in 2002 in the region of highest CA-MRSA frequency and found that 18% of these infections resulted in a prescription for mupirocin (unpub. data). The use of mupirocin in these communities may be driving resistance. A recent study of patients from a Tennessee medical center documented that decreased usage of mupirocin reduces this form of resistance in MRSA (33). We are currently developing educational programs to decrease the use of this drug, which we hope will decrease mupirocin resistance in this region.

The 2 clonal groups of MRSA described in this study continue to circulate in this area of Saskatchewan (data not shown). We are currently undertaking a case-control study to identify risk factors associated with infections caused by these 2 strains in the community. We call for future studies to include mupirocin in their antimicrobial resistance panels because resistance to this agent may affect treatment outcomes.

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Dr. Mulvey is chief of the Antimicrobial Resistance and Nosocomial Infections Laboratory at the National Microbiology Laboratory in Winnipeg, Canada. His research interests include the study of the emergence of antimicrobial resistance and the mechanisms involved in the dissemination of resistance.

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Emergence and Spread of *Streptococcus pneumoniae* with *erm*(B) and *mef*(A) Resistance

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Streptococcus pneumoniae isolates (N = 31,001) were collected from patients with community-acquired respiratory tract infections during the PROTEKT US surveillance study (2000–2003). While the macrolide (erythromycin) resistance rate remained stable at ≈29%, the prevalence of resistant isolates containing both *erm*(B) and *mef*(A) increased from 9.7% in year 1 to 16.4% in year 3, with substantial regional variability. Almost all (99.2%) dual *erm*(B)+*mef*(A) macrolide-resistant isolates exhibited multidrug resistance, whereas 98.6% and 99.0% were levofloxacin- and telithromycin-susceptible, respectively. These strains were most commonly isolated from the ear or middle-ear fluid of children. Of 152 representative *erm*(B)+*mef*(A) isolates, >90% were clonally related to the multidrug-resistant international Taiwan^{19F}-14 clonal complex 271 (CC271). Of 366 *erm*(B)+*mef*(A) isolates from the PROTEKT global study (1999–2003), 83.3% were CC271, with the highest prevalence seen in South Africa, South Korea, and the United States. This study confirms the increasing global emergence and rapidly increasing US prevalence of this multidrug-resistant pneumococcal clone.

Streptococcus pneumoniae is a key pathogen implicated in community-acquired respiratory tract infections, including acute otitis media (1), community-acquired pneumonia (2), acute exacerbations of chronic bronchitis (3), and acute bacterial sinusitis (4). During the last decade, the clinical management of respiratory infections has become increasingly complicated by the emergence and spread of resistance in *S. pneumoniae* to commonly used antibacterial drugs, particularly β-lactams and macrolides, both in the United States (5–10) and worldwide (11–13). PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) is an international, longitudinal surveillance study initiated in 1999 to evaluate the activity of telithromycin, a new ketolide antibacterial drug, against *S. pneumoniae* and other common respiratory pathogens and to compare its activity with other antibacterial drugs (13). In addition, the integration of genotypic testing into PROTEKT has helped elucidate the international molecular epidemiology of resistant strains (14,15).

PROTEKT US is a sister program to the PROTEKT global study that was initiated in 2000, specifically to monitor antibacterial resistance in the United States. Data from PROTEKT US showed an overall pneumococcal macrolide (erythromycin) resistance rate of 31.0% in 2000 and 2001 (9). Macrolide resistance in *S. pneumoniae* is mediated by 2 major mechanisms: methylation of ribosomal macrolide target sites, encoded by the *erm*(B) gene, and drug efflux, encoded by *mef*(A) (14–17). While *erm*(B) is the dominant genotype across much of the world, *mef*(A)-mediated mechanisms of resistance predominate in the United States (14). Recently, PROTEKT and other studies have identified *S. pneumoniae* isolates with both *erm*(B) and *mef*(A) genes in the United States, Canada, South Korea, China, South Africa, Japan, Mexico, and Hungary (14,15,18–22). The initial confirmation of isolates with both *erm*(B) and *mef*(A) was first described in the South African study (19). These dual *erm*(B)+*mef*(A) isolates belong predominantly to 1 major clonal complex (15) and show high rates of resistance to multiple classes of antibacterial drugs; consequently, their potential spread is of serious concern.

We report the prevalence of the multidrug-resistant *erm*(B)+*mef*(A) clonal complex in the United States. In

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addition, molecular epidemiologic data for macrolide-resistant *S. pneumoniae* isolates collected as part of the PROTEKT US study from 2000 to 2003 are compared with data for isolates collected as part of the PROTEKT global study (1999–2003) to assess the spread of the *erm*(B)+*mef*(A) clonal complex.

Methods

For the PROTEKT US study, isolates of *S. pneumoniae* were collected from across the United States from 2000 to 2003. The numbers of collection centers that provided samples were 207 in year 1 (2000–2001), 241 in year 2 (2001–2002), and 247 in year 3 (2002–2003).

Pathogenic respiratory tract isolates of *S. pneumoniae* were collected from adult and pediatric outpatients with community-acquired respiratory tract infections (acute otitis media, pneumonia, acute exacerbations of chronic bronchitis, acute exacerbations of chronic obstructive pulmonary disease, and sinusitis). Also included were isolates cultured from material collected from hospitalized patients within 48 hours of admission. The following sources were considered acceptable: cultures from blood, sputum, bronchoalveolar lavage, middle-ear fluid (collected by tympanocentesis), nasopharyngeal swab or aspirate, and sinus aspirate. Patients with nosocomial respiratory tract infections and those with cystic fibrosis were excluded. Duplicate strains, or strains originating from existing collections, were also not included in the study. Demographic data collected included the age and sex of the patient, infection, culture source, inpatient versus outpatient status, specimen accession number, and date of sample collection. Details of the methods for isolate storage, transportation, and identification have been reported previously (23).

MICs were determined at a central laboratory (CMI, Portland, OR, USA) by using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (24). The following antibacterial agents were tested: amoxicillin-clavulanate (amoxicillin alone was not tested; however, susceptibility can be extrapolated from the amoxicillin-clavulanate results), azithromycin, cefuroxime, clarithromycin, clindamycin, co-trimoxazole, erythromycin, levofloxacin, linezolid, penicillin, telithromycin, and tetracycline. In all cases, CLSI MIC interpretive criteria were used to define susceptibility and resistance (25). Susceptibility to telithromycin was determined by using the CLSI breakpoints (25): susceptible $\leq$1 μg/mL; intermediate 2 μg/mL; resistant $\geq$4 μg/mL.

All erythromycin-resistant (MIC $\geq$1 μg/mL) pneumococcal isolates collected from PROTEKT US years 1–3 were analyzed for the presence of *erm*(B), *erm*(A) subclass *erm*(TR), and *mef*(A) macrolide resistance genes. Isolates in year 1 were analyzed by multiplex rapid-cycle polymerase chain reaction (PCR) with microwell-format probe hybridization, as described previously (26). In years 2 and 3, isolates were analyzed by using a multiplex TaqMan (Applied Biosystems, Foster City, CA, USA) PCR assay that was validated against the previous PCR method (27).

A proportion of dual *erm*(B)+*mef*(A) macrolide-resistant isolates underwent serotyping and multilocus sequence type (MLST) determination at G.R. Micro Ltd (London, UK). Isolates were serotyped by using antisera from the Statens Serum Institute (SSI, Copenhagen, Denmark). MLST was determined as described previously (15).

Serotyping and MLST determination were also conducted on 366/378 dual *erm*(B)+*mef*(A) macrolide-resistant *S. pneumoniae* isolates, respectively, collected from the global PROTEKT study (1999–2003). Sequence type (ST) and alleles were analyzed by UPGMA (unweighted pair group method with arithmetic mean) and BURST (based upon related STs) analysis by using the START program (version 1.0.5 [28]) to assign lineage and clonal complexes.

Results

Macrolide Resistance Mechanisms

From 2000 to 2003, a total of 31,001 *S. pneumoniae* isolates were collected as part of the PROTEKT US study: 10,103 in year 1, 10,012 in year 2, and 10,886 in year 3. The proportion of *S. pneumoniae* isolates resistant to erythromycin was similar across years 1, 2, and 3 of the PROTEKT US study (29.4% overall). The prevalence of *mef*(A) in macrolide-resistant isolates decreased from 68.8% in year 1 to 67.3% in year 2 and to 63.9% in year 3, while the prevalence of *erm*(B) alone appeared stable (16.9% in year 1, 16.5% in year 2, 16.5% in year 3). By contrast, an increase was seen in the prevalence of macrolide-resistant strains carrying both *erm*(B) and *mef*(A) genes; by year 3, 16.4% of isolates were of this genotype (Table 1). When considered as a proportion of all *S. pneumoniae* isolates collected in year 3, a total of 520 (4.8%) of 10,886 were positive for both *erm*(B) and *mef*(A).

Geographic differences were observed in the prevalence of *erm*(B)+*mef*(A)–encoded resistance across the United States, from 10.3% in the Southeast to 23.9% in the North-Central region (year 3). The prevalence of this genotype increased in all regions between years 1 and 3 (Table 1).

The largest increases in *erm*(B)+*mef*(A)-encoded resistance during the 3-year study period occurred in isolates collected from pediatric patients (Table 2). By year 3, isolates exhibiting this genotype made up 254 (22.7%) of 1,119 isolates obtained from pediatric patients ($\leq$14 years of age) compared with 98 (12.3%) of 794 isolates collected from patients >64 years of age. Patients in the 0- to 2-year age group had the highest prevalence (23.9%) of dual *erm*(B)+*mef*(A) resistance (Table 2).

Table 1. Geographic distribution by year of *Streptococcus pneumoniae* collected from years 1 to 3 of the PROTEKT US study with both *erm*(B)- and *mef*(A)-encoded macrolide resistance among genotyped erythromycin-resistant isolates

	No. *erm*(B)+*mef*(A)–positive/no. erythromycin-resistant (%)			
US region*	Year 1	Year 2	Year 3	Years 1–3 combined
North-Central	121/667 (18.1)	117/626 (18.7)	176/735 (23.9)	414/2,028 (20.4)
Northeast	88/985 (8.9)	96/771 (12.5)	140/900 (15.6)	324/2,656 (12.2)
Northwest	8/98 (8.2)	18/119 (15.1)	26/125 (20.8)	52/342 (15.2)
South-Central	23/561 (4.1)	36/463 (7.8)	80/667 (12.0)	139/1,691 (8.2)
Southeast	29/427 (6.8)	31/481 (6.4)	49/475 (10.3)	109/1,383 (7.9)
Southwest	35/395 (8.9)	37/333 (11.1)	49/275 (17.8)	121/1,003 (12.1)
Total	304/3,133 (9.7)	335/2,793 (12.0)	520/3,177 (16.4)	1,159/9,103 (12.7)

*North-Central = Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Wisconsin; Northeast = Connecticut, Delaware, Indiana, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, Washington DC; Northwest = Alaska, Idaho, Montana, Oregon, Washington, Wyoming; South-Central = Alabama, Arkansas, Louisiana, Oklahoma, Tennessee, Texas; Southeast = Florida, Georgia, Kentucky, North Carolina, Puerto Rico, South Carolina, Virginia, West Virginia; Southwest = Arizona, California, Colorado, Nevada, New Mexico, Utah.

Across the 3-year study period, the dual *erm*(B)+*mef*(A) genotype was found most frequently in isolates collected from the ear or middle-ear fluid (Table 3). In year 3, the prevalence of this form of macrolide resistance was >30% in isolates collected from either of these sources. By contrast, isolates cultured from blood samples had the lowest proportion of dual *erm*(B)+*mef*(A)-encoded resistance (92 [4.6%] of 2,014 isolates in the 3 years).

Antimicrobial Resistance in Dual *erm*(B)+*mef*(A) Isolates

In addition to exhibiting almost universal resistance to the macrolides tested (azithromycin, clarithromycin, erythromycin), isolates carrying both *erm*(B) and *mef*(A) were highly resistant (>90%) to penicillin, cefuroxime, tetracycline, and co-trimoxazole (Table 4). Resistance to amoxicillin-clavulanate (and hence amoxicillin) was also common in these isolates (Table 4), and the longitudinal data showed that the rate of resistance to this antibacterial drug increased from 29.9% to 43.9% from year 1 to year 3. Almost all (1,150 [99.2%] of 1,159) of the *erm*(B)+*mef*(A) isolates were multidrug-resistant (i.e., resistant to ≥2 classes of antibacterial drugs).

A total of 16 (1.4%) of the 1,159 dual *erm*(B)+*mef*(A) isolates collected were resistant to levofloxacin; MIC values for these were as follows: 8 μg/mL (2 isolates), 16 μg/mL (9 isolates), 32 μg/mL (4 isolates), and 128 μg/mL (1 isolate). One dual *erm*(B)+*mef*(A) isolate (<0.1% of the total) was resistant to telithromycin (MIC 4 μg/mL).

Molecular Epidemiology

The results of MLST determination on 518 *S. pneumoniae* isolates (366 from PROTEKT global [including 35 from the United States] and 152 from PROTEKT US) with dual *erm*(B)+*mef*(A)–encoded resistance showed 82 ST variants (Figure). Of these, 21 were in the MLST database, and 61 were submitted to the database and assigned a new ST (STs 1407–1467). All of the unique STs were serotyped and, together with the 20 *S. pneumoniae* clones listed by the Pneumococcal Molecular Epidemiology Network (29), were analyzed for clonal relatedness by using UPGMA and BURST (Figure). Both the serotype distribution and range of MLSTs in these isolates were limited (Table 5), with 3 clonal complexes predominating. A phylogenetic analysis of these variations showed that 45 of the 82 STs were closely related, either serotype 19F or 19A (Table 5). These strains were of ancestral ST 271 and hence were designated clonal complex (CC) 271, which is equivalent to CC I (15) and CC 236 (22). Overall, 305 (83.3%) of 366 global isolates had MLST profiles consistent with this clone (Table 6).

Of the 35 isolates collected in the United States from the PROTEKT global study, all exhibited MLST profiles and serotypes characteristic of CC 271 (Table 6). Moreover, analysis of a geographically and chronologically varied sample of 152 *S. pneumoniae* isolates with dual *erm*(B)+*mef*(A)–encoded resistance collected from the PROTEKT US study suggested that >90% of dual-resistant isolates in the United States belong to CC 271.

Table 2. Proportion of erythromycin-resistant *Streptococcus pneumoniae* isolates collected from years 1 to 3 of the PROTEKT US study with the dual *mef*(A)+*erm*(B) genotype according to patient age

	No. *erm*(B)+*mef*(A)–positive/no. erythromycin-resistant (%)		
Patient age (y)	Year 1	Year 2	Year 3
0–2	88/825 (10.7)	118/640 (18.4)	170/710 (23.9)
3–14	52/388 (13.4)	47/365 (12.9)	84/409 (20.5)
15–64	98/1,106 (8.9)	95/972 (9.8)	151/1,173 (12.9)
>64	58/727 (8.0)	70/755 (9.3)	98/794 (12.3)
Not specified	8/87 (9.2)	5/61 (8.2)	17/91 (18.7)
Total	304/3,133 (9.7)	335/2,793 (12.0)	520/3,177 (16.4)

Table 3. Proportion of erythromycin-resistant *Streptococcus pneumoniae* isolates collected from years 1 to 3 of the PROTEKT US study that exhibit the dual *mef*(A)+*erm*(B) genotype according to source of isolate*

	No. *erm*(B)+*mef*(A)–positive/no. erythromycin-resistant (%)		
Source of isolate	Year 1	Year 2	Year 3
BAL	35/346 (10.1)	47/349 (13.5)	74/426 (17.4)
Blood	24/805 (3.0)	31/568 (5.5)	37/641 (5.8)
CSF	–	1/2 (50.0)	–
Ear	67/420 (16.0)	48/246 (19.5)	107/354 (30.1)
Eye	3/16 (18.8)	11/120 (9.2)	2/7 (28.6)
MEF	2/34 (5.9)	6/29 (20.7)	14/40 (35.0)
NAP	34/246 (13.8)	43/242 (17.8)	51/235 (21.7)
Sinus	18/162 (11.1)	15/159 (9.4)	36/180 (20.0)
Sputum	117/1,056 (11.1)	129/1,050 (12.3)	199/1,294 (15.4)
Throat	1/15 (6.7)	1/8 (12.5)	–
Not specified	3/33 (9.1)	3/20 (15.0)	–
Total	304/3,133 (9.7)	335/2,793 (12.0)	520/3,177 (16.4)

*BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; MEF, middle-ear fluid; NAP, nasopharyngeal swab or aspirate; –, no isolates collected.

Discussion

Pneumococcal macrolide resistance in the United States is predominantly mediated by the *mef*(A) gene, which encodes for lower-level, efflux-mediated resistance (14). However, the latest surveillance data from PROTEKT US presented in this article show that the prevalence of this form of resistance is decreasing. This trend coincides with the emergence of multidrug-resistant clones of *S. pneumoniae* that express both *erm*(B) and *mef*(A). These strains increased in prevalence from 9.7% of macrolide-resistant isolates in 2000–2001 to 16.4% in 2002–2003. Moreover, geographic data indicate that dual *erm*(B)+*mef*(A) isolates are currently even more prevalent in some regions of the United States (accounting for >20% of macrolide-resistant strains). By 2002–2003, *S. pneumoniae* strains with this dual mechanism of resistance made up almost 5% of all isolates collected.

The major clinical implication of the present report is the increased potential for treatment failure with most antibacterial drugs currently recommended to empirically treat community-acquired respiratory tract infections (30,31). Ear isolates are more prone to represent treatment failure, and blood isolates represent primary infection; thus, the dramatic increase in CC 271 in ear isolates compared to blood isolates (Table 2) is noteworthy. Almost all dual *erm*(B)+*mef*(A) isolates were highly resistant to multiple antibacterial drugs, including penicillin, macrolides, tetracycline, and co-trimoxazole. This high-level macrolide resistance is presumably mediated by the *erm*(B) gene. Furthermore, resistance to amoxicillin-clavulanate (and hence amoxicillin) increased in these isolates from 29.9% to 43.9% during the 3-year surveillance period, which raises concerns about the potential selection of resistant isolates through widespread use of this agent for community-acquired infections, particularly acute otitis media.

The prevalence of resistance to fluoroquinolones, such as levofloxacin, was low (1.4% overall) in the dual *erm*(B)+*mef*(A) isolates; however, when present, this resistance was often high (MIC 8–128 μg/mL). Telithromycin resistance was rare (<0.1%) in *S. pneumoniae* isolates with dual *erm*(B)+*mef*(A)–encoded macrolide resistance.

Table 4. Susceptibility to various antibacterial drugs among *Streptococcus pneumoniae* isolates collected from years 1–3 of the PROTEKT US study that had both *erm*(B) and *mef*(A) macrolide resistance genes (n = 1,159)

	% susceptibility*		
Drug	Susceptible	Intermediate	Resistant
Amoxicillin–clavulanate†	40.6	22.0	37.4
Azithromycin	0	0.1	99.9
Cefuroxime	5.7	1.7	92.6
Clarithromycin	0	0	100
Co-trimoxazole	3.4	1.2	95.4
Erythromycin	0	0	100
Levofloxacin	98.6	0	1.4
Linezolid	99.8	0	0.2
Penicillin	1.5	6.7	91.8
Telithromycin	99.0	0.9	0.1
Tetracycline	2.7	0.7	96.6

*Susceptibility was defined according to Clinical and Laboratory Standards Institute interpretive criteria (25).
†Amoxicillin alone was not tested; however, susceptibility can be extrapolated from the amoxicillin-clavulanate results.

Previous studies have indicated that a small number of clonal groups account for most penicillin-, macrolide-, and multidrug-resistant *S. pneumoniae* in the United States (18,32). The MLST analysis conducted in the present study shows that the dual *erm*(B)+*mef*(A) macrolide-resistant *S. pneumoniae* isolates collected in the United States from 1999 to 2003 are associated with 3 major global clones, in addition to a wide variety of other MLST variations. Most of these isolates belong to 1 major clonal group; the genotypic profile and serotype distribution of this predominant group show that it is highly related to an international *erm*(B)+*mef*(A) clonal strain, Taiwan^{19F}-14, first found in the Far East (22). The designation of clonal groups is determined by BURST analysis, which assigns ancestral lineage by the most common ST. For this reason, the pneumococcal clone designated CC 271 in the present study was named CC 236 in the study by Ko and Song (22). To avoid confusion, a common CC nomenclature (such as the original designation of the clone, CC 1 [15]) may be more useful.

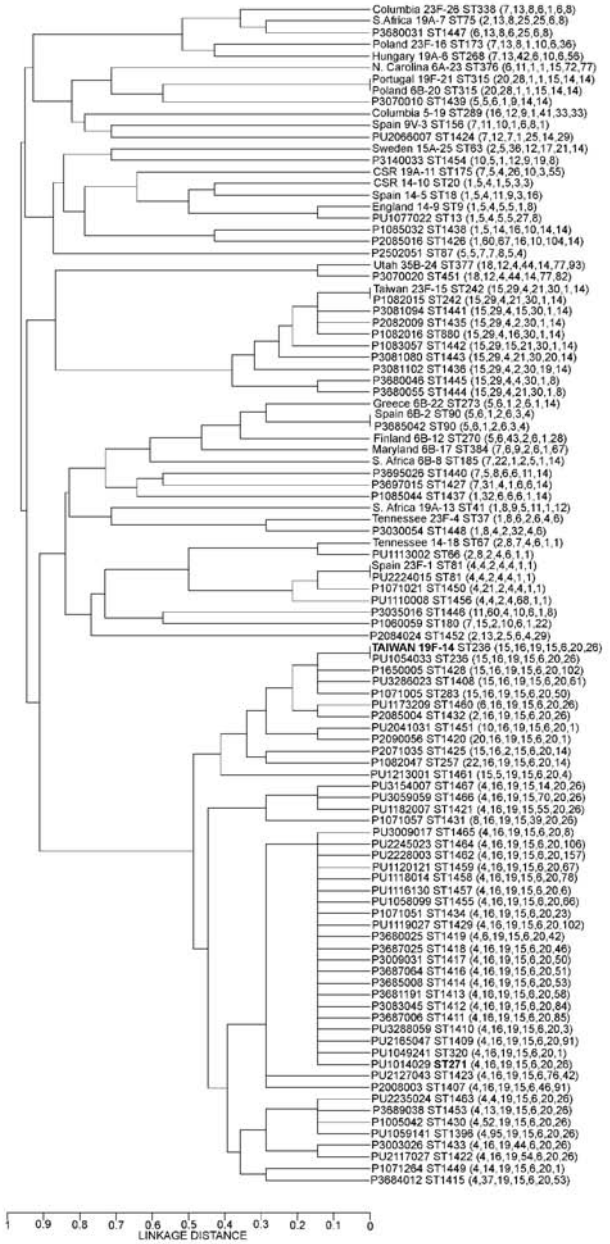

Figure. Phylogenetic relationships of the 82 different sequence type variations found in 518 *Streptococcus pneumoniae* isolates with combined *erm*(B)- and *mef*(A)-mediated macrolide resistance collected during the PROTEKT global study (1999–2003, n = 366) and the PROTEKT US study (2000–2003, n = 152) compared with the 20 PMEN (Pneumococcal Molecular Epidemiology Network [29]) clones.

The pneumococcal clone discussed in this paper was previously identified in the first year (1999–2000) of the PROTEKT global study (15). The most recent data from this survey, which covered the period 1999–2003, confirm that this clone now has a worldwide distribution, with particularly high incidences in South Africa and South Korea, as reported in previous studies (15,19,21). Strains carrying both genes have also been recorded recently in New Zealand (33), Canada (34), Italy (35), and Scotland (36). Together with the regional genotyping data, the epidemiologic analyses we describe show that this multidrug-resistant CC 271 is now widespread and increasing in prevalence across the United States.

The widespread emergence of the *erm*(B)+*mef*(A) genotype into varying lineages at the apparent expense of strains expressing only 1 resistance determinant suggests that *S. pneumoniae* carrying this form of resistance has an evolutionary advantage. Since dual resistant isolates have drug MICs similar to those observed in strains harboring *erm*(B) alone, such an advantage cannot be explained on the basis of increased macrolide resistance alone. This clone has previously been shown to contain 2 mobile genetic elements, Tn*1545* and "mega" (15). While the *erm*(B) gene is most often present on Tn*1545*, "mega" is known to contain the *mef*(E) variant of *mef*(A), and this variant has been shown to be present in CC 271 (15). Hence, acquisition of 2 mobile genetic elements and associated resistance genes is a possible explanation for the successful emergence of this clone over isolates with only *erm*(B) or *mef*(A); it is not solely the acquisition of the efflux or methylase gene but the associated resistance genes on the genetic elements that lead to a multidrug-resistant clone in which prevalence is driven by greater environmental pressures.

Of particular concern is the finding that dual *erm*(B)+*mef*(A)–encoded resistance was most prevalent in isolates collected from pediatric patients. By year 3 of the study, 8.7% of all *S. pneumoniae* isolates collected from children ≤14 years of age and 10.7% of those collected from children ≤2 years of age exhibited this form of

Table 5. Distributions of sequence types and serotypes of 518 dual *erm*(B)+*mef*(A) erythromycin-resistant *Streptococcus pneumoniae* isolates collected during the PROTEKT global study (1999–2003, n = 366) and the PROTEKT US study (2000–2003, n = 152)*

Clonal complex (n)	PMEN clone designation	Sequence types (n)	Serotypes (n)
CC 271 (446)	Taiwan^{19F}-14	236 (48)†, 257 (1), 271 (218), 283 (4), 320 (93), 1396 (6), 1407 (1), 1408 (2), 1409 (4), 1410 (3), 1411 (1), 1412 (3), 1413 (1), 1414 (1), 1415 (1), 1416 (2), 1417 (1),1418 (11), 1419 (4), 1420 (1), 1421 (3), 1422 (1), 1423 (1), 1425 (1), 1428 (3), 1429 (2), 1430 (1), 1431 (1), 1432 (3), 1433 (2), 1434 (1), 1449 (1), 1451 (5), 1453 (1), 1455 (2), 1457 (1), 1458 (1), 1459 (1), 1460 (1), 1461 (2), 1462 (1), 1463 (1), 1464 (2), 1465 (1), 1466 (1)	14 (3), 19A (66), 19F (376), NT (1)
CC 242 (25)	Taiwan^{23F}-15	242 (9), 880 (1), 1435 (2), 1436 (1), 1441 (1), 1442 (1), 1443 (1), 1444 (7), 1445 (2)	23F (25)
CC 81 (12)	Spain^{23F}-1	81 (10), 1450 (1), 1456 (1)	14 (1), 19F (3), 23F (5), 6A (1), NT (2)
Singletons		13 (4), 66 (1), 87 (2), 90 (2), 451 (2), 1424 (3), 1426 (8), 1427 (1), 1437 (2), 1438 (1), 1439 (1), 1440 (1), 1446 (1), 1447 (1), 1448 (1), 1452 (1), 1454 (1), 1467 (2)	14 (4), 34(1), 16F (1), 19A (9), 19F (6), 23F (2), 35B (2), 6A (1), 6B (7), 7F (1), 9N (1)

*PMEN, Pneumococcal Molecular Epidemiology Network; NT, nontypeable.
†Sequence type 236 was previously described as CC 236 by Ko and Song (22) but is described as CC 271 in this study based on BURST analysis.

macrolide resistance. The introduction of the 7-valent pneumococcal vaccine (PCV7) in 2000 was aimed primarily at reducing the incidence of disease in this vulnerable group. While recent evidence suggests that this reduction has occurred (37,38), the vaccine does not provide coverage against all *S. pneumoniae* serotypes. As discussed above, most dual *erm*(B)+*mef*(A) isolates characterized in this study are of serotype 19A (the prevalence of which increased from years 1 to 3) or 19F. Although serotype 19F is represented in the PCV7 vaccine, it affords low levels of protection against upper respiratory infections such as otitis media (39) and has been shown recently to be the least immunogenic of the vaccine serotypes (40). Moreover, little evidence shows that 19F provides cross-protection against serotype 19A. The trends reported in this article indicate that the introduction of routine immunization has not prevented the spread of this nonvaccine serotype multidrug-resistant clone in the pediatric population and may have contributed to the selection of serotype 19A strains.

In summary, although pneumococcal macrolide resistance rates appear to have stabilized in the United States, prevalence of clonal isolates with the combined *erm*(B)+*mef*(A) genotype is increasing. These strains show high-level macrolide and multidrug resistance, and their spread across the United States represents a serious public health concern. These findings also highlight the critical need for continued monitoring of pneumococcal resistance patterns over time, in particular, the spread of these mul-

Table 6. Lineage by country of 366 dual *erm*(B)+*mef*(A) erythromycin-resistant *Streptococcus pneumoniae* isolates collected during the PROTEKT global study (1999–2003)

Country	No. isolates	Clonal complex			
		CC 271	CC 242	CC 81	None*
Australia	6	6	–	–	–
Brazil	2	2	–	–	–
Canada	4	4	–	–	–
China	13	12	–	–	1
France	3	1	–	–	2
Germany	1	1	–	–	–
Hong Kong	2	2	–	–	–
Hungary	2	2	–	–	–
Italy	2	2	–	–	–
Japan	44	12	16	1	15†
Mexico	6	4	–	–	2
South Africa	129	116	9	2	2
South Korea	111	102	–	6	3
Taiwan	5	3	–	–	2
United Kingdom	1	1	–	–	–
United States	35	35	–	–	–
Total, n (%)	366 (100)	305 (83.3)	25 (6.8)	9 (2.5)	27 (7.4)

*No clonal lineage found except for 2 isolates (ST 1467) and 1 isolate (ST 1452), which were clonally related (CC 1467).
†8 isolates were clonally related (ST 1426).

tidrug-resistant clones, and for physicians to be aware of local or regional resistance patterns when selecting empiric antibacterial treatment for community-acquired respiratory tract infections.

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International *Salmonella* Typhimurium DT104 Infections, 1992–2001

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The incidence of multidrug-resistant (MDR) *Salmonella* Typhimurium infections in humans, and in particular MDR definitive phage type 104 (DT104), has increased substantially in many countries in the last 2 decades, often associated with increased illness. To examine the magnitude of this problem, a survey was conducted among countries with available antimicrobial resistance or phage typing surveillance data. A total of 29, primarily industrialized, countries participated in the survey, which covered the years 1992–2001. Overall, the incidence of MDR *S.* Typhimurium and DT104 increased continuously during this period, although the problem affected primarily Europe and North America. The increase appeared to have peaked in the United Kingdom but not in other countries. Also, the incidence of quinolone-resistant *S.* Typhimurium was increasing. This survey implies that MDR *S.* Typhimurium constitutes an increasing public health problem in large parts of the world and emphasizes the importance of surveillance and control programs.

Infections with nontyphoidal *Salmonella* have increased during the last 3–4 decades, and although a decrease has been reported over the last decade, *Salmonella* infections continue to be a major public health concern in many countries (1–3). These salmonellae are zoonotic, and the infections are generally foodborne. Although a large number of *Salmonella* serotypes exist, the overall increase in the number of infections is of relatively few emerging serotypes and phage types. Over periods of several years, certain *Salmonella* types have risen and (sometimes) fallen within large geographic regions. These meta-outbreaks are facilitated through the acquisition, by specific types, of new traits that make them well adapted to spread, as well as through changes to human society, seen, for instance, with modern intensified farming and food production methods and global trade with live breeder animals (2,4,5). Two prominent examples are the international spread of *S.* Enteritidis infections through hens eggs (2,6) and the emergence over the last 2 decades of multidrug-resistant (MDR) *Salmonella* Typhimurium definitive phage type 104 (DT104).

Nontyphoidal *Salmonella* causes mild to severe, including life-threatening, infections. One study estimated that 600 deaths occur per year in the United States alone due to infections with nontyphoidal *Salmonella* serotypes (7). A recent study in Denmark showed that infection with nontyphoidal *Salmonella* was associated with a 2.5-fold increased risk for death within 1 year of infection compared with a matched sample from the general Danish population (8). Salmonellae resistant to antimicrobial drugs appear to pose a particular health risk. Thus, several

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studies have indicated that infection with salmonellae resistant to ≥1 antimicrobial drugs is associated with increased risk for hospitalization, invasive illness, and death (9–15).

In general, antimicrobial drug resistance occurs frequently in zoonotic salmonellae and is largely promoted by using antimicrobial drugs in food animals (4,16–18). *S.* Typhimurium DT104 is commonly resistant to 5 drugs: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). *S.* Typhimurium DT104 was first isolated in the early 1980s in the United Kingdom and later became endemic in bovine animals, from where it spread to the whole food animal production in that country (5,18). Throughout the 1990s, it spread to other parts of the world, and it is now a common *Salmonella* type in many countries, including the United States, the United Kingdom, Germany, and France (2,19–22). DT104 is common in a broad range of food animals, such as poultry, pigs, and sheep (23). This phage type has become a matter of concern because of its rapid international dissemination in the 1990s and its ability to readily acquire additional resistance traits to other, clinically important antimicrobial drug classes, such as quinolones, trimethoprim, and cephalosporins.

A global survey of salmonellosis and *Salmonella* serotyping was published in 2002 (24). However, relatively little information has been compiled on the global spread of DT104 and MDR *S.* Typhimurium (23). Therefore, we have conducted a survey to describe the pandemic of DT104 and MDR *S.* Typhimurium.

Methods

Participating Countries

The survey addressed information on antimicrobial drug resistance testing, phage typing, or both. Since most countries do not routinely apply these typing methods, the questionnaire was not simply sent to all World Health Organization (WHO) member states. Instead, an invitation to participate in the survey was distributed through the WHO Global Salm-Surv (GSS) network (25) and directly to all Enter-Net (26) network countries, plus a group of large countries known or assumed to have resistance testing or phage typing as an integrated part of their national *Salmonella* surveillance system.

A total of 52 questionnaires were sent out in June and July 2002. Of these, 44 were sent directly to countries known or assumed to have resistance testing or phage typing, and 8 were sent out to member states responding to the GSS invitation. Of the 52 questionnaires, 32 were sent to countries in the European region, 6 to the American Region, 7 to the Asian Region, 5 to the African Region, and 2 to the Oceania Region. Country names and names of geographic regions and subregions are used as described in the United Nations classification system (27).

The Questionnaire

Each country was requested to give information on the total annual number of laboratory-verified episodes of nontyphoidal salmonellosis, *S.* Typhimurium, MDR *S.* Typhimurium, and *S.* Typhimurium DT104 from 1992 to 2001. The participating countries were also asked to give details on whether the reported number of *Salmonella* isolates that formed the basis for further phage typing or antimicrobial drug susceptibility testing included all national isolates or if they were a subset of isolates. If only a subset of isolates were tested, the countries were asked to state the proportion of *S.* Typhimurium strains tested and what the criteria for choosing the isolates were. We also asked participants to describe methods used for antimicrobial drug resistance testing and the drugs included in their tests.

Multidrug-resistance was defined as isolates being resistant or intermediately susceptible towards ≥4 separate classes of drugs. This group includes isolates of R-type ACSSuT, the so-called classical penta-resistant phenotype. When information was available, participants were asked to give the numbers of isolates exhibiting R-types extending the ACSSuT-complex and the number of isolates resistant to clinically relevant antimicrobial drugs (quinolones, trimethoprim, and cephalosporins).

In countries where only a subset of *Salmonella* isolates had been submitted for phage typing, antimicrobial drug-resistance testing, or both, the total number of DT104 or MDR *S.* Typhimurium isolates was extrapolated from the reported numbers of isolates and the proportion of tested isolates.

Results

The questionnaire was sent to 52 countries, and a completed questionnaire was received from 29, a response rate of 56% (Figure 1). Of the 52 invited countries, 23 were members of or affiliated with Enter-Net; from these a positive feedback was received from 20 (87%). These countries were Australia, Austria, Belgium, Canada, Denmark, England and Wales, Finland, Germany, Greece, Ireland, Japan, Luxembourg, the Netherlands, New Zealand, Norway, Scotland, South Africa, Spain, Sweden, and Switzerland. The 8 countries and 1 regional center (31% response rate) that participated in the survey that were not associated with Enter-Net when data were collected were Brazil, the Czech Republic, Hungary, Israel, Latvia, Malta, Republic of South Korea, United States, and the Caribbean Epidemiology Centre (CAREC), a regional center that represents 21 countries in the Caribbean (Anguilla, Antigua and Barbuda, Aruba, Bahamas, Barbados, Belize, Bermuda, British Virgin Islands, Cayman Islands,

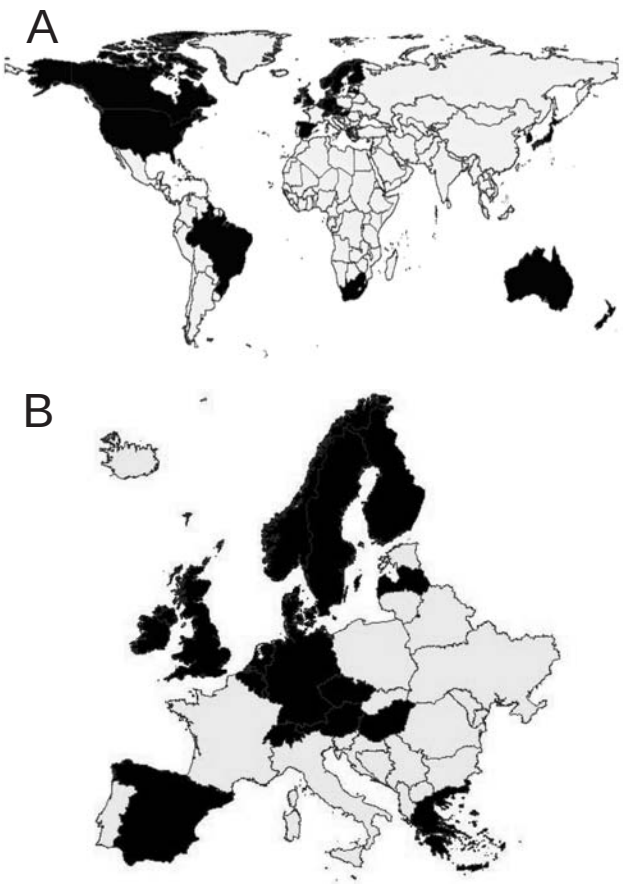

Figure 1. Participating countries in the survey of multidrug-resistant *Salmonella enterica* serotype Typhimurium, 1992–2001, internationally (A) and in Europe (B).

Dominica, Grenada, Guyana, Jamaica, Montserrat, Netherlands Antilles, St. Kitts and Nevis, St. Lucia, St. Vincent and the Grenadines, Suriname, Trinidad and Tobago, and Turks and Caicos). For the purpose of this study, CAREC was treated as 1 unit. The 29 participating countries had a total population of 1.028 billion in 2001.

Surveillance

The proportion of *Salmonella* isolates forwarded from local health laboratories to national or regional institutions varied from 5% to 100%. None of the participating countries restricted the submission of isolates to strains from selected patients, e.g., in case of septicemia or outbreak situations. All 29 participating countries performed serotyping on 85% to 100% of *Salmonella* isolates received at the national reference laboratory. Table 1 shows the number of nontyphoidal *Salmonella* isolates and the proportion hereof that were *S.* Typhimurium in each country from 1992 to 2001 presented as 2-year intervals. The table also shows the estimated incidence of laboratory-confirmed cases of *S.* Typhimurium in 2001. Throughout the study period, the proportion of *S.* Typhimurium among nontyphoidal salmonellae has been relatively stable. In 1992, 16% of isolates were *S.* Typhimurium, compared to 17% in 2001. However, large differences between countries and variations from year to year within each country made comparison difficult. Nevertheless, some trends emerged when the countries were aggregated into geographic regions. In the Caribbean region, South America, Eastern Asia, and Europe, a general decrease in the proportion of *S.* Typhimurium was observed. In North America, on the other hand, the situation has remained relatively stable, whereas both Australia and New Zealand have seen an increase in the number and proportion of *S.* Typhimurium cases from 1992 to 2001.

Phage Typing

In 1992, phage typing was carried out in 6 of the participating countries; by 2001, this number had risen to 22. Five countries (Brazil, Japan, Norway, Switzerland, and the United States) only performed phage typing when an MDR strain was found or in outbreak situations. Although not all countries performed phage typing in accordance with the Colindale Scheme (28), the countries using different standards provided information that allowed for comparison with results obtained using the Colindale Scheme (29–31).

The proportion of *S.* Typhimurium strains that were DT104 and the proportion thereof that were found to be MDR are shown for each country in Table 2. In general, the incidence and proportion of DT104 increased throughout the period. In 1992, 8.7% of *S.* Typhimurium isolates were DT104, but in 2001 this proportion had increased to 33%. Again, large regional differences occurred. In the United Kingdom, the incidence peaked in 1996 and then decreased. In most other European countries and North America, the relative numbers of DT104 strains had increased throughout the period. In Australia and New Zealand, few DT104 isolates (0.7%) were seen among *S.* Typhimurium in 2001. The proportion of *S.* Typhimurium strains that were DT104 is depicted in Figure 2 (the countries are aggregated into 8 regions).

Antimicrobial Drug Resistance

Antimicrobial susceptibility testing became an increasingly common constituent of the surveillance during the study period. In 1992, susceptibility testing was performed in 14 of the 29 participating countries and in all but one in 2000. All countries routinely tested a minimum of 6 antimicrobial drugs, all belonging to different drug classes. The majority of countries (73%) used disc diffusion testing according to NCCLS standards. Listed by frequency, the most common antimicrobial drug classes included in the test panel were (fluoro)quinolones, broad-spectrum penicillins, phenicols, aminoglycosides, tetracyclines,

cephalosporins, sulfonamides, and trimethoprim. In 21 countries, antimicrobial susceptibility testing was performed independently of phage typing results, but in 2 countries (Finland until 1999 and New Zealand), testing was only performed on strains found to be DT104.

Table 2 shows the distribution of MDR *S.* Typhimurium presented as 2-year time-bands. Figure 3 depicts the general trend, with countries divided into 9 different regions. In 1992 15% of all *S.* Typhimurium isolates were MDR; by 2001, this percentage had increased 3-fold to 42%. Once again, large variations occurred between countries and within countries from year to year. In most European countries and North America, MDR *S.* Typhimurium was common and, with the exception of the United Kingdom and Ireland, multidrug resistance increased from the mid-1990s to the end of the study period. For example, in 2001 multidrug resistance ranged from 22% (Greece) to 72% (Ireland). Only limited data were available from Brazil, the Caribbean region, South Africa, and the Republic of Korea. However, in these regions multidrug resistance was far less common, ranging from zero in the Caribbean region to 19% in the

Table 1. Number of patients with culture-confirmed, nontyphoidal *Salmonella* infection and percentage *S.* Typhimurium, international DT104 survey, 1992–2001

Subregion/country	Nontyphoidal *Salmonella* infections (% *S.* Typhimurium)					2001 pop. (IST)*
	1992–1993	1994–1995	1996–1997	1998–1999	2000–2001	
Northern Europe						
England + Wales	62,005 (16.4)	59,725 (20.5)	61,579 (16.8)	41,260 (13.2)	31,309 (15.1)	53 (3.9)
Ireland				1,152 (65.6)	1,147 (36.5)	3.8 (3.9)
Scotland	5,911 (20.1)	6,076 (21.6)	6,615 (19.8)	3,988 (16.0)	3,291 (17.8)	5.1 (5.0)
Denmark	7,184 (34.5)	7,923 (28.0)	8,273 (21.1)	7,148 (17.7)	5,257 (19.5)	5.4 (10.9)
Finland†	6,770 (13.0)	6,070 (14.1)	5,615 (16.9)	5,536 (12.4)	5,358 (10.4)	5.2 (5.0)
Latvia	1,048 (54.2)	1,965 (41.9)	1,393 (29.0)	1,830 (23.1)	1,664 (9.3)	2.4 (2.8)
Norway†	2,181 (18.1)	2,386 (13.3)	2,632 (16.4)	2,933 (14.9)	3,394 (13.5)	4.5 (4.9)
Sweden†	5,296 (17.5)	11,249 (13.3)	10,479 (11.5)	11,398 (13.8)	11,119 (14.1)	8.9 (8.3)
Western Europe						
Austria	11,337 (10.0)	19,790 (5.5)	18,278 (5.2)	16,918 (4.7)	15,135 (5.6)	8.3 (5.6)
Belgium	21,231 (34.7)	22,048 (31.9)	26,247 (26.2)	30,288 (21.7)	25,153 (20.6)	10.3 (23.0)
Germany‡	335,813 (17.1)	248,507 (21.9)	215,524 (26.4)	183,697 (27.1)	163,327 (26.2)	82.2 (8.4)
Luxembourg	490 (27.8)	447 (17.4)	555 (22.5)	652 (22.9)	701 (23.8)	0.44 (19.8)
Netherlands	5,388 (36.2)	5,955 (26.2)	5,445 (32.8)	4,393 (31.1)	4,141 (31.8)	16.0 (6.9)
Switzerland§					2,374 (10.2)	7.2
Eastern Europe						
Czech Republic	85,097 (6.8)	103,342 (2.6)	88,421 (2.3)	95,323 (2.1)	73,529 (1.9)	10.3 (5.3)
Hungary		14,138 (15.0)	11,398 (12.6)	8,188 (20.2)	8,480 (10.7)	10.0 (5.5)
Southern Europe						
Greece	1,198 (13.4)	1,514 (14.5)	1,315 (18.2)	1,091 (24.3)	1,947 (20.5)	10.9 (13.4)
Malta	546 (20.1)	518 (26.6)	236 (19.1)	390 (3.6)	135 (34.8)	0.40 (9.0)
Spain¶	6,374 (25.9)	7,188 (29.0)	8,847 (32.9)	10,806 (25.6)	13,379 (21.1)	39.8 (3.5)
Israel#	15,337 (10.7)	13,674 (8.1)	10,168 (27.2)	9,875 (16.8)	9,353 (15.0)	6.4 (11.4)
North America						
Canada	14,863 (19.2)	13,645 (20.3)	14,596 (23.0)	16,028 (21.0	12,493 (20.9)	31.0 (4.9)
USA	71,605 (22.6)	78,723 (22.5)	73,643 (25.3)	66,753 (25.3)	63,697 (22.1)	281.4 (2.6)
Caribbean region			687 (22.1)	786 (22.8)	379 (10.6)	7.1 (0.3)
Brazil	2,276 (10.5)	7,500 (2.7)	11,832 (1.5)	10,511 (3.8)	15,455 (4.8)	171.8 (0.3)
South Africa					1,341 (51.3)	43.6 (1.2)
Eastern Asia						
Japan	18,385 (8.4)	22,406 (6.1)	27,260 (2.8)	23,359 (3.2)	11,889 (5.3)	127.4 (0.2)
Republic of Korea	1,918 (37.4)	1,567 (32.0)	2,314 (18.7)	3,548 (15.9)	2,404 (11.3)	48.8 (0.3)
Oceania						
Australia	9,496 (28.1)	11,794 (35.2)	13,229 (40.2)	15,104 (35.2)	13,053 (38.9)	19.4 (13.7)
New Zealand	2,579 (56.2)	3,137 (58.3)	2,658 (56.6)	4,472 (63.1)	4,517 (64.7	3.9 (42.7)

*pop., population $\times 10^6$; IST, incidence of *S.* Typhimurium per 10^5 population.
†Most patients in these countries were infected abroad.
‡Data on *S.* Typhimurium and incidence are based on data from the new federal states of Germany and the city of Berlin.
§Submission of strains for serotyping at the central laboratory was not compulsory in Switzerland at the time of data collection. As a result an incidence rate for Switzerland is not presented.
¶Incidence is based on data from 2000.
#According to the United Nations classification, Israel belongs to the western Asian region. In this study, Israel has been grouped with southern European countries.

Republic of Korea. In Australia, multidrug resistance remained low throughout the period, with 1.0% of strains in 1997 and 3.6% in 2001.

MDR DT104 was a frequent subtype of MDR *S.* Typhimurium in most of the countries. In 1992, between 11% (Germany) and 77% (Scotland) of the MDR strains were DT104; in 2001 this ranged from 22% (Australia) to 94% (the Netherlands). Overall, the proportion of MDR DT104 of all DT104 has remained fairly stable; 84% of DT104 were classified as MDR in 2001, compared with 72% in 1992. Among MDR DT104, the classical penta-resistant phenotype, R-type ACSSuT, was by far the most common phenotype throughout the period. It was found in 99% of MDR DT104 strains in 1992 and in 94% of such strains in 2001 (data not shown).

Finally, we looked at trends in development of additional resistance in the classical penta-resistant phenotype (R-type ACSSuT), with focus on 3 clinically important antimicrobial drug classes: quinolones, cephalosporins, and trimethoprim. Both quinolone and trimethoprim resistance increased in MDR DT104 throughout the study period. In 1992, nalidixic acid susceptibility testing was

Table 2. Number (regional level) and proportion (%) of multidrug resistance and definitive phage type 104 (DT104) in *Salmonella* Typhimurium in 29 countries participating in the international DT104 survey*

	% MDR, % DT104 (% DT104 that are MDR)				
Subregion/country	1992–1993	1994–1995	1996–1997	1998–1999	2000–2001
Northern Europe†	479; 2,802; 2,143	910; 7,590; 6,629	1,190; 8,345; 7,520	1,729; 4,698; 2,969	1,380, 3,270, 824
England + Wales	NA, 22.6 (75.5)	NA, 54.5 (87.0)	NA, 67.5 (94.0)	NA, 57.1 (92.0)	NA, 42.3 (NA)
Ireland	40.3, 38.9 (82.5)	66.8, 61.2 (90.9)	76.1, 70.0 (92.6)	70.7, 65.4 (92.3)	63.3, 45.2 (90.6)
Scotland				75.0, 63.1 (73.6)	79.7, 56.3 (89.8)
Denmark		1.5, 1.5 (69.2)	5.0, 3.0 (80.8)	21.9, 15.8 (93.5)	23.1, 12.7 (91.5)
Finland	NA, 4.7 (61.0)	NA, 10.5 (83.3)	NA, 11.5 (78.0)	NA, 11.4 (87.2)	34.8, 27.9 (95.5)
Latvia				79.7, NA (NA)	63.2, NA (NA)
Norway			24.5, NA (NA)	22.0, 12.6 (100.0)	32.0, 24.0 (95.5)
Sweden			NA, 25.3 (NA)	NA, 22.3 (NA)	NA, 23.7 (NA)
Western Europe†	2,807; 885; 609	5,242; 1,944; 1,519	8,407; 6,346; 5,308	7,782; 5,347; 4,539	10,048; 7,626; 6,377
Austria	NA, 17.0 (NA)	NA, 14.6 (NA)	13.7, 32.7 (70.6)	13.1, 28.9 (67.5)	35.8, 29.6 (82.1)
Belgium					39.6, 27.0 (79.9)
Germany	14.3, 3.1 (89.3)	30.2, 9.2 (91.7)	44.3, 32.1 (87.1)	49.0, 32.1 (86.2)	57.1, 44.0 (84.7)
Luxembourg	11.0, NA (NA)	26.9, NA (NA)	43.2, NA (NA)	55.0, NA (NA)	58.1, NA (NA)
Netherlands	10.3, 6.7 (81.5)	8.9, 15.3 (43.1)	26.3, 23.5 (78.1)	29.5, 29.6 (79.8)	33.9, 37.2 (80.8)
Switzerland					48.8, 28.5 (100.0)
Eastern Europe†		NA; 1,034; NA	117; 2,020; 117	778; 1,902; 732	889; 1,182; 764
Czech Republic			5.8, 57.3 (10.1)	11.7, 43.9 (26.1)	28.5, 47.1 (54.6)
Hungary		NA, 48.9 (NA)	NA, 60.4 (NA)	33.0, 62.2 (84.0)	54.1, 57.7 (77.3)
Southern Europe†	121; 612; NA	166; 1,206; NA	1,126; 3,070; 222	112; 1,771; NA	1,360; 1,296; 674
Greece	18.1, NA (NA)	61.6, NA (NA)	44.8, NA (NA)	39.2, NA (NA)	26.3, NA (NA)
Malta	83.6, NA (NA)	22.5, NA (NA)	20.0, NA (NA)	57.1, NA (NA)	4.3, NA (NA)
Spain	NA, 37.1 (NA)	NA, 26.0 (NA)	34.6, 20.2 (80.4)	NA, 23.4 (NA)	45.0, 18.3 (65.0)
Israel‡		NA, 59.5 (NA)	NA, 89.6 (NA)	NA, 67.7 (NA)	85.6, 73.9 (97.7)
North America†	NA; 506; NA	3,507; 755; 237	9,045; 4,209; 3,265	8,315; 4,466; 3,528	6,748; 930; 785
Canada	NA, 17.7 (NA)	NA, 27.3 (42.5)	16.2, 46.1 (51.8)	44.1, 43.8 (82.3)	47.8, 35.5 (84.4)
USA		19.8, NA (NA)	45.7, 29.1 (92.6)	40.5, 34.0 (77.4)	39.0, NA (NA)
Caribbean region†			0; NA; NA	0; NA; NA	0; NA; NA
Caribbean			0.0, NA, (NA)	0.0, NA, (NA)	0.0, NA (NA)
South America†		1; NA; NA	7; NA; NA	52; NA; NA	97; NA; NA
Brazil		0.5, NA (NA)	4.0, NA (NA)	13.0, NA (NA)	13.0, NA (NA)
Southern Africa†					74; NA; NA
South Africa					11.2, NA (NA)
Eastern Asia†	NA; 32; 32	NA; 43; 43	62; 112; 111	44; 121; 121	37; 68; 68
Japan	NA, 2.1 (100.0)	NA, 3.1 (100.0)	NA, 8.8 (100.0)	NA, 13.7 (100.0)	NA, 9.8 (100.0)
Republic of Korea			14.3, 22.2 (97.8)	7.8, 3.2 (100.0)	13.7, 2.2 (100.0)
Oceania†	100; 13; 12	114; 9; 8	80; 4; 4	95; 16; 16	163; 38; 27
Australia	3.8, 0.1 (100.0)	2.7, NA (NA)	1.5, NA (NA)	1.8, 0.2 (100.0)	3.2, 0.7 (68.6)
New Zealand	NA, 0.8 (91.7)	NA, 0.5 (88.9)	NA, 0.3 (100.0)	NA, 0.4 (100.0)	NA, 0.1 (100.0)

*MDR, multidrug-resistant; NA, data not available. The table also shows the % MDR DT104 of all DT104 strains.
†No. of MDR; no. of DT104; and no. of DT104 that are MDR.
‡According to the United Nations classification, Israel belongs to the western Asian region. In this study, Israel has been grouped with southern European countries.

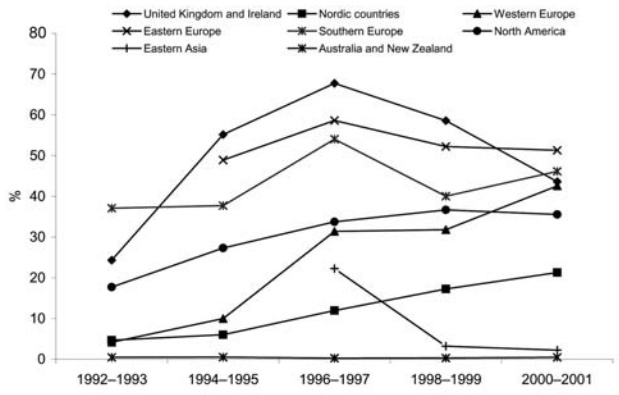

Figure 2. *Salmonella enterica* serovar Typhimurium DT104 as percentage of all *S.* Typhimurium in 8 world regions, 1992–2001. Only countries that had data available for 2 or more 2-year periods are included: United Kingdom and Ireland: England and Wales, Scotland, Ireland; Scandinavia: Denmark, Finland, Norway, Sweden; Western Europe: Austria, Germany, and the Netherlands; Eastern Europe: Czech Republic and Hungary; Southern Europe: Spain and Israel; North America: Canada, United States; Eastern Asia: the Republic of Korea; Oceania: Australia and New Zealand.

performed on a total of 194 MDR DT104 isolates in 4 different countries; no resistant isolates were found. In 2001, nalidixic acid susceptibility testing of 1,812 MDR DT104 strains in 11 countries identified 109 (6.0%) resistant strains. Similarly, trimethoprim resistance was found in 1.2% of the 180 MDR DT104 strains tested in 1992, but in 6.6% of 1,855 MDR DT104 strains tested in 2001. Cephalosporin-resistant MDR DT104 remained rare, with only 0.5% resistant strains in 2001, and no clear trend observable. Figure 4 shows the overall trend of resistance to quinolone, trimethoprim, and cephalosporins from 1992 to 2001. The increase in quinolone resistance seen in 1996 and in 1998 was caused by a general increase in quinolone-resistant MDR DT104 in Scotland and an outbreak of quinolone-resistant MDR DT104 in Denmark (14), respectively. The increase in trimethoprim resistance from 1995 to 1996 was also caused by a general increase in trimethoprim-resistant MDR DT104 in Scotland.

Discussion

The present survey was conducted to gain a better understanding of the global impact of DT104 and MDR *S.* Typhimurium, given the severity of illness than can result from infection with *S.* Typhimurium and MDR strains in particular. The survey's primary findings are that during the period 1992–2001, the total number of MDR *S.* Typhimurium and *S.* Typhimurium DT104 cases increased, while that of other types of *S.* Typhimurium decreased. The total number of nontyphoidal *Salmonella* and *S.* Typhimurium cases also decreased. However, these general findings mask large differences in regional trends. The collected data also may not always accurately describe the real national incidence or be directly comparable between countries.

The total number of isolates of nontyphoidal salmonellae registered at the national level decreased from 1992 to 2001. This result may be biased because of changes in surveillance practices. Since the survey indicated that surveillance systems generally improved throughout the study period, this trend is most likely correct, however. Concurrent with this decrease, an overall decrease in the number of *S.* Typhimurium isolates was observed, thus keeping the proportion of *S.* Typhimurium cases among total *Salmonella* cases constant. The decrease was primarily seen in Europe and North America, whereas Australia and New Zealand saw an increase in both the number of cases of *S.* Typhimurium and of the total number of nontyphoidal *Salmonella* cases. In Germany the total number of nontyphoidal *Salmonella* cases is available for the whole country, but for administrative reasons data on serotypes, antimicrobial susceptibility, and phage types are based only on results from the new federal states of Germany (formerly East Germany) and the city of Berlin. However, German studies have shown that serotype distribution and drug resistance are comparable between former West and East Germany (32) (W. Rabsch, pers. comm.).

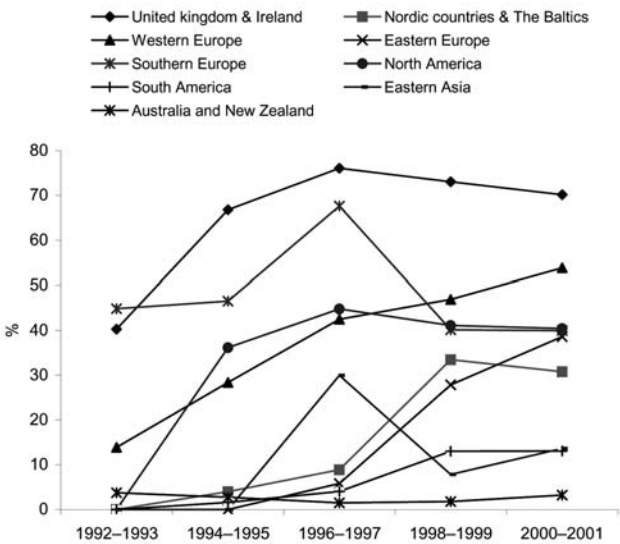

Figure 3. Multidrug-resistant *Salmonella enterica* serovar Typhimurium as a percentage of all *S.* Typhimurium in 9 world regions, 1992–2001. Only countries that had data available for 2 or more 2-year periods are included: United Kingdom and Ireland: Scotland and Ireland; Scandinavia and the Baltics: Denmark, Finland, Norway, and Latvia; Western Europe: Austria, Germany, Luxembourg, and the Netherlands; Eastern Europe: Czech Republic and Hungary; Southern Europe: Greece, Malta, and Spain; North America: Canada, United States; South America: Brazil; Eastern Asia: the Republic of Korea; Oceania: Australia.

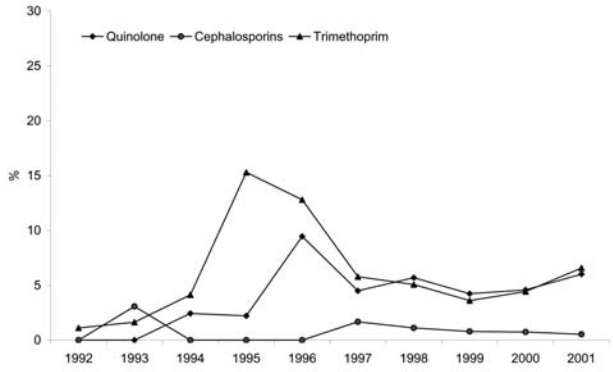

Figure 4. Proportion of multidrug-resistant *Salmonella enterica* serovar Typhimurium with additional resistance to quinolones, cephalosporin, or trimethoprim, 1992–2001.

MDR *S.* Typhimurium has increased in the past decades in almost all the regions covered in this study. Most of this increase is due to the concurrent upsurge of DT104, whereas other phage types, such as U302, DT120, DT12, and DT193, were reported to play a smaller role in this development. A high proportion of MDR *S.* Typhimurium was primarily observed in Europe and North America. Because of the low coverage of participating countries in Asia, South America, and Africa, the situation in these areas was difficult to assess. Both Australia and New Zealand, however, reported high incidence of *S.* Typhimurium, but MDR strains of *S.* Typhimurium and DT104 were largely absent. The isolated increase in MDR *S.* Typhimurium in Australia in 2001 can be explained by a large outbreak of MDR DT104 associated with consumption of *halva* (dessert made of sesame seeds) (33). That DT104 has not spread markedly in Australia and New Zealand may be explained both by geography and the very strict food and livestock import restrictions in force in these countries, which prevent any large-scale introduction and spread of foreign *Salmonella* types in the food animal production chain (34).

Although high and generally increasing levels of MDR *S.* Typhimurium were observed in Europe, the United Kingdom presents a special case. It and Germany had an increase in DT104 in the beginning of the 1990s, before most other countries (5,35). In fact, DT104 was first isolated in the United Kingdom in the early 1980s, years before it was isolated in other countries (5). In the United Kingdom, the incidence of DT104 peaked in 1996 and has since declined ([5] and this study). Possible explanations for this finding include the management of bovine spongiform encephalitis in the United Kingdom, associated general improvements in farm hygiene, and an overall decline in cattle production (36).

Although multidrug-resistance and DT104 were closely linked, large country-specific differences were seen, even between neighboring countries. Most pronounced was the difference between Germany and the Netherlands in 2001, where 64% and 94%, respectively, of MDR isolates were DT104. Such differences probably reflect both real differences and biases resulting from, for instance, different laboratory reporting practices. For example, some of the countries in this survey (New Zealand, Norway, and the United States) performed antimicrobial susceptibility tests on all their DT104 isolates but only on a subset of non-DT104 strains.

In Australia and in Scandinavia, where the incidence of domestically acquired salmonellosis is generally low, many cases appeared to be imported. In a recent study of Australian DT104 isolates, 37% were associated with travel abroad, particularly to Southeast Asia (D. Lightfoot, pers. comm.). Complete data on travel association of MDR *S.* Typhimurium cases were available for Norway and Finland and showed that most MDR *S.* Typhimurium patients were infected abroad. In Sweden, where information on DT104 but not MDR was available, most DT104 patients were infected abroad.

A special issue concerns the possibility of acquisition, with time, of resistance traits additional to the classical penta-resistant pattern. Of particular concern is the additional acquisition of quinolone resistance by MDR DT104, since fluoroquinolones are often the drugs of first choice when treating severe salmonellosis. Our data indicate that the prevalence of quinolone resistance has increased. As mentioned, several studies have now shown that multidrug resistance and quinolone resistance may be associated with particular adverse health effects. When seen in the light of the ability of DT104 to spread and establish itself in a large variety of food animal lines (cattle, pigs, poultry), the increase in the number of MDR *S.* Typhimurium strains that include quinolone resistance becomes particularly problematic. Quinolone, and in particular the fluoroquinolones, have been part of human medicine since the 1980s, resulting in no or very limited resistance in salmonellae. It was not until the license of fluoroquinolones for food animal production in the early 1990s that resistant *Salmonella* strains emerged (37). The use of fluoroquinolones for food production animals should therefore be discontinued or at least severely restricted as quickly as possible.

This survey has several important limitations. First, it was limited to countries with relatively sophisticated surveillance systems in place, since only countries performing phage typing or resistance testing in addition to serotyping were eligible. This meant that Asia, Africa, and South America were barely covered. Therefore, the survey contains no representative data on the situation in these regions. WHO Global Salm-Surv seeks to enhance the capacity of countries to provide such data. Second, the collection of isolates at the national level is likely to vary from

country to country, depending on a number of factors such as sampling frequency at the local level, availability of laboratory reagents, and the degree to which isolates and results are forwarded to the national level. Local practice, the priority given to foodborne illnesses, and financial factors will influence on how often a physician will request a fecal sample. Furthermore, many countries stated that strains and information were not always routinely collected centrally, while in some of the countries strains were never forwarded from certain local laboratories. For these reasons, care should be taken in the interpretation of results of this survey; in particular when comparing incidence rates between countries. However, these limitations are inherent to surveillance in general and do not apply only to this survey. Cross-checking these survey data with available published surveillance data showed them to be in line with each other in the United States (38), the Netherlands (39), and Denmark (40).

In summary, on a global scale, only a small number of countries perform antimicrobial susceptibility testing or phage typing, although the number of countries doing so more than doubled throughout the study period. Despite its limitations, the survey showed that the incidence of both MDR *S.* Typhimurium and MDR *S.* Typhimurium DT104 increased markedly worldwide during the 1990s, although the problem has primarily affected Europe and North America. Of special concern is the increasing incidence of quinolone-resistant *S.* Typhimurium. The survey implies that MDR *S.* Typhimurium poses a serious and increasing public health problem in large parts of the world. Surveillance and control programs such as the Global Salm-Surv international network recently launched by WHO should therefore be reinforced.

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Dr. Helms is a research fellow working on health outcomes in relation to foodborne bacterial infections, in particular, the hazards associated with drug-resistant bacteria in our food supply.

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Methicillin-resistant–*Staphylococcus aureus* Hospitalizations, United States

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly a cause of nosocomial and community-onset infection with unknown national scope and magnitude. We used the National Hospital Discharge Survey to calculate the number of US hospital discharges listing *S. aureus*–specific diagnoses, defined as those having at least 1 International Classification of Diseases (ICD)-9 code specific for *S. aureus* infection. The number of hospital discharges listing *S. aureus*–specific diagnoses was multiplied by the proportion of methicillin resistance for each corresponding infection site to determine the number of MRSA infections. From 1999 to 2000, an estimated 125,969 hospitalizations with a diagnosis of MRSA infection occurred annually, including 31,440 for septicemia, 29,823 for pneumonia, and 64,706 for other infections, accounting for 3.95 per 1,000 hospital discharges. The method used in our analysis may provide a simple way to assess trends of the magnitude of MRSA infection nationally.

Staphylococcus aureus is a major cause of infection in both healthcare and community settings. It is one of the most common causes of healthcare-associated infections reported to the National Nosocomial Infections Surveillance (NNIS) System, including ventilator-associated pneumonia, surgical site infection, and catheter-associated bloodstream infection (1). *S. aureus* is also a frequent cause of community-associated infections, particularly skin and soft tissue infections. Although most community-onset infections are treated in the outpatient setting, some invasive infections, including bacteremia, septic arthritis, toxic shock syndrome, osteomyelitis, and endocarditis, have devastating complications and may require hospitalization (2).

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Antimicrobial resistance in *S. aureus* emerged soon after penicillin came into common use in the 1940s. During the next 2 decades, resistance of this pathogen to penicillin became widespread, followed by increasing resistance to the new semisynthetic penicillinase-resistant antimicrobial drugs (e.g., methicillin, oxacillin, nafcillin) (3). In the last 20 years, methicillin-resistant *S. aureus* (MRSA) has spread throughout the world in healthcare settings, leading to an increased reliance on vancomycin for empiric treatment (4). Recently, *S. aureus* resistance to vancomycin, the last commonly used antimicrobial drug to which this organism was considered uniformly susceptible, has emerged (5). In addition, serious MRSA infection has been increasingly reported in persons without identified predisposing risk, including recent healthcare exposure (6).

MRSA infections are thought to cause substantial illness and contribute to healthcare costs in the United States. However, published estimates vary widely and have been based on single-center or local data with limited applicability (4,7). Accurate estimates of the incidence of MRSA infection are essential to determine effects on health and healthcare expenditures. Since most patients with serious MRSA infections are hospitalized, we focused our estimate on hospitalized patients.

Methods

The incidence of *S. aureus* infection was estimated from the number of hospitalizations with *S. aureus*–related discharge diagnoses in a national surveillance database. We used 1999 and 2000 public-use data from the National Hospital Discharge Survey (NHDS) to calculate the number of hospital discharges with at least 1 *S. aureus*–related discharge diagnosis. All acute-care hospitalizations, except infants whose hospital stay began at their own birth, were

included. The NHDS is a nationally representative annual sample of discharge records from ≈475 nonfederal short-stay hospitals (8). The survey is based on a stratified, multistage probability design; the sampled hospital discharge records are weighted to produce national estimates. The database includes ≤7 principal discharge diagnoses. We identified *S. aureus*–related discharge diagnoses by using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes specific for *S. aureus* infection: 038.11 (*S. aureus* septicemia), 482.41 (*S. aureus* pneumonia), and 041.11 (*S. aureus* infection in conditions classified elsewhere or of unspecified site). A discharge record listing multiple *S. aureus*-related diagnoses was counted only once. Septicemia was preferentially included, followed by *S. aureus*–related pneumonia.

Next, the percentage of isolates resistant to oxacillin was determined. To simplify terminology, resistance to methicillin and oxacillin hereafter are used interchangeably. Oxacillin is used as a proxy for testing of susceptibility to all β-lactam antimicrobials, including methicillin. The Surveillance Network (TSN) Database-USA (Focus Technologies, Herndon, VA, USA) was the source of antimicrobial susceptibility testing results. TSN is a repository of quantitative and qualitative susceptibility results collected from >200 microbiology laboratories in the United States. These laboratories make up a nationally representative sample based on associated hospital bed size, patient population, and geographic region as determined by the US Bureau of the Census (9,10). Susceptibility testing of patient isolates is conducted on site by each participating laboratory as part of routine diagnostic testing; only isolates judged as clinically significant are included. Data are generated by using Food and Drug Administration–approved testing methods. *S. aureus* antimicrobial susceptibility to oxacillin was classified as susceptible, intermediate, or resistant according to NCCLS breakpoint criteria; we classified intermediate isolates as methicillin-susceptible *S. aureus* for purposes of this analysis. Data were stratified by site of infection, i.e., bloodstream, lung, and other sites. Duplicate isolates were removed if the initial and subsequent isolates were cultured within 30 days of each other.

The number of hospital discharges listing *S. aureus*–specific diagnoses was multiplied by the proportion of methicillin resistance at each corresponding infection site to determine the total number of MRSA infections. Infections also were stratified by geographic region and age. The frequency of primary diagnosis and the 10 most frequent secondary (all-listed) diagnoses were abstracted from hospitalizations that included *S. aureus*–specific diagnoses.

Results for the years 1999–2000 were determined by calculating data specific to each year and then averaging. Data on resistance rates were stratified first by region and then by age; for each stratification, a chi-square test was used to determine whether differences were significant. The Cochran-Armitage test, a nonparametric method, was used to determine the trend in MRSA hospitalization rate by age category.

The effects of region and age on the incidence rate of MRSA were assessed by calculating relative rates and their associated 95% confidence intervals, with the lowest rates designated as comparison groups. Since the rate of *S. aureus* and the MRSA proportion were estimated separately and then multiplied to obtain the MRSA hospitalization rate, the variance of the MRSA rate was calculated by using the delta method (11). The variance of the methicillin resistance proportions was determined under the assumption that the antimicrobial susceptibility data reflected those that would have been derived from a random sample of all *S. aureus* isolates in the United States in that time period. Variance estimates were calculated using SUDAAN software (Research Triangle Institute, Research Triangle Park, NC, USA). For both *S. aureus* rates and methicillin resistance proportions, variances were estimated separately for 1999 and 2000, and the larger of the variance estimates was used in subsequent calculation of 95% confidence intervals for relative rates.

Results

We estimate that 291,542 hospital discharges with *S. aureus* infection-related diagnoses occurred annually from 1999 to 2000 (Table 1). A diagnosis of *S. aureus* infection occurred in 9.13 of every 1,000 hospital discharges. The overall rate of methicillin resistance for all *S. aureus* infections was reported to be 43.2%. MRSA rates for septicemia, pneumonia, and other infections increased with patient age. An estimated 125,969 hospitalizations with 1 or more discharge diagnoses associated with MRSA infection occurred annually, accounting for 3.95 of every 1,000 hospital discharges. For all sites, most diagnoses occurred in persons ≥65 years of age.

In hospitalizations in which *S. aureus* septicemia and pneumonia were listed as discharge diagnoses, these conditions were primary diagnoses in 34.3% and 49.3% of discharges, respectively. For *S. aureus* infection in conditions classified elsewhere and in an unspecified site, a diagnosis intended only for secondary listing, the most frequent primary diagnoses were postoperative (e.g., wound) infection (10.1%), cellulitis or abscess (9.9%), infection from an implanted device or graft (7.3%), and urinary tract infection (3.6%).

The largest proportion of *S. aureus*–related discharge diagnoses occurred in patients from the South, followed by the Midwest, Northeast, and West (Table 2). For both, the rate of *S. aureus* discharge diagnoses and methicillin

Table 1. *Staphylococcus aureus*–related discharge diagnoses, United States, 1999–2000, by patient age and infection site*

Discharge diagnosis	Age (y) ≤14	15–44	45–64	≥65	Total†
S. aureus septicemias	2,918	12,272	20,028	38,948	74,166
Proportion of methicillin-resistant isolates from blood culture	0.144	0.317	0.392	0.495	0.424
MRSA septicemias	420	3,890	7,851	19,279	31,440
S. aureus pneumonias	2,328	5,582	6,926	41,427	56,263
Proportion of methicillin-resistant isolates from lower respiratory culture	0.195	0.333	0.467	0.586	0.530
MRSA pneumonias	454	1,859	3,234	24,276	29,823
Other *S. aureus* infections	14,290	39,222	40,496	67,105	161,113
Proportion of methicillin-resistant isolates from other culture sites	0.160	0.279	0.378	0.539	0.402
Other MRSA infections	2,286	10,943	15,307	36,170	64,706

* MRSA, methicillin-resistant *S. aureus.*
†Due to rounding of methicillin-resistant proportions, total MRSA infections may differ slightly when estimates are calculated across category groups by row (i.e., age) compared with column (i.e., infection site).

resistance proportion, significant differences were seen by geographic region. *S. aureus* discharge diagnoses were significantly higher for the South than the Northeast, while for methicillin resistance proportion, the Northeast, Midwest, and South were significantly higher than the West ($p<0.05$ for all comparisons). The South had the highest MRSA hospitalization rate, reflecting both the *S. aureus* rate and methicillin resistance proportion, which was significantly higher than the MRSA rate estimated for the West (South vs. West, relative risk 1.57, 95% confidence interval 1.29–1.91).

Most *S. aureus*–related discharge diagnoses occurred in patients ≥65 years of age. When *S. aureus* diagnoses by rate were examined, a bimodal distribution was seen, with highest rates occurring in children and the elderly (Table 3). Patients ≤14 and 15–44 years of age had higher MRSA hospitalization rates compared with patients 45–64 and ≥65 years of age ($p<0.01$). Overall, the MRSA rate increased with patient age ($p<0.05$ for trend).

Discussion

Infectious diseases cause many hospitalizations each year in the United States; these diseases include syndromes commonly associated with *S. aureus*. In 1994, the rate of hospitalization for infectious disease was 15 per 1,000 US population, with a total of 4 million hospitalizations, including 1,480,000 pneumonias, 335,000 skin infections, and 302,000 septicemias; yearly rates for these disease syndromes were similar from 1999 to 2000 (12–14). Gram-positive organisms are an increasingly recognized cause of systemic infection, including sepsis (12,15). More than half of all sepsis cases are estimated to be caused by gram-positive organisms, including *S. aureus* (16). In the Calgary Health Region in Canada, the annual incidence of invasive *S. aureus* infection was estimated to be 28.4 cases per 100,000 population from 1999 to 2000, which is comparable with the rate of invasive pneumococcal disease and exceeds the rate of invasive streptococcal infection (17).

Drug resistance in *S. aureus*, including the emergence of MRSA in healthcare and community settings, is an increasingly reported event that makes treating serious infection difficult. Extrapolating from our estimates and those of Simonsen et al. (12), a rate of ≈47 diagnoses per 100,000 population, making up 3% of all infectious disease hospitalizations, were associated with laboratory-confirmed MRSA infection from 1999 to 2000, and ≈10% of septicemias were caused by MRSA.

Although the burden of MRSA infection has not been systematically estimated nationally, past estimates have been based on single-center or selected population-based studies in the United States. Based on ICD-9-CM data from the New York City metropolitan area, an estimated 1.0% of hospital discharges are associated with *S. aureus* infection, and 0.21% of discharges are estimated to be associated with MRSA (18). In 1995, based on extrapolation of hospital discharge data from NHDS and nosocomial infection data from the NNIS System, an estimated 206,504 *S. aureus* infections (0.58% of admissions) and 70,270 MRSA infections (0.20% of admissions) were acquired in the healthcare setting (Centers for Disease

Table 2. *Staphylococcus aureus*–related hospitalizations, United States, 1999–2000, by geographic region*

Region	Discharge diagnosis *S. aureus* (%)	*S. aureus* rate†	MR (%)	MRSA rate†
West	17.4	9.04	31.4	2.84
Northeast	20.5	8.51	41.3	3.52
Midwest	22.6	9.06	43.5	3.94
South	39.5	9.58	46.5	4.45

*MR, methicillin resistant; MRSA, methicillin-resistant *S. aureus.*
†Rate, hospitalizations with *S. aureus*– or MRSA–related discharge diagnoses per 1,000 discharges.

Table 3. *Staphylococcus aureus*–related hospitalizations, United States, 1999–2000, by patient age*

	Discharge diagnosis				
Age (y)	*S. aureus* (%)	*S. aureus* rate†	MR (%)	MRSA rate†	MRSA RR (95% CI)
≤14	6.7	80.8	16.2	13.1	Referent
15–44	19.6	56.9	29.3	16.7	1.2 (0.94–1.6)
45–64	23.1	97.4	39.1	38.1	2.9 (2.2–3.8)
≥65	50.6	117.6	54.1	63.6	4.8 (3.7–6.2)

* MR, methicillin resistant; MRSA, methicillin-resistant *S. aureus*; RR, relative risk; CI, confidence interval.
†Rate, hospitalizations with *S. aureus* or MRSA-related discharge diagnoses per 1,000 discharges.

Control and Prevention, unpub. data). Our estimates for 1999 to 2000 are similar for *S. aureus* infections but are higher for MRSA.

Although ICD-9-CM coding accuracy for *S. aureus* infections has not been specifically examined, the accuracy of coding for sepsis from all causes has been reviewed, and has demonstrated a sensitivity >75% for any septicemia or bacteremia code and positive and negative predictive values >80% for the code specific for *Staphylococcus* spp. septicemia (ICD-9-CM 038) (16,19). However, the relationship between true *S. aureus* infections and ICD-9 discharge coding should be further assessed to validate this method as a tool for monitoring national trends.

We found associations between MRSA rate and both region and age. This finding is consistent with previously published data showing an association between age and both the incidence of invasive *S. aureus* infection and the rate of methicillin resistance (17,20). We also demonstrated a significant difference in MRSA discharge rates between the South and West. Although past microbiologic surveys also have reported higher rates of methicillin resistance in the South compared with other regions, the reasons for this variation are unclear (21,22). These differences may need to be assessed as community-associated MRSA infection becomes more common.

Our estimate is subject to a number of limitations that most likely underestimated hospitalizations associated with MRSA infection. First, *S. aureus* infections may not have been accurately represented by the ICD-9-CM discharge code; colonization may have been inadvertently included; and more likely, true infections may not have been identified, since these diagnoses require laboratory culture confirmation. Since only 7 principal diagnoses are included in NHDS, infections listed less prominently may have been excluded. Duplicate isolates were excluded when identified within 30 days of each other; thus, unusual scenarios, such as multiple infections during a hospitalization or infections present for >30 days, were not included. We were not able to distinguish between community- and healthcare-acquired infection. However, this analysis was designed to measure the overall incidence of disease associated with acute care hospitalization, regardless of acquisition site, and did not include disease managed in the outpatient setting. Although previously published region and age stratification groups were used, which reduces risk of bias, unmeasured confounders may have affected calculated trends. Finally, although both NHDS and TSN data aim to represent nationally representative samples based on similar factors, methods may have differed, which could have skewed our results. For all data used, institutional settings, such as long-term care or correctional facilities, were not included.

In summary, our estimates indicate that the national burden of serious MRSA disease is quantifiable and substantial. Measurement of trends in *S. aureus* disease, such as the increasing incidence of antimicrobial resistance associated with certain age groups and geographic regions, will have implications in the development of prevention programs, both in the healthcare and community settings. Our method provides a simple way to estimate trends of magnitude of hospitalization associated with *S. aureus* infection in the United States and could complement methods currently in place for national surveillance.

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Dr. Kuehnert is a medical epidemiologist at the National Center for Infectious Diseases, Centers for Disease Control and Prevention. His research interests have included antimicrobial resistance surveillance and now focus on improvement of blood, organ, and other tissue safety.

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Integrating *Escherichia coli* Antimicrobial Susceptibility Data from Multiple Surveillance Programs

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Collaboration between networks presents opportunities to increase analytical power and cross-validate findings. Multivariate analyses of 2 large, international datasets (MYSTIC and SENTRY) from the Global Advisory on Antibiotic Resistance Data program explored temporal, geographic, and demographic trends in *Escherichia coli* resistance from 1997 to 2001. Elevated rates of nonsusceptibility were seen in Latin America, southern Europe, and the western Pacific, and lower rates were seen in North America. For most antimicrobial drugs considered, nonsusceptibility was higher in isolates from men, older patients, and intensive care unit patients. Nonsusceptibility to ciprofloxacin was higher in younger patients, rose with time, and was not associated with intensive care unit status. In univariate analyses, estimates of nonsusceptibility from MYSTIC were consistently higher than those from SENTRY, but these differences disappeared in multivariate analyses, which supports the epidemiologic relevance of findings from the 2 programs, despite differences in surveillance strategies.

The World Health Organization (WHO) highlights the establishment of "effective, epidemiologically sound surveillance of antimicrobial resistance among common pathogens in the community, hospitals, and other health care facilities" as 1 of 2 fundamental public health priorities in efforts to confront antimicrobial drug–resistant organisms (1). At present, most data published in the international literature on antimicrobial resistance are derived from short-term surveys of specific organisms and agents in defined areas. Consequences of this nonsystematic, discontinuous approach are the inability to establish meaningful baseline trends; low sensitivity in detecting new threats; inadequate information to evaluate interventions; and lack of data on organisms, antimicrobial drugs, and patient populations not included in the surveys.

Surveillance groups must coordinate efforts to provide the broadest set of data to policymakers and researchers and to assess the reliability of findings from individual systems. Recognizing the urgency of the problem and the value of joint surveillance collaborations, the Alliance for the Prudent Use of Antibiotics (APUA), a nonprofit organization, established the Global Advisory for Antibiotic Resistance Data (GAARD) (2) in 1999 to involve several of the world's largest multinational enterprises tracking global trends in resistance as well as the Centers for Disease Control and Prevention, WHO, and the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, which serve in advisory roles. Currently, AstraZeneca International (supporting the Meropenem Yearly Susceptibility Test Information Collection [MYSTIC] surveillance project), Bayer AG (TARGETed), Bristol-Myers Squibb Company (SENTRY), GlaxoSmithKline (Alexander Project), and Ortho-McNeil Pharmaceuticals (TRUST) work with APUA to provide data for GAARD studies. In 2002, data were collected from then-participating GAARD members on *Streptococcus pneumoniae* (3), *Haemophilus influenzae* (4), and *Escherichia coli*. The focus of this article is the analysis of submitted *E. coli* results from GAARD-participating systems tracking *E. coli* at that time, i.e., MYSTIC and SENTRY.

E. coli is the most common cause of infections by gram-negative bacilli (5) and the bacterial organism most often isolated from blood cultures (6–9). It is a frequent cause of outpatient urinary tract infections in women

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worldwide, of hospitalization due to pyelonephritis and septicemia, and of nosocomial infections among hospitalized patients. Meningitis caused by *E. coli* in neonates is frequently fatal. Resistance to recommended first- and second-line agents, such as penicillins, cephalosporins, sulfa drugs (5,7,10), and fluoroquinolones (11,12) is high in many countries and is commonly associated with treatment failure (13,14).

Methods

Antimicrobial susceptibility data on *E. coli* collected by the MYSTIC and SENTRY systems were forwarded to GAARD coordinators at APUA for descriptive and inferential analysis of temporal, demographic, and geographic trends. MYSTIC was launched by AstraZeneca in 1997 to study bacterial resistance in specialist and general hospital units in hospitals using meropenem (15). At present, 52 sites from 19 countries are contributing results. Each center isolates up to 100 gram-positive and 100 gram-negative aerobic bacteria per year from routine diagnostic samples from hospitalized patients, excluding repeat patient isolates. Antimicrobial susceptibility tests are performed by broth microdilution by using NCCLS reference methods (16) either on-site (for non-US laboratories) or by a reference laboratory (for US participants). More than 9,000 isolates are processed annually, with at least 9 antimicrobial drugs tested per strain.

Bristol-Myers Squibb established the SENTRY program in 1997 as a global program for the surveillance of resistance in bacterial and fungal populations (17). SENTRY has expanded from 75 sites in 1997 to 94 laboratories in 35 countries in 2003. Bacterial isolates are obtained from diagnostic specimens taken in the course of routine clinical management of both hospitalized and community patients. Each site collects a defined number of consecutively identified strains within a number of distinct protocols, e.g., blood isolates, urine isolates, and respiratory isolates, excluding repeat patient isolates. Strains, including basic patient demographic data, are shipped to a coordinating laboratory for centralized identification and susceptibility testing by broth microdilution panels according to NCCLS reference guidelines (16). Forty-five to 50 antimicrobial drugs are monitored each year, with ≈30 tested per strain; >200,000 strains are processed annually.

Available Data

Data on *E. coli* from 1997 to 2001 were available from 24 countries from the MYSTIC program (4,818 isolates) and 34 countries from SENTRY (14,819 isolates). Because 20 countries are tested by both systems, this figure represents 38 countries, as shown in Table 1. Numbers in the table indicate the number of centers that contributed data at any point during the 5-year period. Descriptive analyses and multivariate regressions included data from all countries, except when data were insufficient (defined as <30 isolates in 2000 and 2001) (18): MYSTIC data from Bulgaria, Malta, Russia, Switzerland, Hong Kong, and Thailand and SENTRY data from Austria, the Netherlands, Portugal, Russia, Mexico, Uruguay, and China. Data from both networks were available for 16 countries, but direct univariate comparisons of findings between the 2 networks were limited to the 10 countries, shown in Figure 1, with

Table 1. Countries participating in the MYSTIC and SENTRY programs*

System	North America	Latin America	Northern Europe	Southern Europe + South Africa	Western Pacific
MYSTIC (24 countries)	Canada (14, 97), United States (18, 816)	Argentina (3, 41), Brazil (3, 75), Colombia (1, 20), Mexico (4, 170)	Belgium (9, 572), Czech Republic (1, 90), Germany (7, 668), Poland (1, 70), Russia (1, 7),† Sweden (3, 153),† Switzerland (1, 40),† United Kingdom (8, 294)	Bulgaria (1, 10),† Greece (2, 37), Israel (1, 96), Italy (5, 369), Malta (1, 11),† Spain (5, 517), Turkey (9, 529)	Australia (1, 46), Hong Kong (1, 20),† Thailand (1, 70)†
Total (101 sites, 4,818 isolates)	32 sites, 913 isolates	11 sites, 306 isolates	31 sites, 1,894 isolates	24 sites, 1,569 isolates	3 sites, 136 isolates
SENTRY (34 countries)	Canada (8, 1,334), United States (36, 5,438)	Argentina (2, 282), Brazil (5, 488), Chile (2, 610), Colombia (1, 181), Mexico (3, 166),† Uruguay (1, 17),† Venezuela (1, 72)	Austria (1, 105),† Belgium (1, 171), Germany (6, 440), Ireland (1, 52), Netherlands (1, 107),† Poland (1, 141), Russia (1, 6),† Sweden (1, 112), Switzerland (1, 380), United Kingdom(1, 260)	France (9, 1,086), Greece (1, 212), Israel (1, 128), Italy (4, 431), Portugal (1, 91),† South Africa (1, 76), Spain (3, 1,007), Turkey (3, 217)	Australia (4, 480), China (3, 62),† Hong Kong (1, 228), Japan (3, 93), Philippines (1, 130), Singapore (2, 118), Taiwan (3, 98)
Total (114 sites, 14,819 isolates)	44 sites, 6,772 isolates	15 sites, 1,816 isolates	15 sites, 1,774 isolates	23 sites, 3,248 isolates	17 sites, 1,209 isolates

*The number of participating centers at any point from 1997 to 2001 and number of isolates by country are indicated in parentheses.
†Countries excluded from analyses for insufficient data, as defined in the text.

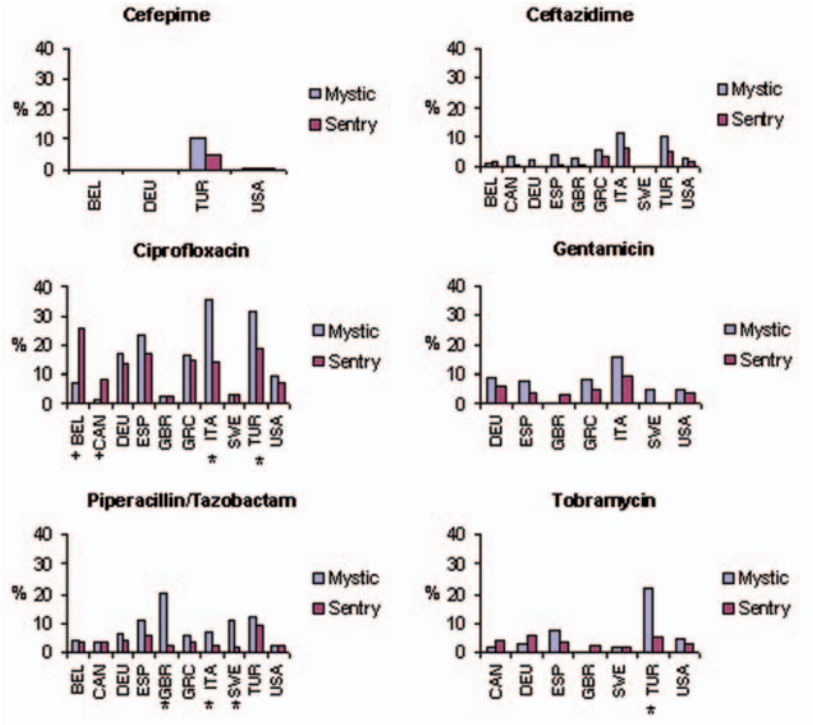

Figure 1. Comparison of MYSTIC and SENTRY rates of *Escherichia coli* nonsusceptibility rates in 2001 to antimicrobial drugs tested by both networks. Significant findings are indicated with an asterisk where the MYSTIC estimate is higher than the SENTRY result and with a plus sign when the SENTRY estimate is higher. Country codes are the official 3-letter codes designated by the International Organization for Standardization: BEL, Belgium; CAN, Canada; DEU, Germany; ESP, Spain; GBR, United Kingdom; GRC, Greece; ITA, Italy; SWE, Sweden; TUR, Turkey; and USA, United States. Little resistance was seen with imipenem and meropenem, and an expanded version of this figure, including those agents, is available from http://www.cdc.gov/ncidod/EID/vol11no06/04-1160-G1.htm.

at least 20 isolates in each of the years displayed. These 10 "comparison" countries, principally representing North America and Europe, are Belgium, Canada, Germany, Greece, Italy, Spain, Sweden, Turkey, the United Kingdom, and the United States. The United States provided 17% of the MYSTIC isolates and 46% of the SENTRY isolates.

Antimicrobial Drugs

For *E. coli* in the MYSTIC project, either 12 (United States isolates) or 11 (non-US isolates) antimicrobial drugs were tested. In SENTRY, 26 antimicrobial drugs were examined. The following 8 compounds were tested by both programs and will be referred to as the core antimicrobial agents for comparisons between the 2 networks: cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin/tazobactam, and tobramycin. Because a primary objective of this study is to highlight the value in contrasting findings from different surveillance programs, most subsequent regression analyses will focus on these 8 agents.

With the exception of ciprofloxacin, these compounds are primarily administered as second-line therapy to hospitalized patients and not routinely to outpatients. Because monitoring resistance to first-line agents is essential to guide empiric treatment decisions, data from the SENTRY network are also presented for the following compounds not tested by MYSTIC laboratories: amoxicillin/clavulanic acid, ampicillin, nalidixic acid, nitrofurantoin, tetracycline, and trimethoprim/sulfamethoxazole.

Data Analysis

Similar demographic data were available from both systems and included patient country, age, and sex; intensive care unit (ICU) or non-ICU location; and specimen type. Susceptibility test data were recorded as MIC values. Resistant, intermediate, and susceptible categories were interpreted according to 2003 NCCLS guidelines (19). During the period studied, NCCLS breakpoints did not change for the drugs studied. Strains with a clinical interpretation of resistant or intermediate were considered nonsusceptible in further analyses.

Available data on *E. coli* from 1997 through 2001 were sent in Microsoft Excel (Microsoft Corp., Redmond, WA, USA) format by MYSTIC and SENTRY coordinators to APUA for analysis. For descriptive data analysis, files were imported into WHONET 5.2 (World Health Organization, Geneva, Switzerland) (20). Univariate analyses by chi-square testing and multivariate logistic regressions were carried out with Intercooled STATA v. 7 (StataCorp LP, College Station, TX, USA), with null hypotheses rejected for values of $p<0.05$ and without correction for multiple comparisons. Age was categorized in 10-year intervals, and countries were categorized by geographic region defined in Table 1.

Results

Univariate Comparison of Surveillance Networks

A comparison of the MYSTIC and SENTRY results for 2001 is shown in Figure 1 for the 10 comparison countries.

Excluding ciprofloxacin, resistance rates were ≤10% in 2001 for the core antimicrobial drugs among the comparison countries, with the following exceptions: ceftazidime (11.4%) and gentamicin (15.7%) in Italy (MYSTIC); tobramycin (21.9%) in Turkey (MYSTIC); and piperacillin/tazobactam in Spain (10.8%), Sweden (10.9%), Turkey (11.9%), and the United Kingdom (20.9%) (MYSTIC). No isolates confirmed resistant to meropenem or imipenem were found by SENTRY. In the MYSTIC dataset, 2 isolates (from Mexico and Turkey) were found to be nonsusceptible to meropenem and 23 (from Belgium, Brazil, Germany, Mexico, Malta, Turkey, and the United Kingdom) to imipenem. As part of an ongoing protocol for quality assurance, several of these isolates were subsequently confirmed through centralized testing.

Nonsusceptibility estimates in MYSTIC data were consistently higher than in SENTRY. For the 2001 data, country-specific comparisons of MYSTIC to SENTRY nonsusceptibility rates were examined for each antimicrobial drug. From the 46 possible comparisons, MYSTIC estimates were higher than in SENTRY 37 times (80.4%, sign test p<0.001). Excluding comparisons in which either rate was equal to 0%, MYSTIC estimates were on average 2.2 times higher than SENTRY values. Subsequent analysis suggests that the principal contributor to the differences between the surveillance systems would be the higher proportion of ICU patients in MYSTIC (38.0%, n = 1,468) than in SENTRY (19.5%, n = 2,642). Significant differences are depicted in Figure 1.

Univariate Temporal Trends in *E. coli* Nonsusceptibility

Temporal trends from several of the comparison countries are shown in Figures 2, 3, and 4. With the exception of ciprofloxacin, the antimicrobial drugs tested by both systems are principally reserved for intravenous use in hospitalized patients in most countries, and nonsusceptibility rates for these second-line agents were low worldwide, with some exceptions. Countries with nonsusceptibility rates ≥20% to at least 3 of the core agents by at least 1 of the systems in 2000 or 2001 include Israel, Poland, Mexico, Venezuela, Hong Kong, and the Philippines.

Significant trends (chi-square test for trend without correction for multiple comparisons, p<0.05) evident in the SENTRY dataset include increasing susceptibility to piperacillin/tazobactam in Argentina, Australia, Brazil, Chile, Israel, and the Philippines; increasing susceptibility to cefepime in Argentina and Brazil but decreasing susceptibility in Israel; increasing susceptibility to gentamicin in Brazil and Hong Kong; increasing susceptibility to tobramycin in Australia and Brazil; and decreasing susceptibility to ciprofloxacin in Belgium, Canada, Colombia, and the United States. MYSTIC data showed a significant decreasing trend in nonsusceptibility to ciprofloxacin in Belgium; susceptibility to piperacillin/tazobactam decreased in the United Kingdom; and susceptibility to gentamicin and tobramycin decreased in Israel.

Figure 4 shows trends in nonsusceptibility data in comparison countries for a number of antimicrobial drugs test-

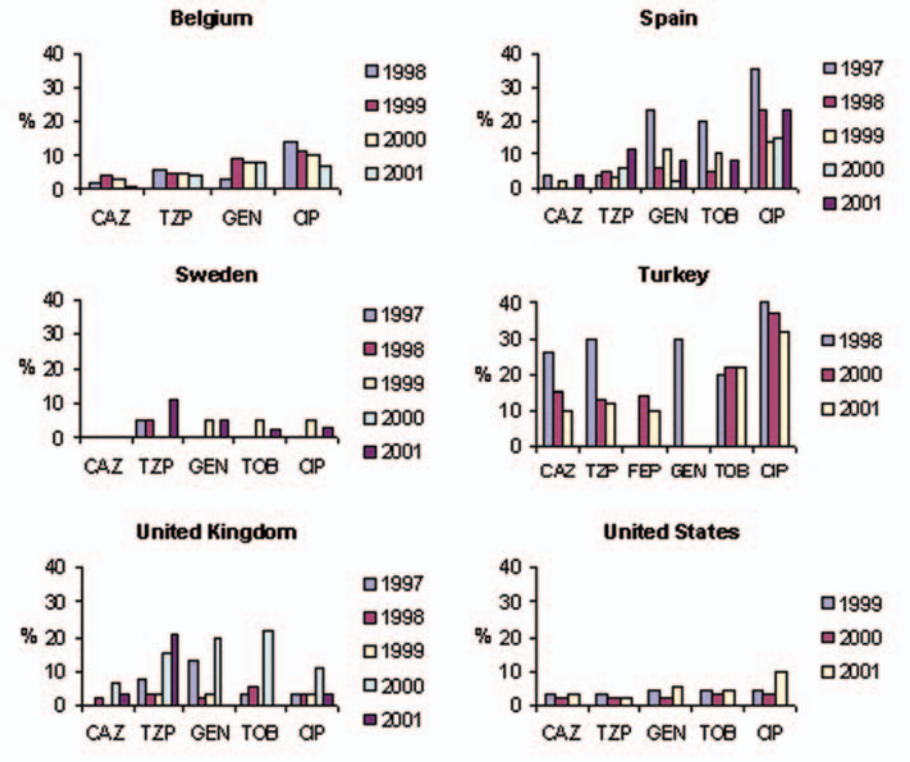

Figure 2. MYSTIC results for comparison countries. Annual nonsusceptibility rates of *Escherichia coli* isolates, 1997–2001. p<0.05. CAZ, ceftazidime; TZP, piperacillin/tazobactam; GEN, gentamicin; CIP, cipro-floxacin; TOB, tobramycin; FEP, cefepime. An expanded version of this figure, including data from Canada, Germany, Greece, and Italy, is available from http://www.cdc.gov/ncidod/EID/vol11no06/04-1160-G2.htm.

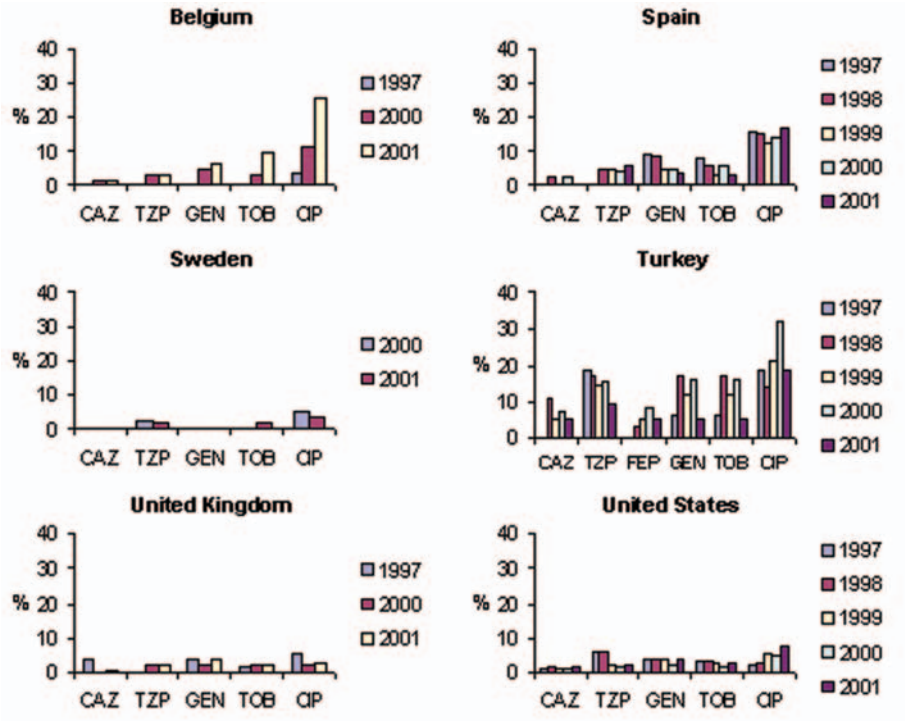

Figure 3. SENTRY results for antimicrobial agents tested in common with MYSTIC. Annual nonsusceptibility rates of *Escherichia coli*, 1997–2001. p<0.05. CAZ, ceftazidime; TZP, piperacillin/tazobactam; GEN, gentamicin; CIP, ciprofloxacin; TOB, tobramycin; FEP, cefepime. An expanded version of this figure, including data from Canada, Germany, Greece, and Italy, is available from http://www.cdc.gov/ncidod/EID/vol11no06/04-1160-G3.htm.

ed only by the SENTRY system commonly prescribed in the outpatient setting. Nonsusceptibility for multiple first-line agents was high (approaching or exceeding 50%) in South Africa, Turkey, Brazil, Chile, Colombia, Venezuela, Hong Kong, the Philippines, Singapore, and Taiwan. Noteworthy trends (p<0.05, chi-square for trends without correction for multiple comparisons) were noted for a number of these agents. Increasing susceptibility to amoxicillin/clavulanic acid was seen in Argentina, Brazil, Canada, Chile, Italy, the United Kingdom, and the United States. Increasing susceptibility to trimethoprim/sulfamethoxazole was seen in Singapore, Chile, Australia, the United States, and Italy, but decreasing susceptibility was seen in Germany; susceptibility to ampicillin decreased in Germany, Colombia, and the Philippines but increased in Chile. Susceptibility to nalidixic acid decreased in Belgium, Canada, Germany, and the United States; susceptibility to nitrofurantoin increased in Canada, Spain, and Chile. Susceptibility to tetracycline increased in Italy and the United Kingdom but decreased in Germany.

Multivariate Trends in *E. coli* Nonsusceptibility

Multivariate logistic regression was performed to simultaneously control for the effect of potentially confounding variables on nonsusceptibility rates. Independent variables included region, age group, sex, specimen year, ICU specimen source, and surveillance system. Table 2 highlights the significant factors. Because of the rarity of meropenem- and imipenem-resistant isolates in the database, these agents were not studied by logistic regression.

Certain regions (southern Europe, Latin America, and western Pacific), male sex, older age, and ICU isolates were consistently (for at least 4 of the 6 drugs) associated with higher nonsusceptibility rates. North American isolates had lower nonsusceptibility rates (for 5 of the 6 drugs), while isolates from northern Europe had higher rates only for ciprofloxacin. Significant temporal trends were identified only with ciprofloxacin (decreased susceptibility over time, odds ratio [OR] 1.14, 95% confidence interval [CI] 1.07–1.21, p<0.001) and piperacillin/tazobactam (increased susceptibility, OR 0.74, 95% CI 0.68–0.81, p<0.001). For ciprofloxacin, in contrast to findings with other agents, younger age was associated with a higher risk for nonsusceptibility (OR 0.39, 95% CI 0.29–0.52, p<0.001), and nonsusceptibility was not associated with ICU status. An important finding of the multivariate analysis is that the surveillance system (MYSTIC vs. SENTRY) was not associated with nonsusceptibility for any of the compounds, in contrast to the findings of the univariate analyses.

Discussion

Through integrated analysis of data from multiple sources, the GAARD project seeks to realize a number of benefits: 1) increased statistical power in detecting evolutionary events of public health importance and elucidating risk factors for resistance emergence and spread; 2) greater geographic, demographic, and temporal coverage

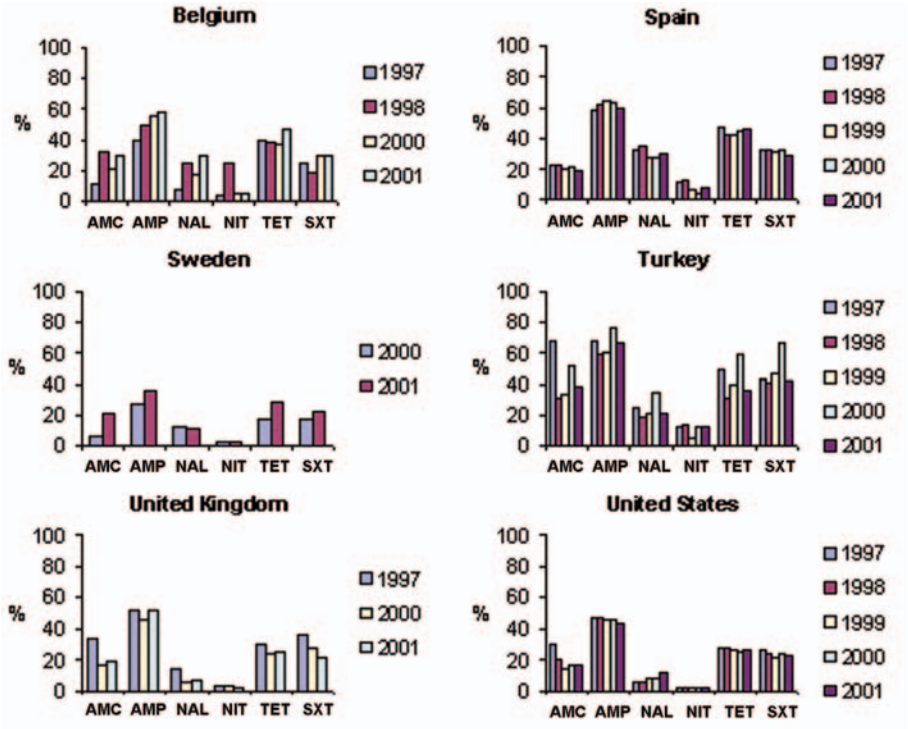

Figure 4. SENTRY results for supplemental antimicrobial drugs tested only by SENTRY. Annual nonsusceptibility rates of *Escherichia coli*, 1997-2001. p<0.05. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; NAL, nalidixic acid; NIT, nitrofurantoin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole. An expanded version of this figure, including data from Canada, Germany, Greece, and Italy, is available from http://www.cdc.gov/ncidod/EID/vol11no06/04-1160-G4.htm.

of bacterial populations than is possible under any single system with limited resources; and 3) cross-validation of findings from complementary data sources with distinct strategies for site recruitment, patient identification, specimen collection, and laboratory testing, which should prompt deeper investigation of seemingly discordant findings (21).

For countries in which a direct comparison of results from the 2 systems was possible, resistance frequencies from MYSTIC were typically higher than from SENTRY. In only 2 instances were higher SENTRY estimates significant (ciprofloxacin in Belgium and Canada). Observation of such incongruent findings should prompt a focused review for possible rationales, such as laboratory testing errors, differences among patient populations sampled, criteria for specimen selection, antimicrobial use patterns, or local outbreaks of resistant organisms. Because SENTRY estimates for Belgium reflect the experience of a single institution while MYSTIC data include results from 9 sites, the MYSTIC results may better reflect the situation in that country.

One of the most substantial findings of the multivariate analysis is that the surveillance system was not associated with nonsusceptibility in any of these compounds, in contrast to the findings of the univariate analyses. Thus, the finding that MYSTIC estimates of nonsusceptibility were consistently higher than SENTRY isolates in paired comparisons may be completely attributable to differences in the demographics of the patient subpopulations sampled. In this study, the principal contributor identified was the proportion of ICU patients represented in the 2 systems. Such findings should increase confidence in the reliability and validity of findings reported separately from the 2 programs. The observation of consistent differences in uncontrolled comparisons of results between systems also highlights the importance of including relevant demographic information in reports on antimicrobial susceptibility rates.

An arbitrary categorization of countries into relatively low, medium, and high resistance is shown in Table 3 for a few key first- and second-line antimicrobial drugs used to treat *E. coli* infections. The intervals indicated were selected to provide some degree of separation between groups of countries using the observed estimates and should not be interpreted as having a direct clinical implication for therapy decisions. The high rates of resistance to both ampicillin and trimethoprim/sulfamethoxazole in many countries observed in this study should prompt close review of treatment success rates in settings in which they are commonly used in empiric therapy (22).

The use of surveillance data to guide antimicrobial therapy guidelines is a complicated issue that must address the constraints of available resources and therapeutic alternatives, local resistance and antimicrobial use patterns, and potential epidemiologic biases in available data. A number of studies have addressed empiric and quantitative approaches for using surveillance data in treatment guidelines for urinary tract infections and pyelonephritis, includ-

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) from multivariate analysis of risk factors for nonsusceptibility in *Escherichia coli*, 1997–2001*

Drug	Factor	OR (95% CI)	p value
Cefepime (18,239 isolates)	Southern Europe	2.23 (1.08–4.69)	0.034
	Latin America	4.82 (2.58–9.012)	<0.001
	North America	0.35 (0.16–0.76)	0.008
	Western Pacific	6.39 (1.98–20.56)	0.002
	Age group	1.74 (1.09–2.79)	0.021
	Intensive care unit	2.84 (1.93–4.17)	<0.001
Ceftazidime (19,404 isolates)	Southern Europe	2.20 (1.20–4.06)	0.011
	Latin America	4.79 (2.83–8.12)	<0.001
	Age group	1.94 (1.38–2.75)	<0.001
	Intensive care unit	2.25 (1.69–3.01)	<0.001
Ciprofloxacin (19,320 isolates)	Northern Europe	1.62 (1.18–2.23)	0.003
	Southern Europe	2.99 (2.27–3.93)	<0.001
	Latin America	3.76 (2.93–4.84)	<0.001
	North America	0.77 (0.60–0.99)	0.046
	Western Pacific	3.07 (1.63–5.76)	<0.001
	Male	1.46 (1.26–1.68)	<0.001
	Age group	0.39 (0.29–0.52)	<0.001
	Year	1.14 (1.07–1.21)	<0.001
Gentamicin (18,773 isolates)	Latin America	2.44 (1.86–3.20)	<0.001
	North America	0.74 (0.56–0.97)	0.027
	Western Pacific	4.64 (2.66–8.09)	<0.001
	Male	1.28 (1.09–1.52)	0.004
	Age group	1.47 (1.15–1.88)	0.002
	Intensive care unit	1.23 (1.01–1.51)	0.042
Piperacillin/tazobactam (19,261 isolates)	Southern Europe	2.01 (1.38–2.92)	<0.001
	Latin America	2.18 (1.60–2.96)	<0.001
	Western Pacific	2.11 (1.01–4.40)	0.046
	North America	0.73 (0.54–0.99)	0.040
	Male	1.33 (1.09–1.61)	0.004
	Year	0.74 (0.68–0.81)	<0.001
	Intensive care unit	1.51 (1.24–1.92)	<0.001
Tobramycin (18,416 isolates)	Southern Europe	1.43 (1.00–2.05)	0.047
	Latin America	3.09 (2.31–4.13)	<0.001
	Western Pacific	3.42 (1.77–6.63)	<0.001
	North America	0.70 (0.52–0.94)	0.019
	Male	1.31 (1.10–1.57)	0.003
	Age group	1.66 (1.30–2.13)	<0.001
	Intensive care unit	1.37 (1.11–1.69)	0.003

*Logistic regression models simultaneously controlled for geographic region, age categories, sex, intensive care unit status, year of specimen, and reporting system. Only significant associations are presented. No significant relationships between nonsusceptibility and reporting system (MYSTIC vs. SENTRY) were found.

ing cost-effectiveness studies and establishing resistance thresholds to guide therapy decisions (23–27).

Several significant results were noted in the univariate analyses of temporal trends. Such changes over time could be due to real shifts in the bacterial populations, changes in the number or type of participating institutions, changes in specimen collection practices, or spurious correlations, as no statistical corrections were made for multiple comparisons. The significant decrease to 4 or more agents in Brazil, Chile, and Italy in particular is worth highlighting for further exploration; Chile has successfully implemented and enforced new national legislation banning the sale of antimicrobial drugs without a prescription since 1999, and this legislation has produced substantial reductions in total antimicrobial drug use in the country (28).

Significant findings from the multivariate analysis of core antimicrobial drugs were mentioned above: higher rates of nonsusceptibility in isolates from ICU patients, older patients, and male patients and in isolates from Latin America, the western Pacific, and southern Europe. When all other variables were controlled for, nonsusceptibility to ciprofloxacin showed a statistical increase in over time, while nonsusceptibility to piperacillin/tazobactam decreased. This decrease in nonsusceptibility to

Table 3. Nonsusceptibility rates of *Escherichia coli* by region, 2001*

Drug	North America	Latin America	Northern Europe	Southern Europe + South Africa	Western Pacific
Ampicillin					
20%–40%	Canada (35%)		Sweden (31%)	Italy (40%)	Japan (30%)
40%–60%	United States (44%)	Argentina, Brazil, Chile, Venezuela (54%–57%)	Belgium, France, Germany, Ireland, Switzerland, United Kingdom (46%–57%)	Greece (51%)	Australia, Singapore (50%–54%)
>60%		Colombia, Mexico (71%–76%)	Poland (62%–84%)	Israel, South Africa, Spain, Turkey (62%–84%)	Hong Kong, Philippines, Taiwan (64%–82%)
Trimethoprim/ sulfamethoxazole					
0%–20%				Italy (19%)	Australia, Japan (11%–17%)
20%–40%	Canada, United States (20%–23%)	Argentina, Chile (28%–39%)	Belgium, Ireland, Poland, Sweden, Switzerland, United Kingdom (20%–31%)	France, Greece, Spain (20%–31%)	
40%–60%		Brazil, Colombia, Mexico, Venezuela (51%–57%)	Germany (40%)	Israel, South Africa, Turkey (42%–59%)	Hong Kong, Philippines, Singapore, Taiwan (40%–60%)
Ceftazidime					
≤5%	Canada, United States (MYS 3%, SEN 1%–2%)	Brazil, Chile (SEN 2%–4%)	Belgium, Czech Republic, Germany, Ireland, Poland, Sweden, Switzerland, United Kingdom (MYS 0%–3%, SEN 0%–3%)	Greece (SEN), France, South Africa Spain, Turkey (SEN) (MYS 4%, SEN 0%–5%)	Australia, Hong Kong, Japan, Singapore (MYS 0%, SEN 2%–3%)
>5%		Argentina, Colombia, Mexico, Venezuela (MYS 7%–13%, SEN 6%–11%)		Greece (MYS), Israel, Italy, Turkey (MYS) (MYS 6%–11%, SEN 6%–8%)	Philippines, Taiwan, Thailand (MYS 19%, SEN 6%)
Ciprofloxacin					
≤10%	United States, Canada (MYS 2%–10%, SEN 7%–9%)	Argentina (MYS), Brazil (SEN) (MYS 4%, SEN 10%)	Belgium (MYS), Czech Republic, Ireland, Poland, Sweden, Switzerland, United Kingdom (MYS 0%–7%, SEN 0%–9%)	France, South Africa (SEN 2%–6%)	Australia, Japan (MYS 0%, SEN 0%–2%)
>10%		Argentina (SEN), Brazil (MYS), Chile, Colombia, Mexico, Venezuela (MYS 14%–17%, SEN 12%–26%)	Belgium (SEN), Germany (MYS 18%, SEN 14%–26%)	Greece, Israel, Italy, Spain, Turkey (MYS 14%–39%, SEN 14%–30%)	Hong Kong, Philippines, Singapore, Taiwan (SEN 12%–31%)

*For countries with <30 isolates in 2001, data from 2000 and 2001 were combined. Ranges of nonsusceptibility rates are indicated in parentheses. For ampicillin and trimethoprim/sulfamethoxazole, data are only available from the SENTRY system. MYS, MYSTIC; SEN, SENTRY.

piperacillin/tazobactam was significant in 11 countries in univariate analyses and merits further investigation into contributory factors. While temporal trends in the multivariate analysis may reflect, to some degree, the high proportion of US isolates in the SENTRY database, demographic characteristics of SENTRY isolates within and outside the United States were comparable, with only a small but significant difference seen for sex (44.2% [n = 1,058] male in the United States vs. 48.1% [n = 2,331] male outside the United States for 2001 data, p = 0.034).

The higher rate of nonsusceptibility among isolates from male patients has been previously noted for ciprofloxacin resistance (10,12,29) and ascribed to epidemiologic differences between men and women with *E. coli* infections. Urinary tract infections in male patients are more frequently complicated or healthcare-associated than those in the typical female patient, and infection in men may be associated with higher rates of previous antimicrobial drug usage and time in the hospital setting (29).

The finding of higher resistance in isolates from ICU patients to most agents is not unexpected, given the high selection pressure exerted by intensive antimicrobial use in this setting and the ease of transmission of resistant pathogens on the hands of healthcare workers. The observation that ICU isolates did not have higher rates of resistance to ciprofloxacin, most frequently used in the outpatient setting, suggests that risk factors for ciprofloxacin resistance are distinct from those of the

other, principally second-line, agents studied. This dichotomy was also observed with respect to age. For ciprofloxacin, in contrast to the other core antimicrobial drugs, older age was associated with a significant protective effect, i.e., lower nonsusceptibility (OR 0.39, 95% CI 0.29–0.52, p<0.001), than seen in younger patients. One hypothesis holds that resistance in certain antimicrobial drugs, such as intravenous or second-line agents, is more closely associated with patterns of prescribing in hospitals and in older patients, while resistance in others, such as ciprofloxacin, is more correlated with patterns of antimicrobial drug use in the community. This hypothesis merits further investigation in a variety of geographic and clinical settings (30,31). Given the ubiquity of *E. coli* as a commensal pathogen in the human gut and in animal populations, resistance in *E. coli* may be a sensitive indicator of distinct therapeutic and nontherapeutic, appropriate and inappropriate uses of antimicrobial drugs (32). Another APUA-coordinated project, Reservoirs on Antibiotic Resistance, is a 5-year scientific collaboration that addresses this issue by exploring the movement of resistance determinants within commensal bacterial populations and between commensals and human pathogens (33).

Both the MYSTIC and SENTRY surveillance networks rely on routinely generated test results, a strategy with advantages over purely research-oriented, resource-intensive special surveys. These advantages include sustainability, more complete organism and geographic coverage, monitoring of baseline trends, infection control alerts, and outbreak detection. However, potential biases may be introduced that must be considered, such as selectively testing patients whose infections did not respond to treatment or who had more severe disease. Such biases may be amplified in the outpatient setting and in low-resource countries where treatment is frequently empiric with limited diagnostic testing. Results from routinely generated sample collections could usefully be compared to findings from periodic validation surveys in which greater resources are expended in identifying and testing representative patient populations (34–36).

With antimicrobial resistance continuing to evolve and present a global public health challenge, appropriately designed and implemented surveillance systems are a priority. Collaboration among existing surveillance systems can improve the quality, breadth, and impact of data for guiding and evaluating clinical and public health policy.

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Nonprescribed Antimicrobial Drugs in Latino Community, South Carolina

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We investigated in a sample of Latinos the practices of antimicrobial drug importation and use of nonprescribed antimicrobial drugs. In interviews conducted with 219 adults, we assessed health beliefs and past and present behaviors consistent with acquiring antimicrobial drugs without a prescription in the United States. Many (30.6%) believed that antimicrobial drugs should be available in the United States without a prescription. Furthermore, 16.4% had transported nonprescribed antimicrobial drugs into the United States, and 19.2% had acquired antimicrobial agents in the United States without a prescription. A stepwise logistic regression analysis showed that the best predictors of having acquired nonprescribed antimicrobial drugs in the United States were beliefs and behavior consistent with limited regulations on such drugs. Many persons within the Latino community self-medicate with antimicrobial drugs obtained without a prescription both inside and outside the United States, which adds to the reservoir of antimicrobial drugs in the United States.

The amount of antimicrobial drugs consumed in a community is directly related to the amount of antimicrobial drug resistance found in the community (1,2). Inappropriate use of antimicrobial drugs, particularly for respiratory infections, has contributed to the major public health problem of antimicrobial resistance. Interventions in the United States have decreased inappropriate antimicrobial drug prescribing in general and for respiratory infections in particular (3–5).

In many nonindustrialized countries, antimicrobial resistance is an even greater problem than it is in the United States; however, many of these countries have few restrictions on the sale of antimicrobial agents, or laws are unevenly enforced (6–9). The dispensation of antimicrobial drugs without a prescription is particularly problematic in Latin America (9,10). Persons in countries with few regulations on antimicrobial agents tend to have cultural norms that encompass self-diagnosis and buying of antimicrobial drugs in subtherapeutic quantities (8,10,11). More than 320 million persons cross the border into the United States each year from Latin American countries where antimicrobial drugs are available without a prescription (12).

Health beliefs and practices are integrated into one's ethnic and cultural orientation (13–15). Limited evidence indicates that even after moving to the United States, persons from countries with limited or no restrictions on antimicrobial drugs are more likely than persons born in the United States to use them for inappropriate indications, such as common colds, and use them without a prescription (16,17).

Initiatives to increase judicious use of antimicrobial drugs to address antimicrobial resistance have focused primarily on changing prescribing patterns of US physicians and have failed to account for antimicrobial drug importation and concomitant inappropriate self-medication within immigrant communities. The pool of nonprescribed antimicrobial drugs entering the United States is currently unknown as is the extent of acquisition of nonprescribed antimicrobial drugs within communities whose health beliefs and practices may be inconsistent with those of the US public health community. Thus, the purpose of this study was to investigate a sample of Latinos living in the United States in terms of their practices of antimicrobial drug importation and acquisition and use of nonprescribed antimicrobial drugs.

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Methods

South Carolina, like many other cities and states that have historically had few Latinos, has experienced recent surges in immigration and now has small informal enclaves and social structures. Many Latinos in these new communities may lack documentation for US residency. Consequently, our sampling design took into account the fact that many probability-based sampling designs (e.g., random-digit dialing telephone survey, mail survey from a published list of addresses) would not adequately access this hard-to-reach population. Adult (≥18 years of age) Latinos attending 2 clinics in the greater Charleston, South Carolina, area that serve a primarily Latino population were approached for participation in a face-to-face, structured, confidential interview in which no names were recorded. This Latino population was employed primarily in agriculture.

Eligible persons included both those seeking care as well as those accompanying patients since the goal was to collect data from Latino adults regarding health behavior that was not necessarily linked to a current illness. Persons in the waiting rooms of the clinic were approached by an interviewer, who was introduced to the prospective respondent by clinic personnel. If persons did not consider themselves to be Latino, they were not included in the study. Data were collected in July and August 2004. The study was approved by the Medical University of South Carolina Institutional Review Board.

Instrument

Previously used and validated surveys designed for use with Latino populations were accessed from the scientific literature and were consulted for question wording relevant to the present study's aims. An instrument was created in English, pretested initially in English for flow and comprehension, and following modifications was then translated into Spanish. Once in Spanish, the instrument was put through 2 levels of pretests. The first pretest was with medical center personnel who were fluent with Spanish and dealt with Latino populations. Following modifications from the first pretest, the second pretest was conducted with Latino persons seen at a clinic different from the ones used in the study to provide pretest experience with the instrument with a similar population. After the second pretest, the instrument was translated back into English so that the wording of the questions could be checked for consistency in both English and Spanish. Two trained bilingual interviewers administered the survey in a structured format in the participant's preferred language (Spanish or English) and reinforced to the potential respondents the anonymous nature of the survey.

Variables

The study focused on 3 general sets of information. First, questions were included that assessed acquiring antimicrobial drugs without a prescription while outside the United States and corresponding self-medication. These questions were asked to get an idea of health behavior in their home culture and society. In addition, we asked whether the respondents believed that antimicrobial drugs should be available in the United States without a prescription. Second, questions were included that provided an assessment of the importation of nonprescription antimicrobial drugs into the United States and the context and circumstances related to this behavior. Third, a set of questions was included that assessed acquisition of nonprescribed antimicrobial drugs within the United States. We were specifically interested in the scope of acquisition of nonprescribed antimicrobial drugs from *bodegas*, *pharmacias*, or other stores since this behavior had been suggested in a study in New York City (17). For both importing nonprescribed antimicrobial drugs and acquiring them within the United States, a series of questions was asked, based on the pretest information, to gain an understanding of why the respondents would engage in these practices.

Analysis

Initially, descriptive statistics were computed to gain an understanding of the extent of the acquisition and importation of nonprescribed antimicrobial drugs. We also computed chi-square values to examine bivariate relationships between importation of nonprescribed antimicrobial drugs and acquisition of nonprescribed antimicrobial drugs in the United States by health beliefs and behavior in the home country, access to care in the United States, and demographic characteristics. Finally, a stepwise logistic regression model was computed on the dependent variable of acquisition of nonprescribed antimicrobial drugs with the same set of variables (acquired antimicrobial drugs without a prescription outside the United States, believe antimicrobial drugs should be available in the United States without a prescription, health insurance, age, sex, education, time in United States, country of birth, health status) to determine the best predictors of this behavior. Because no one born in the United States had acquired nonprescribed antimicrobial drugs in the United States, the category variable of country of birth was not used as a predictor of acquiring nonprescribed antimicrobial drugs. Instead, country of birth was coded for inclusion in the regression as 1) born in Mexico or 2) born elsewhere.

Results

A total of 277 adults were approached for participation. Four persons who were initially approached indicated that they did not consider themselves to be Latino. Of the

remaining 273, 54 refused or provided incomplete information, leaving a sample of 219. The demographic characteristics of the sample are presented in Table 1.

A large proportion of the sample (30.6%) believed that antimicrobial drugs should be available in the United States without a prescription. The behavior of acquiring antimicrobial drugs without a prescription while outside of the United States was quite common, with 45.2% indicating that they had done this.

A substantial number of persons had transported nonprescribed antimicrobial drugs into the United States (16.4%). The primary illnesses for which they bought the antimicrobial drugs were primarily for what we believe to have been viral respiratory infections. Among respondents who reported bringing back antimicrobial drugs that they purchased without seeing a doctor first, the reported conditions they were trying to treat included cough (88.9%), ear infections (88.9%), sore throat (69.4%), and colds (58.3%). When asked whether, on their next trip outside the United States, they would purchase antimicrobial drugs without seeing a doctor first and bring them back into the country, 23.7% of the sample reported "likely" or "very likely." Among persons who transported nonprescribed antimicrobial drugs into the United States, the primary reason reported for doing so was because they had a mistrust of medicines in the United States and were more comfortable with medicines from the home country (30.6%); other reasons included the following: to pay less for medicines bought in the home country than they would for those bought in the United States (19.4%), to avoid going to the doctor while in the United States (16.7%), to avoid the language barrier to care in the United States (13.9%), to prepare for future illness (13.9%), and to treat someone else's medical problem (5.6%).

Acquiring antimicrobial drugs not prescribed for the respondent within the United States was also a common behavior (19.2%). Among those who acquired antimicrobial drugs in the United States without a prescription, 92.9% reported that they had acquired them without prescription at stores in the United States. As with transportation of nonprescribed antimicrobial drugs into the United States, the primary illnesses for which they acquired the drugs without a prescription were what we believe to have been viral respiratory infections. Among respondents who reported acquiring antimicrobial drugs without a prescription, they reported attempting to treat "gripe" (flu) (97.6%), ear infections (97.6%), cough (83.3%), and sore throat (80.9%). Additionally, 97.6% reported acquiring antimicrobial drugs to treat diarrhea. Among persons who had acquired antimicrobial drugs in the United States without a prescription, 64.3% suggested that doing so was preferable to going to the doctor, while 26.2% reported that it was cheaper than paying for the doctor visit in addition to paying for the prescription. Only 7.1% of this group reported acquiring antimicrobial drugs in this way because of language barriers.

Table 1. Characteristics of sample*

Characteristic	No. persons (%)
Country of birth	
Mexico	164 (74.8)
Other Central American country	24 (11.0)
South American country	24 (11.0)
United States (US)	7 (3.2)
Age, y (mean ± SD)	29.8 ± 8.1
Health status	
Excellent	30 (13.7)
Very good	64 (29.2)
Good	96 (43.8)
Fair	22 (10.1)
Poor	7 (3.2)
Sex	
Male	76 (34.7)
Female	143 (65.3)
Years in US	
<1	18 (8.2)
1–3	71 (32.4)
4–6	68 (31.1)
>6	62 (28.3)
Insurance	
None	200 (91.3)
Medicaid	14 (6.4)
Other	5 (2.3)
Education	
Did not graduate from high school	107 (48.9)
High school graduate or more	112 (51.1)

*N = 219.

Tables 2 and 3 show the relationship between home country behaviors, health beliefs, access to care variables, and demographic characteristics to importing nonprescribed antimicrobial drugs and acquiring nonprescribed antimicrobial drugs in the United States. Persons who acquired nonprescribed antimicrobial drugs in the United States had beliefs and practices consistent with limited regulations on antimicrobial drugs.

The best predictors of acquiring antimicrobial drugs in the United States without a prescription are shown in Table 4. Only 4 variables were significantly related. The strongest predictors were health beliefs and practices consistent with limited regulations on antimicrobial drugs.

Discussion

This study confirms the existence of a large reservoir of nonprescription antimicrobial drugs in the United States that are used for likely inappropriate self-medication in the Latino community. Besides being imported, many of these nonprescription drugs are acquired from small stores, showing an organized system of nonprescription antimicrobial drug distribution within the Latino community in the United States

Table 2. Relationship between home country behavior, health beliefs, and access to care variables to importation of nonprescribed antimicrobial drugs into United States*

Variable	Brought antimicrobial drugs into United States, n (%)		p
	Yes	No	
Bought outside the United States without prescription			<0.01
Yes	27 (27.3)	72 (72.7)	
No	9 (7.5)	111 (92.5)	
Should antimicrobial drugs be available without prescription?			0.11
Yes	15 (22.4)	52 (77.6)	
No	21 (13.8)	131 (86.2)	
Age (y)			<0.01
<30	11 (9.2)	109 (90.8)	
≥30	25 (25.2)	74 (74.8)	
Health status			
Excellent-good	30 (15.8)	160 (84.2)	0.51
Fair-poor	6 (20.7)	23 (79.3)	
Sex			0.85
Male	13 (17.1)	63 (82.9)	
Female	23 (16.1)	120 (83.9)	
Years in United States			0.33
<4	12 (13.5)	77 (86.5)	
≥4	24 (18.5)	106 (81.5)	
Insurance			
None	33 (16.5)	167 (83.5)	0.94
Insured	3 (15.8)	16 (84.2)	
Education			
Did not graduate from high school	13 (12.2)	94 (87.8)	0.09
High school graduate or more	23 (20.5)	89 (79.5)	
Country of birth			
Mexico	24 (14.6)	140 (85.4)	0.13
Other Central American country	3 (12.5)	21 (87.5)	
South American country	8 (33.3)	16 (66.7)	
United States	1 (14.3)	6 (85.7)	

*N = 219.

The cultural beliefs and practice of obtaining antimicrobial drugs without prescriptions, particularly for what are likely viral respiratory infections, is reflected in the antimicrobial drug use patterns of the Latino community in this United States. Health beliefs and practices that were instilled in their countries of origin appear to be maintained even after living in the United States, as the relatively high frequency of acquisition of nonprescription antimicrobial drugs in the United States demonstrates. As previous research has shown, persons born in the United States are less likely to acquire antimicrobial drugs without a prescription within the United States (16). In fact, none of the Latino respondents born in the United States had acquired antimicrobial drugs without a prescription in the United States. Thus, a special emphasis with patient education should be made to target this population to instill health beliefs that are more consistent with those proposed by the US medical and public health communities.

An additional issue that may play a role in the ability to encourage appropriate use of antimicrobial drugs in this community are problems associated with access to health care. More than 90% of the respondents reported that they had no health insurance. Although health insurance was not a distinguishing variable, common reasons given by the respondents for both importation and acquisition of nonprescribed antimicrobial drugs in the United States revolved around the economics of doctor visits and costs of medication in the United States. A lack of health insurance may encourage Latinos to self-medicate with antimicrobial drugs.

Our findings should be interpreted within the context of several limitations to our study. First, the sample was recruited from a single mid-sized community, thereby limiting the generalizability of the results. However, many areas in the United States have seen recent large increases in the Latino population. These new communities may reflect different practices than communities that have had generations of Latino immigrants (e.g., Miami, New York, San Antonio). A second limitation concerns the location of data collection. By focusing on persons who sought treatment at a health clinic, Latinos who may not access care in the formal health care system would not be represented in this study. Those persons could be even more likely to acquire antimicrobial drugs without a prescription. A

Table 3. Relationship between home country behavior, health beliefs, and access to care variables to acquisition of nonprescribed antimicrobial drugs in United States*

Variable	Obtained in US without prescription, n (%)		p
	Yes	No	
Bought outside the United States without prescription			
Yes	31 (31.3)	68 (68.7)	<0.01
No	11 (9.2)	109 (90.8)	
Should antimicrobial drugs be available without prescription?			
Yes	25 (37.3)	42 (62.7)	<0.01
No	17 (11.2)	135 (88.8)	
Age (y)			
<30	29 (24.2)	91 (75.8)	0.04
≥30	13 (13.1)	86 (86.9)	
Health status			
Excellent-good	39 (20.5)	151 (79.5)	0.19
Fair-poor	3 (10.3)	26 (89.7)	
Sex			
Male	16 (21.0)	60 (79.0)	0.61
Female	26 (18.2)	117 (81.8)	
Years in United States			
<4	11 (12.4)	78 (87.6)	0.03
≥4	31 (23.8)	99 (76.2)	
Insurance			
None	40 (20.0)	160 (80.0)	0.32
Insured	2 (10.5)	17 (89.5)	
Education			
Did not graduate from high school	15 (14.0)	92 (86.0)	0.06
High school graduate or more	27 (24.1)	85 (75.9)	
County of birth			
Mexico	35 (21.3)	129 (78.7)	0.26
Other Central American country	2 (8.3)	22 (91.7)	
South American country	5 (20.5)	19 (79.2)	
United States	0	7 (100)	

*N = 219.

direction for future research would be to investigate these issues about antimicrobial drug use in a broader community sampling frame.

Third, the results are based on self-reports of behavior, some of which may be somewhat threatening to relate to an interviewer. Thus, the reports of antimicrobial drug importation and acquisition of nonprescribed antimicrobial drugs may be an underreport of actual behavior. Several strategies were used to try and obtain valid reports, including multiple pretests and having the clinic staff introduce the interviewers to the potential participants.

Persons may also have been confused about which medications are antimicrobial drugs. Following the pretests, the interview was modified to contain several instances of descriptions of antimicrobial drugs and names that would be recognizable to the Latino community. Moreover, each time a respondent reported acquiring antimicrobial drugs, the respondent was asked by the interviewer to name the drug to make sure that the respondent had actually acquired antimicrobial drugs. If in fact the results represent underreporting, the findings are even more dramatic because nearly 20% acknowledged getting antimicrobial drugs without a prescription in the United States.

Our findings suggest that a large public health problem is at hand. However, because this study is one of the first to document the problem and the first, to our knowledge, to specifically focus on both importation and US acquisition of nonprescribed antimicrobial drugs, government agencies have not yet responded to this problem. The South Carolina Department of Health and Environmental Control has a judicious antibiotic use initiative (South Carolina Careful Antibiotic Use, http://www.scdhec.gov/health/disease/sccause). Their initiatives are similar to many other initiatives that focus on both physician and patient education, but thus far have not focused on Latinos and nonprescription antimicrobial drug importation and local acquisition.

A substantial number of persons within the US Latino community self-medicate with antimicrobial drugs obtained without a prescription both inside and outside the United States, which adds to the reservoir of these drugs in the United States. The public health system and clinicians should be aware of the different health belief systems and

Table 4. Significant predictors from stepwise logistic regression regarding whether person had obtained nonprescribed antimicrobial drugs in United States

Variable	Odds ratio	95% CI*
Should antimicrobial drugs be available without a prescription?		
Yes	5.70	2.52–12.92
No	1.00	–
Bought outside the United States without prescription		
Yes	5.41	2.33–12.58
No	1.00	–
Years in United States		–
<4	1.00	
≥4	4.28	1.72–10.68
Age (y)		
<30	3.00	1.28–7.01
≥30	1.00	–

*CI, confidence interval.

practices in ethnic minority communities. Patient education materials should communicate in a culturally competent way the dangers of self-medication and antimicrobial misuse to both recent immigrants and others in the Latino community. US health policies also may need to be revised, with consideration given to tightening regulations to reduce the presence of nonprescribed antimicrobial drugs and to increasing access to care for many Latinos.

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Fluoroquinolone-resistant *Escherichia coli* Carriage in Long-term Care Facility

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We conducted a cross-sectional study to determine the prevalence of, and risk factors for, colonization with fluoroquinolone (FQ)-resistant *Escherichia coli* in residents in a long-term care facility. FQ-resistant *E. coli* were identified from rectal swabs for 25 (51%) of 49 participants at study entry. On multivariable analyses, prior FQ use was the only independent risk factor for FQ-resistant *E. coli* carriage and was consistent for FQ exposures in the previous 3, 6, 9, or 12 months. Pulsed-field gel electrophoresis of FQ-resistant *E. coli* identified clonal spread of 1 strain among 16 residents. Loss (6 residents) or acquisition (7 residents) of FQ-resistant *E. coli* was documented and was associated with de novo colonization with genetically distinct strains. Unlike the case in the hospital setting, FQ-resistant *E. coli* carriage in long-term care facilities is associated with clonal spread.

The increasing prevalence of antimicrobial resistance affecting hospitalized and ambulatory populations has gained national prominence. Although this setting is less well studied, evidence is mounting that antimicrobial resistance is also an increasing problem in long-term care facilities (1–5). Most research on colonization with resistant bacteria in the long-term care setting has focused on gram-positive organisms, in particular, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (6–9); substantially fewer data address the prevalence of antimicrobial resistance among gram-negative bacteria.

Past studies in such facilities found that resistance in gram-negative bacteria was not uncommon, whereas resistance among isolates of *Escherichia coli* was unusual (1,4,10,11). More recent investigations reported that among hospitalized patients, residence in a long-term care facility was a risk factor for colonization or infection with *E. coli* that was resistant to higher generation cephalosporins and to the fluoroquinolone (FQ) antimicrobial agents (12–15). Moreover, Weiner et al. reported that nursing home residents were likely to be colonized with such isolates at the time of hospital admission (15). Finally, we recently noted significant increases over a 5-year period in FQ-resistant *E. coli* in clinical isolates from 4 long-term care facilities in Pennsylvania (16). *E. coli* is the most common species causing infections in the elderly long-term care resident, primarily as a consequence of the prevalence of urinary tract infections. FQs are the most frequently prescribed antimicrobial class in this setting, accounting for ≈25% of all antimicrobial prescriptions (17,18).

While evidence suggests that the prevalence of FQ-resistant *E. coli* carriage among such residents is increasing, no patient level study of risk factors for FQ-resistant *E. coli* colonization has been performed in this setting (16). We conducted this study to determine the prevalence of fecal carriage with FQ-resistant *E. coli* among residents of a single long-term care facility, to identify risk factors associated with colonization, and to describe the ecology of carriage of FQ-resistant *E. coli* over time.

Methods

Study Site and Patient Population

This study was conducted at a single Veterans Affairs Medical Center nursing home. This 240-bed facility, adjacent to a 150-bed acute-care hospital, opened in 1990 (9) and maintains an average daily census of >95% of capacity. The demographics of the facility parallel that of the adjacent tertiary medical center: 1% female and 50%

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minority residents. More than 80% of residents are admitted from the adjacent medical center. Approximately 20 beds are used for patients requiring admission for skilled nursing care.

Residents were recruited for this study from March to July 2002 (19). All residents were considered eligible for inclusion if informed consent was obtained. For residents who were cognitively impaired, informed consent was obtained from a legal guardian or medical proxy. The study was reviewed and approved by the local institutional review board.

For enrolled participants, rectal swabs were obtained at study entry. FQ-resistant *E. coli* were detected by a 1-step screening procedure (19). Species identification and FQ resistance were confirmed by automated testing (Vitek, bioMérieux, Hazelwood, MO, USA). Because a recent study noted excellent sensitivity and specificity (>90%) for rectal swab specimens compared to stool culture for detecting FQ-resistant *E. coli* (20), subsequent rectal swab samples were obtained at monthly intervals to identify changes in colonization status.

Case-patients were defined as those for whom FQ-resistant *E. coli* was identified at the initial sampling. Controls were defined as patients without FQ-resistant *E. coli* at the initial sampling. Any study participant colonized with both a FQ-resistant *E. coli* and a FQ-susceptible *E. coli* was considered a case-patient. Patients with new colonization with FQ-resistant *E. coli* were defined as those for whom the initial study sample yielded only FQ-susceptible *E. coli* with a sample at a later time point yielding FQ-resistant *E. coli*. Patients clearing colonization with FQ-resistant *E. coli* were defined as those for whom this organism was detected at the time of initial sampling with 2 subsequent consecutive samples that yielded only FQ-resistant *E. coli*.

Data Collection

Computerized medical records were reviewed for all patients. Patients admitted to the long-term care facility are evaluated by a nurse practitioner and physician with a comprehensive assessment documented at admission and at yearly intervals. Quarterly assessments are performed for minimal data set, functional, and mental evaluations. Interval notes by the nurse practitioner and physician were entered at times of clinical events. Data collection was assisted by the fact that patients requiring hospital admission were cared for in the adjacent medical center. Medical records for both facilities are maintained jointly. The nursing home admission note and yearly review notes contained detailed problem lists. For patients who had received care at other Veterans Affairs institutions, medical records were available through the Veterans Affairs Intranet. Demographic data obtained included age, sex, race, date of admission to the facility, and dates of prior hospitalizations at the time of study enrollment.

Records were also reviewed to identify potential risk factors for carriage of FQ-resistant *E. coli*. Devices and conditions that would interfere with normal mucosal defense mechanisms (21) were ascertained, including indwelling catheters, intravenous catheters, feeding tubes, decubitus ulcers, and surgical wounds. Data on coexisting conditions included renal insufficiency (defined as a serum creatinine level of >2.0), liver failure, hepatitis C, cirrhosis, congestive heart failure, chronic obstructive lung disease, malignancy, and HIV. Disorders associated with cognitive impairment included dementia, history of cerebral vascular accident, and psychiatric disorders such as depression and schizophrenia. Low ambulatory status was defined as requiring a wheelchair for ambulation or documentation of the patient's being bed-bound. Pharmacy records were reviewed for all antimicrobial use in the year before study entry and during the period of prospective fecal sample collection.

Genotypic Analysis of *E. coli*

Up to 25 colonies of *E. coli* as available were sampled from the initial patient sample, and ≤10 colonies were obtained from subsequent cultures. Individual colonies were subjected to pulsed-field gel electrophoresis (PFGE) to determine macrorestriction polymorphisms after *Xba*I restriction digestion of chromosomal DNA as described (22,23). Clonal analysis was performed (24) per the criteria of Tenover et al. (25); isolates that differed by ≤3 bands were considered clonal and isolates that differed by 4 to 6 bands were considered related.

Statistical Methods

We conducted a cross-sectional study to identify risk factors for FQ-resistant *E. coli* colonization. Bivariable analysis was conducted to determine the association between potential risk factors and such colonization; the primary risk factor variable of interest was prior FQ use. Although we used FQ use in the past year as the primary measure, we also explored different cutpoints of prior FQ use (i.e., 3, 6, and 9 months). Categorical variables were compared by using the Fisher exact test. An odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the strength of associations. Continuous variables were compared with the Student *t* test or the Wilcoxon rank-sum test, depending on the validity of the normality assumption (26).

Stratified analyses were then performed to identify where confounding and interaction were likely to exist for the primary comparison of interest (i.e., FQ exposure and FQ-resistant *E. coli* colonization). Stratification was performed with the following variables: duration of residence

in the facility before study enrollment (divided into quartiles) and hospitalization in the prior year. The Mantel-Haenszel test for summary statistics was used to evaluate possible confounding (27); interaction was assumed when the test for heterogeneity between the OR for different strata was significant ($p<0.05$).

Multivariable analysis was performed with multiple logistic regression (28). Building the multivariable model began with inclusion of key variables based on a priori hypotheses (i.e., prior FQ use). All variables with a p value <0.20 on bivariable analysis were also considered for inclusion in a multivariable explanatory model (29). Variables were also considered for inclusion in the model if they were noted to be involved in confounding on stratified analysis. Finally, we evaluated the impact of the variable indicating "time at risk" (i.e., duration of residence in the facility before study enrollment) in the multivariable model. The interaction between risk factor variables in the final model was also investigated. A 2-tailed p value <0.05 was considered significant. All statistical calculations were performed with standard programs in STATA v. 8.0 (Stata Corp, College Station, TX, USA).

Results

Of 75 randomly selected residents who were consecutively approached for enrollment, 60 (80%) gave informed consent for inclusion in the study. Five residents were discharged or died before having an initial rectal sample obtained for study; 6 other residents had stool samples that did not yield *E. coli* despite multiple samplings. Thus, samples from 49 residents yielded *E. coli* isolates and were included in the study. The median age of participants was 69 years (range 38–98 years). Two (4.1%) participants were female, 18 (36.7%) were African American, and 1 (2.0%) was Hispanic. Three residents were admitted only for skilled nursing care. Patient functional scores exhibited a bimodal pattern: one third of patients were considered full care, one third as fully independent, and the remaining patients evenly spread through a range of functional levels. Approximately 50% of patients were incontinent. Multiple sclerosis was documented for 5 patients.

FQ-resistant *E. coli* was detected in the stool of 25 (51%) residents (19). The median age of case-patients was 73 years (range 38–87 years) and 65.5 years (range 42–98 years) for controls ($p = 0.99$). One case-patient and 1 control were women ($p>0.99$). Ten case-patients and 8 controls were African American ($p = 0.77$). The results of bivariable analysis are shown in Table 1. Duration of nursing home residence, hospitalization within the 12 months before study entry, low ambulatory status, FQ use within the past year, and prior metronidazole use were associated with FQ-resistant *E. coli* colonization. Association of FQ exposure and colonization with FQ-resistant *E. coli* was noted at all quartiles except for exposure within the 3 months before study entry: 6 (25%) of 24 controls and 18 (72%) of 25 case-patients received FQ in the 9 months before study entry ($p = 0.002$); 5 (21%) of 24 controls and 14 (56%) of 25 case-patients received FQ in the 6 months before study entry ($p = 0.02$); and 3 (13%) of 24 controls and 7 (28%) 25 received FQ in the 3 months before study entry ($p = 0.29$).

On multivariable analysis, only prior FQ use remained an independent risk factor for FQ-resistant *E. coli* colonization (Table 2). A borderline significant association was seen between FQ-resistant *E. coli* colonization and duration of prior long-term care residence as well as prior metronidazole use.

Genotypic analysis of 25 colonies from each study participant was performed by PFGE. Multiple strains were detected in initial fecal samples from 22 (44.9%) participants. For those with FQ-resistant *E. coli* in stool, 16 (64%) had multiple *E. coli* strains. In contrast, multiple strains were less common for those not colonized with FQ-resistant *E. coli* as only 6 (25%) harbored multiple strains of *E. coli* (OR 5.33, 95% CI 1.34–22.23, $p = 0.006$). Both FQ-resistant and FQ-susceptible *E. coli* were detected in fecal samples from 15 participants. Comparison of strains between participants documented 2 clusters of clonally related strains of FQ-resistant *E. coli* detected for multiple persons. Clone A was detected in fecal samples from 16 participants: 7 were colonized with clone A alone and 9 with strain A and ≥1 unique strains of FQ-resistant *E. coli*, FQ-susceptible *E. coli*, or both (Table 3). A second resistant clone, clone C, was detected in the stools from 2 participants (Table 3). Unique stains of FQ-resistant *E. coli* (i.e., other than clone A or C) were detected in the stools of 14 (56%) persons. FQ-susceptible strains were genetically unique in different participants. FQ exposure in patients colonized with clone A compared with other strains of FQ-resistant *E. coli* did not differ (data not shown). Thus, person-to-person clonal spread of FQ-resistant *E. coli* was common and occurred in the absence of FQ exposure.

Of the 49 participants enrolled in the study, 45 (92%) had follow-up cultures. For the 25 participants initially colonized with FQ-resistant *E. coli*, 22 (88%) had sequential follow-up cultures (median follow-up 6 months, range 1–10 months). Rectal swabs from 16 (73%) study participants continued to yield FQ-resistant *E. coli* at each monthly sample, while swabs from 6 patients demonstrated clearance of this organism. The median time for clearance of FQ-resistant *E. coli* was 5 months (range 2–10 months). For the 24 participants not initially colonized with FQ-resistant *E. coli*, 23 (96%) had follow-up rectal swab samples. New colonization with FQ-resistant *E. coli* was detected in samples from 7 (30%) persons at a median of 6 months from study entry (range 1–8 months). Three

Table 1. Comparison of cases and controls, fluoroquinolone-resistant *Escherichia coli* colonization study, long-term care facility*

Variable	Controls (n = 24) (%)	Cases (n = 25) (%)	OR (95% CI)	p value†
Prior hospitalization	18/24 (75.0)	13/25 (52.0)	0.36 (0.09, 1.41)	0.08
Duration of residence in facility (d)‡§	209.5 (22–2,571)	411 (81–2,580)		0.13
Decubitus ulcer	6/24 (25.0)	2/25 (8.0)	0.26 (0.02, 1.73)	0.14
Low ambulatory status	15/24 (62.5)	10/25 (40.0)	0.40 (0.11, 1.46)	0.16
Fluoroquinolone use	6/24 (25.0)	18/25 (72.0)	7.71 (1.86, 33.60)	0.002
Metronidazole use	1/24 (4.2)	7/25 (28.0)	8.90 (0.96, 420.17)	0.05

*OR, odds ratio; 95% CI, 95% confidence interval.
†Fisher exact test (categorical variables); Wilcoxon-rank sum test (continuous variables).
‡Median (range).
§Days from admission into facility until study enrollment.

of the 45 participants included in this follow-up phase were prescribed antimicrobial agents after study entry, but the antimicrobial agent was not an FQ. For these 3 patients, no change in carriage of FQ-resistant *E. coli* occurred. No study participant was hospitalized in the follow-up period. No demographic or clinical factors were associated with a change in colonization status. Thus, resistance patterns were altered in a large number of study participants (13 [29%] of 45), independent of antimicrobial treatment in the 1-year follow-up period.

For the 7 study participants with newly acquired FQ-resistant *E. coli*, PFGE genotypes of all strains of FQ-susceptible *E. coli* cultured from the initial study sample were compared to 5 colonies of FQ-resistant *E. coli* randomly chosen from the first sample yielding FQ resistance. For each patient, PFGE genotypes differed between initial and subsequent samples, a demonstration of de novo colonization with resistant bacteria. Similarly, clearance of FQ-resistant *E. coli* was associated with de novo colonization with genetically distinct strains in 5 of 6 cases. For 1 case-patient, a resistant strain cleared; colonization with a susceptible strain present at the initial study visit continued.

Discussion

FQ use was the only independent risk factor for FQ-resistant *E. coli* colonization. Borderline significant associations existed between carriage of such organisms and duration of residence in the long-term care facility before study enrollment and prior metronidazole use. Most study participants harboring FQ-resistant *E. coli* were colonized with clonally related strains. Change in colonization status, either acquisition or clearance of FQ-resistant *E. coli*, was common in the 1-year period of follow-up and did not appear to be related to antimicrobial therapy.

Although never previously investigated in a long-term care facility, the association between FQ exposure and colonization or infection with FQ-resistant *E. coli* has been documented (30–36). Other investigators have, however, found prior FQ exposure to represent a modest (37) or no risk (38) for colonization with FQ-resistant *E. coli*. Exposure effect was found to be relatively short-lived among cancer patients prescribed FQ antimicrobial agents as part of prophylaxis during chemotherapy: >75% of patients had clearance of FQ-resistant *E. coli* within 3 months of ceasing FQ use (31,34). While our data corroborate the relationship between FQ exposure and FQ-resistant *E. coli*, we also found that temporally more distant FQ exposures (>3 months) may also represent risks for colonization with resistant bacteria. And, in contrast to the findings with cancer patients, colonization with FQ-resistant *E. coli* may persist over long periods.

We addressed the question of clearance and acquisition of FQ-resistant *E. coli* in the long-term care setting. Of the 45 patients with follow-up cultures, 13 (29%) demonstrated acquisition or clearance with FQ-resistant *E. coli* over the 1-year follow-up period. No patient who changed colonization status was treated with FQ during the 1-year study period or in the year before study entry (data not shown). Thus, in this closed setting, colonization with FQ-resistant *E. coli* appears to be a dynamic process and may be less affected by prior FQ therapy than it would be in the acute-care setting.

Molecular analysis shed further light on this process. In all cases but one, alteration in colonization status (whether from resistant to susceptible or susceptible to resistant) was marked by de novo colonization with bacteria genetically distinct from those patients had at study entry. For the remaining patient, a resistant strain was

Table 2. Multivariable comparison of cases and controls, fluoroquinolone-resistant *Escherichia coli* colonization, long-term care facility study*

Variable	Unadjusted OR	Adjusted OR (95% CI)	p value
Fluoroquinolone use	7.71	9.16 (2.08, 40.41)	0.003
Duration of residence in facility†‡		1.00 (1.00, 1.01)	0.07
Metronidazole use	8.90	5.90 (0.52, 66.50)	0.15

*OR, odds ratio; 95% CI, 95% confidence interval.
†Days from admission into facility until study enrollment.
‡Odds ratio (OR) reflects the odds associated with each increase in 1 day of residence in the facility.

Table 3. Genotypic analysis of patient samples of fluoroquinolone-resistant *Escherichia coli**

Analysis	No. of patients
Strain A detected	15
Strain A only detected	7
Strain A + FQSEC	3
Strain A + FQSEC + unique FQREC	5
Strain B detected	2
Strain B + FQSEC	1
Strain B + FQSEC + unique FQREC	1
Unique FQREC detected	7
Unique FQREC only	2
Unique FQREC + FQSEC	5

*FQSEC, fluoroquinolone-susceptible *E. coli*; FQREC, fluoroquinolone-resistant *E. coli.*

cleared, and a susceptible strain detected at study entry persisted.

Most (64%) study participants colonized with FQ-resistant *E. coli* harbored a single clonally related strain (clone A); 56% were colonized with strains other than clone A. Thus, the emergence of FQ-resistant *E. coli* colonization in this patient population appeared to arise from patient-to-patient spread as well as de novo resistance. Clonal spread of FQ-resistant *E. coli* has not been adequately addressed, although 2 studies of cancer patients suggest that it is uncommon in other clinical settings (34,39).

Our study had several potential limitations. Our small sample size may have hampered our ability to identify smaller effect sizes for risk factors of interest. The possibility of selection bias is of concern, given that only 25% of the total population of the facility was enrolled in the study. Since we only sampled participants monthly, the longitudinal component of the study is limited regarding fully characterizing the dynamics of how frequently resistance profiles of nursing home residents are altered. These factors also limit our ability to assess outcomes and risks for subsequent infections. Changes in resistance profiles were also associated with colonization with different *E. coli* strains. Whether this represents possible antimicrobial effects on non–*E. coli* affecting the ability of new strains of *E. coli* to colonize the gut is unknown. Since environmental cultures were not performed, we cannot exclude a common source exposure (e.g., food or showers) to explain a single clone's being detected among different patients. Finally, whether our study results can be generalized to other institutions is not known.

Our study represents the first investigation of patient-level risk factors for FQ-resistant *E. coli* colonization in the long-term care setting. We found that FQ-resistant *E. coli* carriage is common in such residents and that prior FQ exposure is the only independent risk factor for such carriage. These finding emphasize the importance of limiting antimicrobial drug use in general and FQ use in particular in this setting. Unlike the hospital setting, carriage of FQ-resistant *E. coli* in long-term care facilities is associated with clonal spread. Finally, carriage of FQ-resistant *E. coli* in long-term care facilities appears to represent a fluid process, with frequent loss or acquisition of FQ-resistant *E. coli*.

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Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant Enterococci in Rural Communities, Western United States

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The impact and prevalence of antimicrobial drug resistance in rural community healthcare settings is uncertain. Prospective surveillance in 51 rural hospitals in Idaho and Utah examined the epidemiologic features of clinical cases of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Thirty-two cases of VRE were reported; for 6, the patient had no prior healthcare exposure or coexisting condition. Among the 724 MRSA cases available for evaluation, 405 (56%) were healthcare-associated (HA-MRSA), and 319 (44%) were community-associated (CA-MRSA). The characteristics of HA-MRSA and CA-MRSA patients with coexisting factors were similar, which suggests community transmission of healthcare strains. CA-MRSA cases without coexisting factors, however, demonstrated features previously reported for community strains. MRSA infections were substantially more frequent than VRE in rural communities in the western United States. Based on epidemiologic criteria, a large proportion of MRSA cases were community-associated. CA-MRSA rates were predictive of institutional MRSA rates.

Antimicrobial resistance is steadily rising among bacterial pathogens associated with both community- and healthcare-associated infections (1,2). Among the most important of these pathogens are vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) (3). Risk factors for VRE acquisition include chronic dialysis, multiple and prolonged hospitalizations, main admitting diagnosis, coexisting factors (diabetes mellitus, organ transplant, or hepatobiliary disease), previous infection or colonization with MRSA or *Clostridium difficile*, and prior treatment with antimicrobial agents (4–6). Most acquisition of VRE in the United States has occurred in the hospital or intensive care setting (4,7–9).

Risk for colonization or infection with *S. aureus* is highest in patients with diabetes mellitus, intravenous drug users, patients undergoing hemodialysis, surgical patients, and patients with AIDS (10). Additional patient risk factors for nosocomial MRSA infections, when compared to methicillin-susceptible *S. aureus*, include increased number of coexisting factors; increased length of hospital stay; exposure to antimicrobial drug agents, especially fluoroquinolones; enteral feedings; and surgery (11). In the past few years, however, reports of patients with serious MRSA infections who had no known risk factors or exposure to healthcare settings have been increasing (12–20). The distinctive properties of community-associated (CA) MRSA strains compared to nosocomial strains include a much more susceptible antimicrobial phenotype (due to the presence of a much smaller staphylococcal cassette chromosome [SCC] *mecA* [type IV]) (21) and the presence of different exotoxin gene profiles, including Panton-Valentine leukocidin (17,22,23). Patients tend to be younger and have skin and soft tissue infections or other necrotizing infections (17,21). Hospital-acquired (HA)-MRSA typically have a much larger SCC*mecA* (types I, II, and III) with a much more resistant antimicrobial phenotype (12,24).

The epidemiology of MRSA and VRE transmission may be different in the rural setting than that reported for

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the urban environment. For instance, inpatient acuity is substantially less in rural community hospitals than tertiary care facilities. Some of the reports of CA-MRSA infections included patients from rural communities, often native North American populations (15,16,19,23,25–28). A large study of community MRSA infection examined the characteristics of MRSA infections predominantly reported from urban and suburban regional laboratories but representing urban, suburban, and rural populations; overall, 12% of MRSA infections were community-associated by epidemiologic criteria (17).

To address the extent to which nosocomial MRSA and VRE are substantive problems in rural hospitals, we conducted a prospective study in rural Utah and Idaho. Epidemiologic and clinical data on VRE and MRSA clinical cases were collected during a 15-month period in 51 rural communities. The hospitals participating in this epidemiologic study had been surveyed about their infection control practices in 2000 (29). All had policies in place to institute contact isolation for patients with clinically recognized MRSA and VRE infection. However, none had performed active surveillance cultures to detect patients needing isolation.

Methods

Participating Hospitals and Study Population

Fifty-one rural hospitals in Idaho and Utah were recruited to participate in a surveillance project for antimicrobial drug resistance funded by the Centers for Disease Control and Prevention (CDC). The participating institutions represented 89% of the total number of rural hospitals in the 2 states.

All hospitals except 2 met the Office of Management and Budget definition of rural location (30). Based on this definition, a rural county was considered any county that did not have a metropolitan center with a population exceeding 50,000 persons. Hospitals within such counties were considered to meet this rural definition. Among the 2 participating hospitals not meeting this definition, 1 hospital was in an isolated county slightly exceeding the 50,000 metropolitan population limit but was considered to serve a primarily rural population. The second was a small hospital (50 beds) located within but at the border of an urban county.

Surveillance System

Clinical cases of VRE and MRSA identified by the clinical laboratories of participating hospitals were reported to the respective infection control practitioners, who compiled demographic, medical history, and other epidemiologically relevant data on each case. Individual level race and ethnicity were not captured.

In some cases, the microbiology staff contributed to data collection. The primary source of information was the patient's medical record. In most cases direct confirmation of healthcare exposure through patient or family interview was not possible. These data were recorded on a standardized data collection form and submitted on a regular, usually monthly, schedule. Data evaluated in this analysis were collected from October 1, 2002, to December 31, 2003. All data collected and analyzed were for this 15-month period, except for incidence rate calculations, which were for calendar year 2003, as described below. The following approaches were used to improve the validity of the data: 1) a data dictionary and operations manual were created with explicit instructions for completion of the data collection forms; 2) the data collection protocol was discussed during conference calls along with frequent one-on-one communication; and 3) anomalous data in the data reports were routinely searched for and corrected.

Epidemiologic Definitions

Definitions used in this study focused on the location of the patient at the time of initial culture and the presence or absence of exposure to the healthcare environment. The emphasis, therefore, is on healthcare or community association rather than definitive identification of the site of acquisition. These definitions are consistent with those of CDC and others (16–18,20).

Healthcare-associated VRE (HA-VRE) or MRSA (HA-MRSA)

This category included VRE or MRSA cultured from patients >48 hours after hospital admission, or while a patient of another hospital, resident of a long-term care facility, or transitional care unit. Also included in this category were infected patients with history of prior hospitalization or outpatient surgery, prior residence in a long-term care facility or transitional care unit, or prior care from home health agency or with documented indwelling catheters. This group also included patients with a postoperative wound infection, even if the surgery was performed as an outpatient. Patients identified with any of the above healthcare exposures were included in this category; the period from healthcare exposure to inclusion was generally 6 months. Patients known to have previous VRE or MRSA infections or positive cultures were excluded from analysis.

Community-associated VRE (CA-VRE) or MRSA (CA-MRSA)

This category included VRE or MRSA cultured from patient <48 hours after hospital admission, or as an outpatient. Excluded from this category were patients with previous history of positive VRE or MRSA culture or

infection, prior hospitalization or outpatient surgery, prior residence in a long-term care facility or transitional care unit, or prior home health.

Coexisting Factors

This category included medical or other factors possibly associated with healthcare exposure (diabetes mellitus, renal failure, prior antimicrobial drug therapy, and immunosuppression). Such factors were assessed for all groups of VRE and MRSA cases.

CA-VRE or CA-MRSA with Coexisting Factors

This category included VRE or MRSA clinical cases satisfying the definition for community-associated infection in which the patient had identifiable coexisting factors.

CA-VRE or CA-MRSA without Coexisting Factors.

This category included VRE or MRSA case satisfying the definition for community-associated infection in which the patient had no identifiable coexisting factors.

Incidence Rates

The incidence of CA- and HA-VRE and MRSA were calculated for each hospital reporting positive cultures from January 1, 2003, to December 31, 2003. Individual institutions were excluded from this analysis if the respective hospital infection control and microbiology staff could not verbally attest to the completeness of reporting after the study ended, based on a retrospective review of all cases during the study period. Thus, hospitals with partial or incomplete reporting of all cases were not included in the incidence rate calculations or Poisson regression model. The described hospital located at the border of the urban county was also excluded from the incident rate calculations. The denominator for healthcare-associated cases was the total inpatient census for 2003, including long-term care facility census if a hospital had such an attached facility. These data were obtained from published hospital statistics (31) or by direct communication with hospital staff. The denominator for community-associated infections was the most recent estimated county population data derived from the 2000 census in Idaho (Idaho Department of Health, http://www2.state.id.us/dhw/vital_stats/health_district_report.pdf) and Utah (Utah Department of Health, http://health.utah.gov/vitalrecords/pub_vs/ia02/02bx.pdf). In both cases, the incidence rates were expressed as the number of hospital cases per 10,000 patient-days or number of community cases per 10,000 person-years, based on county population. Incidence rates for CA-MRSA were also calculated by using the community population size as the denominator. For comparison, MRSA incidence rates for CDC National Nosocomial Infections Surveillance (NNIS) hospitals during 2003 were obtained (T. Horan and J. Edwards, pers. comm.).

Susceptibility Testing

Antimicrobial susceptibility results, collected retrospectively, were not available for all MRSA cases. Available data were aggregated and compared among the different groups of MRSA patients. Specifically, clinical and epidemiologic characteristics of MRSA cases for which the infecting organisms were resistant to both clindamycin and ciprofloxacin ("resistant group") and MRSA cases for which the infecting organisms were sensitive to both clindamycin and ciprofloxacin ("susceptible group") were compared. Phenotypic susceptibility to these 2 antimicrobial agents in MRSA is most often associated with community acquisition (17,24).

Data Analysis

All data were entered into an Access (Microsoft Corporation, Redmond, WA, USA) relational database for analysis. Proportions of total cases meeting specific epidemiologic criteria were calculated, and characteristics of each category were compared by using Fisher exact testing. Hospital bed size, census data, and age for MRSA patients were available for most but not all entries. Census data and ages of patients in each category were compared with Kruskal-Wallis equality of populations rank test. The relationship of community MRSA rates and other covariates on the hospital MRSA rates were modeled by using random effects Poisson regression. In this model, each specific hospital was considered a unit and was treated as a random effect; its MRSA cases were assumed to be correlated. That is, the hospitals were considered to be a random sample of all rural Utah and Idaho hospitals. This assumption permitted inferences to be made to this target population, rather than limiting inferences to only those hospitals included in the model. Continuous predictor variables were converted to ordered categoric variables and included in the models as tertiles, quartiles, or quintiles to verify that risk was "linearly" increasing. Multivariable models were fitted by using backwards stepwise variable selection. All statistical testing was performed with STATA, version 8 (Stata Corporation, College Station, TX, USA). All statistical analyses were 2-sided and significance was set at $p<0.05$.

Results

Case Ascertainment

A total of 34 unique VRE and 799 unique MRSA cases were reported by participating rural healthcare institutions in Idaho and Utah from October 1, 2002, to December 31, 2003. Twenty-six of 51 institutions reported ≥1 MRSA or

VRE case during this interval. Infection control practitioners or microbiology staff from 28 institutions confirmed that reporting was complete; 9 of the 28 institutions with confirmed complete reporting had no MRSA cases, and 23 of the 28 institutions had no VRE cases during the interval. The 22 institutions that did not attest to complete reporting contributed 17 MRSA and 2 VRE cases to the descriptive case series analysis but were removed from the incidence rate analysis. The average bed size of institutions with complete reporting was greater than the average bed size of institutions that did not attest to complete reporting (53 beds vs. 29 beds, p = 0.03).

Two VRE (6%) and 75 MRSA (8%) cases were excluded from the case-series analysis because of incomplete data. Of the 32 VRE cases with complete data, criteria for HA-VRE infection and CA-VRE infection were met by 25 (78%) and 7 (22%), respectively. Of the remaining 724 MRSA cases, 405 (56%) were HA-MRSA infection and 44% were CA-MRSA infection. Among the CA-MRSA cases, 79 (25%) were from patients with known coexisting factors, and 240 (75%) came from patients without any such factors reported (Table 1).

Characteristics of VRE and MRSA Clinical Cases

Eight institutions reported at least 1 VRE case. The major healthcare location of patients with HA-VRE was the transitional care unit (12/32, 38%); other locations are outlined in Table 1. Six patients (19%) had no reported risk factors or known exposure to the healthcare setting. The most common clinical source of VRE isolates was urine (15/32, 47%). The major location for patients at the time of MRSA culture was the community (391/724, 54%) with the long-term care facility (147/724, 20%) representing the most common healthcare location (Table 1). The most common clinical source of MRSA cultures was skin and soft tissue (400/724, 55%).

The clinical sites of infection for all MRSA cases were compared (Table 2). Comparison of clinical sources for MRSA infection between groups of HA-MRSA and CA-MRSA patients with coexisting factors showed no significant differences (data not shown). Patients in the CA-MRSA group without coexisting factors, however, had the highest proportion of skin/soft tissue infections (156/240, 65%, p<0.0001). Patients in this group were also much younger (mean age 41.5 years, n = 178) than the

Table 1. Characteristics of VRE and MRSA clinical cases*

	VRE		MRSA	
Characterization	No. (N = 32)	%	No. (N = 724)	%
Healthcare-associated	25	78	405	56
Community-associated	7	22	319	44
without coexisting factors	6	19	240	33
with coexisting factors	1	3	79	11
Location of time of culture				
Community	9†	28	391‡	54
Ward	4	13	113	16
Intensive care unit	0	0	24	3
Long-term care facility	6	19	147	20
Transitional care unit	12	38	19	3
Other hospital	1	3	29	4
Clinical sources				
Skin and soft tissue	4	13	400	55
Urine	15	47	104	14
Blood	2	6	38	5
Sputum	0	0	116	16
Other	11	34	64	9
Unknown	0	0	2	1
Coexisting factors				
>65 years of age	19	59	353	49
Diabetes mellitus	4	13	142	20
Renal failure	3	9	61	8
Prior antimicrobial therapy	5	16	210	29
Immunosuppression	6	19	77	11
Sex				
Male	14	44	382	53
Female	18	56	342	47

*VRE, vancomycin-resistant enterococci; MRSA, methicillin-resistant *Staphylococcus aureus*.
†There were 2 VRE cases in which cultures were obtained in the community setting in patients with previous history of healthcare exposure (recent hospitalization, residence in long-term care facility).
‡There were 72 MRSA cases in which cultures were obtained in the community setting in patients with previous history of healthcare exposure. The location at time of culture was unknown in one MRSA case.

Table 2. Clinical sources for MRSA cultures*

Clinical source	No. (%) HA-MRSA, N = 405	CA-MRSA with CFs, N = 79	CA-MRSA without CFs, N = 240	p value†
Skin and soft tissue	197 (49)	47 (60)	156 (65)	<0.0001
Urine	61 (15)	11 (14)	32 (13)	NS
Blood	33 (8)	2 (3)	3 (1)	<0.0001
Sputum	89 (22)	12 (15)	15 (6)	<0.0001
Other	23 (6)	7 (9)	34 (14)	0.001
Unknown	2 (1)	0 (0)	0 (0)	NS

*MRSA, methicillin-resistant *Staphylococcus aureus*; HA, healthcare-associated; CA, community-associated; CFs, coexisting factors; NS, not significant.
†Based on Fisher exact test.

other 2 groups (mean ages 68.8, n = 357 and 59.0 years, n = 66) (p = 0.0001); the proportion of patients <20 years of age in this group was 24% (43/178) compared to 2% (6/351) and 8% (5/66) in the other 2 groups (p<0.0001). Among hospitals with complete reporting, the fraction of MRSA cases that were CA-MRSA (with and without coexisting factors) was slightly higher in smaller hospitals compared to larger hospitals, but this difference was not significant (48% if bed size <40 and 41% if bed size >40, p = 0.323).

Susceptibility to antimicrobial agents was compared among these 3 epidemiologic groups of MRSA infections (Table 3). Susceptibility to 3 key non-β-lactam antimicrobial agents (erythromycin, clindamycin, and ciprofloxacin) was significantly higher in the group of CA-MRSA patients without coexisting factors than in the other 2 groups. No statistical difference in the susceptibility to erythromycin, clindamycin, or ciprofloxacin existed between HA-MRSA and CA-MRSA groups with coexisting factors (data not shown).

MRSA cases in which the isolate was resistant to both clindamycin and ciprofloxacin (n = 142) were compared to cases in which both antimicrobial agents were susceptible (n = 32) (Table 4). The proportion of patients with skin/soft tissue infections in the susceptible group (81% vs. 52%, p = 0.003) increased significantly. Most of the cases in the susceptible group were community-associated (75% vs. 27%, p<0.0001), and the mean age of the susceptible group was significantly lower (32 vs. 69, p = 0.0001).

Comparison of MRSA and VRE Incidence Rates

Incidence rates of VRE and MRSA infections, particularly of HA-MRSA, varied substantially across institutions (Figure). Rates of CA-MRSA correlated strongly with HA-MRSA rates, regardless of whether CA-MRSA rates were denominated by community or county population size (Table 5). The rate of HA-MRSA in hospitals belonging to the third quintile of CA-MRSA rates was 11-fold higher than in hospitals with no CA-MRSA (first and second quintiles). The rate of HA-MRSA in hospitals belonging to the fourth and fifth quintiles of CA-MRSA rates was >30-fold higher than in hospitals with no CA-MRSA. This association was independent of hospital bed size.

MRSA incidence was also examined in relation to proximity to Native American reservations. HA-MRSA and CA-MRSA incidence rates at the 7 communities that were in proximity to Native American reservations were comparable to other communities. Only 1 of these 7 had CA-MRSA rates in the highest quintile.

Discussion

Much of our current understanding of MRSA and VRE in rural communities comes from reports of outbreaks or smaller case series (15,16,19,23,25–28). In this study, epidemiologic data on MRSA and VRE cases were collected from a large number of rural hospitals in Idaho and Utah during a 15-month period. VRE incidence was low in all but 3 institutions. The overall incidence of MRSA infection was substantially greater and varied widely across

Table 3. Comparison of antimicrobial susceptibilities by category

Agent	No. susceptible (%) HA-MRSA	CA-MRSA with CFs	CA-MRSA without CFs	p value†
Oxacillin	0/204 (0)	0/34 (0)	0/106 (0)	NS
Erythromycin	10/193 (5)	3/34 (9)	16/98 (16)	0.007
Clindamycin	42/195 (22)	11/34 (32)	61/100 (61)	<0.0001
Ciprofloxacin	9/138 (7)	2/19 (11)	23/73 (32)	<0.0001
Gentamicin	196/204 (96)	33/34 (97)	99/105 (94)	NS
Trimethoprim-sulfamethoxazole	202/203 (99)	34/34 (100)	102/106 (96)	NS
Rifampin	157/166 (95)	23/24 (96)	79/82 (96)	NS
Tetracycline	183/190 (96)	25/27 (93)	93/101 (92)	NS
Vancomycin	203/203 (100)	33/33 (100)	105/106 (99)‡	NS

*HA, healthcare-associated; MRSA, methicillin-resistant *Staphylococcus aureus*; CA, community-associated; CFs, coexisting factors; NS, not significant.
†Based on Fisher exact test.
‡One isolate reported as vancomycin resistant. Isolate not available for confirmatory testing.

Table 4. Comparison of MRSA cases with resistant and susceptible phenotypes*

	No. (%)		
	Resistant group†, N = 142	Susceptible group‡, N = 32	p value§
Skin and soft tissue	74 (52)	26 (81)	0.003
Urine	15 (11)	0 (0)	NS
Blood	9 (6)	1 (3)	NS
Sputum	30 (21)	1 (3)	0.019
Other source	14 (10)	4 (13)	NS
Community-associated	38 (27)	24 (75)	<0.0001
Sex			
Male	79 (56)	17 (53)	–
Female	63 (44)	15 (47)	–
Mean age (y)	69	32	0.0001
Age >65 y	89 (63)	3 (9)	<0.0001
Diabetes mellitus	38 (27)	1 (3)	0.002
Renal failure	13 (9)	0 (0)	NS
Prior antimicrobial therapy	61 (43)	6 (19)	0.015
Immunosuppression	14 (10)	0 (0)	NS

*MRSA, methicillin-resistant *Staphylococcus aureus*; NS, not significant.
†MRSA isolates resistant to both clindamycin and ciprofloxacin.
‡MRSA isolates susceptible to both clindamycin and ciprofloxacin.
§Based on Fisher exact test. Comparison of age tested by Kruskal-Wallis equality of populations rank test.

different institutions. Rates of HA-MRSA were significantly higher in communities classified as having a high incidence of CA-MRSA. The incidence rates of HA-MRSA in communities that did report cases were in a range comparable to reports from hospitals participating in the CDC NNIS system (HA-MRSA incidence rates from NNIS hospitals ranged from 12.6 to 19.5 per 10,000 patient days) (T. Horan and J. Edwards, pers. comm.). The lack of reporting of MRSA and VRE from many rural hospitals suggests that these resistance types may still be infrequent in some locations. However, confirming this finding by performing active surveillance cultures to determine whether the prevalence of carriage is correspondingly rare in communities without clinical cases would be useful.

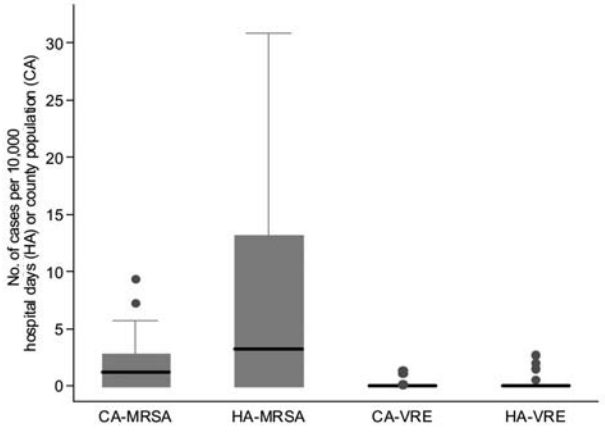

Figure. Box plot of incidence rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcal (VRE) infections. CA, community-associated; HA, healthcare-associated.

Forty-four percent of the MRSA cases met epidemiologic criteria for being community-associated. These cases were comparable to CA-MRSA cases reported by other investigators with respect to the infrequency of coexisting factors, the predominance of skin and soft tissue infection, and the increased susceptibility to other antimicrobial drug classes (12–18). Some cases in the CA-MRSA group without coexisting factors had a more resistant phenotype, which suggests that, even in these patients without obvious risk factors, healthcare exposure to MRSA occurred, or descendants of hospital strains of MRSA were available in the community for transmission. The subset of CA-MRSA cases with coexisting factors had characteristics similar to cases meeting the epidemiologic criteria for healthcare acquisition. Transmission of healthcare-associated strains in these cases may have occurred during contact in ambulatory rather than hospital settings. These hypotheses are supported by the work of other investigators (18,32–34). In 1 study, molecular analysis of CA-MRSA isolates in a nonoutbreak setting demonstrated that the hospital was the main source of community MRSA (33).

We found that the rate of hospital-associated MRSA was significantly greater in communities classified as having a high incidence of CA-MRSA. Several plausible reasons for this association exist. One possibility is that the sensitivity of laboratory detection and reporting was better in communities that had increased rates of both CA- and HA-MRSA. Another potential explanation is that CA- and HA-MRSA cases are dynamically interdependent (35). CA-MRSA cases may contribute to nosocomial dissemination of MRSA within hospitals because of increased prevalence of MRSA carriage at the time of admission, followed by transmission to other hospitalized patients (36,37).

Table 5. Random effects Poisson regression model for HA-MRSA* rate†

Predictors	No. of institutions	Incidence rate ratio	95% CI	p value
Quintile of CA-MRSA rate‡				
1st–2nd: 0	11	Reference		
3rd: 0.3 to 1.6	6	11	2.8–4.5	0.001
4th: 1.7 to 3.4	6	35	10–122	<0.0001
5th: 3.5 to 9.4	5	33	9.2–115	<0.0001
Hospital bed size				
13–25	10	Reference		
26–50	9	0.8	0.3–2.0	0.596
51–235	9	1.3	0.6–3.0	0.530
State				
Idaho	17	Reference		
Utah	11	2.3	1.3–4.2	0.005

*HA-MRSA, heathcare-associated methicillin resistant *Staphylococcus aureus*; 95% CI, 95% confidence ratio; CA-MRSA, community-associated MRSA.
† No. of HA-MRSA cases/10,000 occupied bed-days.
‡ No. of CA-MRSA cases/10,000 person-years, based on county population.

Increased HA-MRSA incidence may in turn foster dissemination of MRSA in community populations.

A smaller but substantial proportion of the VRE cases (19%) also met criteria for community association, without other risk factors. VRE transmission from farm animals to humans has been reported in Europe, and community transmission has been suggested as a possible but yet undocumented mechanism in the United States (7,9).

This study has several limitations. Clinical microbiology laboratories, particularly those in rural hospitals, may have difficulty detecting MRSA, VRE, and other resistant organisms (38–40). We did not directly evaluate proficiency testing in the current study but have examined this issue in prior investigations. Laboratory practices in rural hospitals in Idaho and Utah were examined by survey in July 2000 (40). Five institutions in the current study that had inadequate MRSA confirmation procedures according to the survey conducted in 2000 had rates of HA-MRSA that were comparable to institutions with adequate procedures for confirmation of MRSA. In a follow-up study, laboratory proficiency was assessed by distribution of unknown specimens for blinded testing to a subset of 28 facilities in Idaho and Utah (K.B. Stevenson et al., unpub. data). Reporting of interpretative category for MRSA and VRE was correct in 100% and 61.5% of hospitals, respectively. These results highlight the potential for problems in microbiology proficiency to contribute to either underdetection or overdetection of resistant organisms.

Another potential drawback of this study was that original records such as hospital charts were not independently reviewed to assess the reliability of epidemiologic data collection by local infection control practitioners. Our use of an explicit data collection protocol and data dictionary was designed to mitigate this limitation. Similar methods of medical record review have been used successfully in other studies of CA-MRSA (15,16).

This study focused on patients with clinical infection. Because serial surveillance cultures were not obtained, the timing or location of VRE or MRSA acquisition could not be precisely determined. Surveillance cultures also identify patients with clinically unrecognized carriage of resistant organisms. MRSA isolates were not collected prospectively during the study period, which limited our ability to confirm antimicrobial drug–susceptibility patterns and perform molecular analysis. Examining the clonal patterns of isolates by pulsed-field gel electrophoresis and determining the type of SCC*mecA* would have been useful for supporting the interpretations of the epidemiologic analysis and overcoming the recognized limitations. Molecular analyses of MRSA isolates that have been recently collected from these rural communities are planned.

Finally, the number of persons corresponding to the source population for CA-MRSA and CA-VRE cases could not be precisely determined. Therefore, we could not derive reliable estimates of CA-MRSA and CA-VRE infection rates for purposes of comparison with rates from other geographic locations.

In summary, infection control practitioners and clinicians working in rural areas are likely to confront problems of hospital- and community-associated MRSA infection. In some rural areas, the MRSA incidence approaches or exceeds what has been reported from larger hospitals in urban areas. The role of more aggressive prevention strategies, such as active surveillance culturing, in these rural healthcare settings is still uncertain. Further studies of the epidemiologic factors that influence MRSA and VRE transmission and of infection control interventions in rural communities are needed.

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Clinician Knowledge and Beliefs after Statewide Program to Promote Appropriate Antimicrobial Drug Use

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In 1999, Wisconsin initiated an educational campaign for primary care clinicians and the public to promote judicious antimicrobial drug use. We evaluated its impact on clinician knowledge and beliefs; Minnesota served as a control state. Results of pre- (1999) and post- (2002) campaign questionnaires indicated that Wisconsin clinicians perceived a significant decline in the proportion of patients requesting antimicrobial drugs (50% in 1999 to 30% in 2002; p<0.001) and in antimicrobial drug requests from parents for children (25% in 1999 to 20% in 2002; p = 0.004). Wisconsin clinicians were less influenced by nonpredictive clinical findings (purulent nasal discharge [p = 0.044], productive cough [p = 0.010]) in terms of antimicrobial drug prescribing. In 2002, clinicians from both states were less likely to recommend antimicrobial agent treatment for the adult case scenarios of viral respiratory illness. For the comparable pediatric case scenarios, only Wisconsin clinicians improved significantly from 1999 to 2002. Although clinicians in both states improved on several survey responses, greater overall improvement occurred in Wisconsin.

In the United States, a substantial proportion of antimicrobial agents are prescribed for acute respiratory infections, including colds, upper respiratory infections (URIs), acute bronchitis, pharyngitis, sinusitis, and otitis media (1–9). Many of these illnesses are viral, and antimicrobial agents offer no benefit. However, widespread and inappropriate use of antimicrobial agents for viral illnesses has contributed to the emergence of infections caused by antimicrobial drug-resistant organisms such as *Streptococcus pneumoniae* (10–12). The proportion of invasive infections caused by penicillin-nonsusceptible *S. pneumoniae* increased nationally from 1% in 1992 to 27% in 2000 (10). Multidrug resistance has also occurred with increasing frequency: the proportion of *S. pneumoniae* isolates nonsusceptible to ≥3 classes of antimicrobial drugs increased from 7% in 1995 to 19% in 2000 (10). Multiple studies have shown a strong and consistent association between recent antimicrobial drug use and infection with a drug-resistant strain of pneumococcus (13–18). More recently, rapidly increasing rates of fluoroquinolone use have also been implicated in the emergence of fluoroquinolone-resistant pneumococcal infections (19–22). The increase in antimicrobial drug–resistant infections has economic as well as clinical implications; the annual cost of unnecessary antimicrobial drug prescribing for acute respiratory infections has been estimated to be ≈$726 million (5).

Throughout the previous decade, multiple interventions aimed at patients and clinicians have been implemented to promote appropriate antimicrobial drug use and prevent the development of antimicrobial resistance. In Wisconsin, a multifaceted educational campaign focusing on clinicians and the public was launched in late 1999 by the Wisconsin Antibiotic Resistance Network (WARN). Clinician education included presentations at professional meetings, conferences, and grand rounds; continuing medical education satellite conferences; distribution of slide presentations on CD-ROM; and multiple mailings of educational materials to all primary care clinicians. The public education component consisted of multilingual brochures and posters, tear-off sheets, coloring sheets, stickers, magnets, and handouts. These items were distributed statewide to clinics, managed care organizations, pharmacies, childcare facilities, and community groups. Mass media activities included radio advertisements statewide and paid television advertisements in selected

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markets. A more detailed account of WARN campaign activities is provided in the accompanying article (23). The purpose of this study was to assess the impact of the WARN campaign on the knowledge, beliefs, and decision-making of Wisconsin primary care clinicians regarding appropriate antimicrobial drug use for upper respiratory infections.

Methods

Design and Study Population

The study consisted of serial cross-sectional surveys in 2 states with pre- and postintervention measurements. Minnesota served as a control state to distinguish intervention-related changes from the regional secular trend. Minnesota was selected for geographic proximity and similarity in terms of population size and racial/ethnic distribution. Before 2002, educational activities on appropriate antimicrobial drug use were limited in Minnesota. Approval for this study was obtained from the institutional review board of CDC.

Eligible participants for the survey included physicians, nurse practitioners, and physician assistants. Practice specialties for physicians and physician assistants included family practice, pediatrics, internal medicine, emergency medicine, and general practice. Specialties for nurse practitioners included family practice and pediatrics. In 1999 and 2002, independent random samples were selected from Wisconsin and Minnesota licensing databases. The 1999 sampling frame was 7,113 in Wisconsin and 6,335 in Minnesota; the 2002 sampling frame was 6,218 in Wisconsin and 5,800 in Minnesota. The survey sample included 400 Wisconsin and 400 Minnesota clinicians in 1999, and 600 Wisconsin and 400 Minnesota clinicians in 2002. The baseline sample size was selected to provide >80% power to detect a 15% increase in the proportion of clinicians giving the correct or desired response to a specific survey question ($\alpha = 0.05$). Wisconsin clinicians were oversampled in 2002 to facilitate a within-state analysis of the impact of a television advertising campaign (not reported here). The probability of the same clinician being sampled in both 1999 and 2002 was low, and the samples were considered independent in the analyses.

Questionnaire

The preintervention questionnaire was mailed to Wisconsin clinicians in April 1999 and to Minnesota clinicians in November 1999. During March–May 2002, the postintervention questionnaire was mailed to clinicians in both states. The questionnaires contained a cover letter explaining the purpose of the survey; 2 follow-up reminders were sent to maximize compliance. The preintervention and postintervention questionnaires were identical in their measures of knowledge, beliefs, and decision making and differed only in the addition of questions to the preintervention questionnaire regarding clinician opinion for effective campaign materials (for planning purposes) and the addition of questions to Wisconsin's postintervention questionnaire about the television advertisements.

After determining practice setting and basic demographics, clinicians caring for adults were asked to estimate the proportion of adult patients who requested antimicrobial agents for cough, cold, or flulike symptoms. Likewise, those caring for children were asked to estimate the proportion of parents who requested antimicrobial agents for their child. The survey questionnaire (Figure 1) then asked a series of questions to assess 1) the influence of 2 nonpredictive clinical factors (i.e., clinical symptoms or signs characteristic of both viral and bacterial infections, which therefore did not necessarily warrant antimicrobial drug therapy) and 1 social factor on the decision to prescribe antimicrobial drugs; 2) the likelihood of antimicrobial agent prescribing in adult and pediatric clinical case scenarios for URIs and bronchitis; and 3) perceptions and beliefs regarding patient expectations and peer-established norms. In addition, questions regarding exposure to and perceived impact of the WARN campaign were asked on the postintervention questionnaire (Wisconsin clinicians only). For most questions, the responses were based on a 5-point Likert-scale (e.g., "strongly disagree to strongly agree"). The Likert responses were dichotomized into desired and undesired responses (Figure 1); responses were classified as "desired" if they were consistent with national pediatric and adult clinical practice guidelines or

Question	*Likert-scale Response Options*	*Desired Response*
Knowledge-based questions: clinical and social factors influencing decision to prescribe antibiotics		
How much does each of the following factors increase the likelihood that you will prescribe an antibiotic for an acute respiratory infection when the etiologic agent (viral versus bacterial) is uncertain?	1 (no influence) to 5 (major influence)	"No" or "little" influence on prescribing (1 or 2)
1. Purulent nasal discharge		
2. Productive cough with purulent sputum		
3. Patient/parent states that he/she received an antibiotic for similar symptoms in the past		
Clinical case scenarios		
Likely to prescribe antibiotics for	1 (definitely prescribe) to 5 (definitely not prescribe)	No antibiotic prescription (4 or 5)
1. Adult/child with acute bronchitis × 5 days, no fever, normal lung examination		
2. Adult/child with purulent nasal discharge unresolved after 5 days, no fever, normal lung exam		
Beliefs and attitudes		
1. Most of my patients think I should prescribe antibiotics for cough, cold, or flu-like symptoms	1 (strongly agree) to 5 (strongly disagree)	"Disagree" or "strongly disagree" (4 or 5) to statement
2. It is hard for me to withhold antibiotics for cough, cold, or flulike symptoms because other clinicians in my community prescribe antibiotics for these illnesses.		

Figure 1. Representation of survey items assessed in 1999 and 2002 among Wisconsin and Minnesota clinicians.

the educational goals of the WARN campaign. The influence of social factors, patient expectations, and peer-established norms on clinical decision-making was considered "undesired" since each was an inappropriate reason for antimicrobial drug prescribing.

Statistical Analysis

For the clinician-reported estimates of the percentage of patients or parents who requested an antimicrobial drug, we compared the distribution of responses for each year and state. We used the 1-sided Jonckheere-Terpstra test, a generalization of the nonparametric Mann-Whitney U test, to compare within-state distributions for 1999 and 2002. The null hypothesis was that the distributions did not differ between these 2 periods.

For each Likert-scale question, we calculated a ratio based on the proportion of clinicians with a desired response in 2002 (numerator) divided by the proportion with a desired response in 1999 (denominator). A ratio >1.0 indicated improvement in 2002 versus 1999. After calculating this ratio for each state, we compared the ratios between Wisconsin and Minnesota. All ratios were adjusted for clinician sex, years in practice, practice setting, and clinician type. All adjusted ratios and corresponding statistical test results were obtained directly from multivariable models similar to logistic regression models but with a log (rather than logit) link function (24). Such models permit comparison of proportions rather than odds. The models included terms for state, year, their interaction, and the control variables (clinician sex, years in practice, practice setting, and clinician type). Examining appropriate combinations of the estimated parameters from these models permitted within-year comparisons of Wisconsin versus Minnesota (e.g., baseline comparisons), within-state comparisons of 2002 versus 1999, and between-state comparisons of within-state ratios (i.e., comparisons of the 2002/1999 ratios for Wisconsin to those in Minnesota for estimating the effect in Wisconsin beyond that observed in Minnesota).

Ten percent of questionnaire responses were entered in duplicate for quality assurance. Statistical analyses were performed by using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA) and EpiInfo 6 (CDC, Atlanta, GA, USA).

Results

The survey response rates ranged from 65% to 71%. Most respondents were physicians (Table 1). The most common practice specialty was family practice. The sex distribution and years in practice in each group did not differ by state or year of survey.

Baseline Survey

Baseline responses were compared for Wisconsin and Minnesota in 1999, before initiation of the WARN campaign in Wisconsin. Clinicians in Wisconsin and Minnesota perceived similar proportions of their adult patients requesting antimicrobial agents ($p = 0.217$) (Figure 2). The median percentage of patients perceived to request antimicrobial agents was 50% in Wisconsin and 40% in Minnesota; this difference was not significant. The perceived demand by parents for antimicrobial agents to treat their child's respiratory illness was also similar between the 2 states ($p = 0.473$) (Figure 3); the median reported percentage of parents requesting antimicrobial agents was 25% in both states.

Clinicians in Wisconsin and Minnesota gave similar baseline responses regarding the influence of a social factor (e.g., patient states antimicrobial agents given for similar symptoms in the past) and a nonpredictive clinical factor (e.g., purulent nasal discharge) (Table 2, baseline p values not presented). However, Minnesota clinicians were significantly more likely to report that productive cough with purulent sputum would not influence their decision to prescribe an antimicrobial agent (Wisconsin 14%, Minnesota 20%, $p = 0.027$). For the bronchitis and viral URI case scenarios, the overall proportion of clinicians who would withhold antimicrobial agents was similar in each state. The proportion who would withhold antimicrobial agents was greater for the pediatric case scenarios than

Table 1. Response rate and respondent characteristics, Wisconsin and Minnesota clinicians, 1999 and 2002*

	Wisconsin		Minnesota	
Characteristic	1999	2002	1999	2002
Response rate (%)	71	65	69	70
Practice setting (%)				
ER/urgent care	11	22	13	14
Family practice	46	42	49	48
Pediatrics	17	13	12	16
Internal medicine	18	16	17	14
Other	8	7	9	8
Physician (%)	73	72	74	78
Male (%)	61	51	55	53
Mean y in practice	12.6	12.7	13.2	13.8

*ER, emergency room.

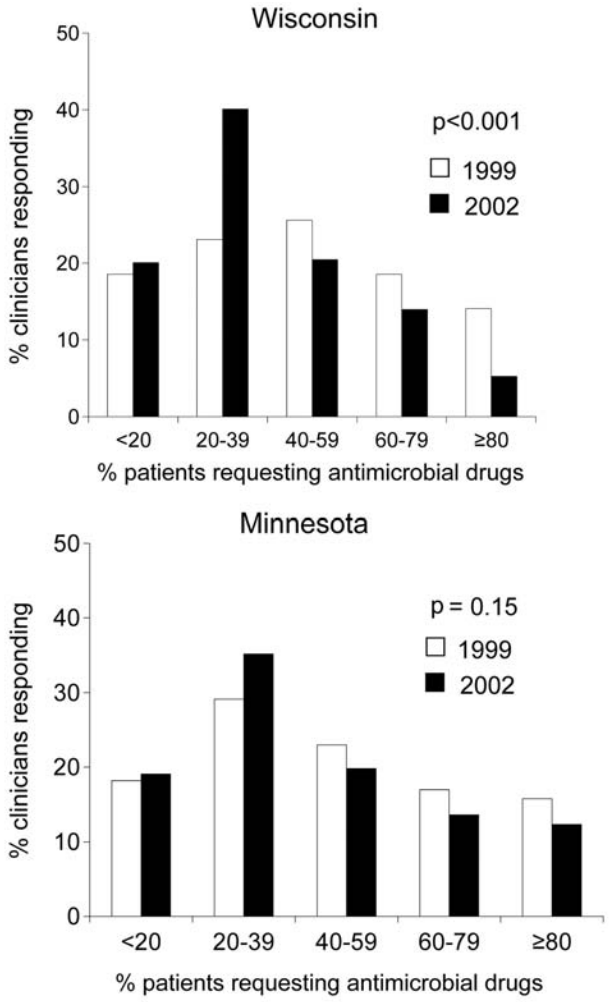

Figure 2. Proportion of clinicians reporting various estimates of the percentage of their adult patients who requested an antimicrobial agent for cough, cold, or flulike symptoms in 1999 and 2002.

for the adult case scenarios (Table 3). Responses to the belief questions regarding patient expectations and clinician peer norms were similar between the states during the baseline period (Table 4).

We also compared baseline responses between states for clinicians in practice ≤10 years and those in practice >10 years. For those in practice ≤10 years, a higher proportion of Minnesota clinicians indicated that their decision to use antimicrobial agents was not influenced by purulent nasal discharge (Wisconsin 36%, Minnesota 51%, p = 0.024) or cough with productive sputum (Wisconsin 12%, Minnesota 25%, p = 0.005). Responses to the influence of the social factor and responses to the adult and pediatric case scenarios were similar between the states. For clinicians in practice >10 years, responses were similar between the 2 states regarding the influence of nonpredictive clinical factors and the social factor. Compared to Wisconsin clinicians practicing >10 years, a higher proportion of Minnesota clinicians in long-term practice indicated they would withhold antimicrobial agents in the pediatric case scenarios (p = 0.048) and the adult case scenarios (p = 0.118).

Within Wisconsin, some baseline responses differed according to length of time in practice. A significantly higher proportion of clinicians practicing ≤10 years gave the desired responses for the pediatric (p = 0.027) and adult (p = 0.002) case scenarios. They were also more likely to give the desired response regarding the influence of a social factor (i.e., patient states antimicrobial agents were given for similar symptoms in the past) (p = 0.043). Wisconsin clinicians in practice ≤10 years and those in practice >10 years gave similar responses regarding the influence of the nonpredictive clinical factors (i.e., purulent nasal discharge and productive cough).

When specialties were compared, a higher proportion of pediatric clinicians gave desired responses than clinicians in other specialties on most outcome measure in both Wisconsin and Minnesota. Baseline comparisons between physicians and nonphysicians did not show a consistent tendency for 1 group to perform better than the other.

Follow-up Survey

In 2002, Wisconsin clinicians perceived less demand for antimicrobial agents among adult patients compared with 1999 (p<0.001) (Figure 2). Based on clinician estimates, the median percentage of patients who requested an antimicrobial agent for cough, cold, or flu symptoms decreased from 50% in 1999 to 30% in 2002. Minnesota clinicians

Table 2. Influence of 2 nonpredictive clinical factors and 1 social factor on antimicrobial agent prescribing, 2002 versus 1999

	Proportion giving desired response (%)*					
	Wisconsin (WI)		Minnesota (MN)		WI % 2002/WI % 1999 (adjusted)†‡	MN % 2002/MN % 1999 (adjusted)†‡
Response	1999	2002	1999	2002		
Purulent nasal discharge	34	61	42	54	1.71 (p<0.001)	1.24 (p = 0.054)
Productive cough with purulent sputum	14	36	20	31	2.61 (p<0.001)	1.31
Received antimicrobial agents for similar symptoms in past	57	72	63	70	1.20 (p = 0.015)	1.10

*Desired response: presence of the factor had little or no influence on the decision to prescribe.
†Only significant p values presented; p values for baseline comparisons not presented.
‡Ratios and corresponding p values adjusted for sex, years in practice, practice setting, and clinician type.

Table 3. Responses to clinical case scenarios for viral upper respiratory infection and bronchitis, 2002 versus 1999

	Proportion giving desired responses for both scenarios (%)*					
	Wisconsin (WI)		Minnesota (MN)		WI% 2002/WI%1999	MN% 2002/MN%
Response	1999	2002	1999	2002	(adjusted)†‡	1999 (adjusted)†‡
Adult case scenarios	43	64	46	59	1.45 (p = 0.001)	1.28 (p = 0.023)
Pediatric case scenarios	62	74	66	68	1.16 (p = 0.058)	0.98

*Desired response to all scenarios—not prescribe or definitely not prescribe.
†Only significant p values presented; p values for baseline comparisons not presented.
‡Ratios and corresponding p values adjusted for gender, years in practice, practice setting, and clinician type.

also perceived a decrease in the percentage of patients who requested antimicrobial agents, but the difference was not significant (p = 0.152) (Figure 2); the median percentage of Minnesota patients requesting antimicrobial agents decreased from 40% in 1999 to 30% in 2002.

In both states, a decline was noted in the perceived parental demand for antimicrobial agents to treat pediatric respiratory illness (Figure 3). The temporal change was significant in Wisconsin (p = 0.004) and approaching significance in Minnesota (p = 0.064). The median reported percentage of parents who requested an antimicrobial agent decreased from 25% in 1999 to 20% in 2002 in both states, but the distribution around the medians differed significantly between the states.

In Wisconsin, significant improvement occurred in the responses to the 2 questions about nonpredictive clinical factors and the social factor that may increase the likelihood of prescribing antimicrobial agents (i.e., purulent nasal discharge, productive cough, and patient or parent statement that antimicrobial agents were prescribed for similar symptoms in the past) (Table 2). Wisconsin clinicians were significantly more likely to report that each factor did not influence antimicrobial agent prescribing practices in 2002 compared with 1999. In Minnesota, a significant improvement occurred in responses regarding the influence of purulent nasal discharge, but no significant change occurred for the other 2 factors. Overall, Wisconsin clinicians demonstrated significant improvement regarding the influence of purulent nasal discharge (p = 0.044) and productive cough (p = 0.010) after accounting for temporal changes in Minnesota.

Both Minnesota and Wisconsin clinicians improved in their responses to the adult case scenarios for URI and bronchitis (Table 3). The magnitude of improvement was greater for Wisconsin clinicians, but the improvement in Wisconsin was not significant after accounting for the secular trend in Minnesota. In the pediatric case scenarios, Wisconsin clinicians improved from 1999 to 2002 (p = 0.058), while the responses of Minnesota clinicians were essentially unchanged (p = 0.807).

Wisconsin clinicians demonstrated a modest improvement from 1999 to 2002 in response to questions concerning perceived clinician peer norms and patient expectations, but the changes in Wisconsin were not significant after accounting for temporal changes in Minnesota (p = 0.103 and 0.519, respectively, Table 4).

Subgroup Analysis

Responses were analyzed separately for clinicians who had practiced >10 years (1999, n = 198; 2002, n = 243) and those in practice ≤10 years (1999, n = 187; 2002, n = 239). In Wisconsin, clinicians who were in practice for the longer period demonstrated significant improvements regarding the likelihood of prescribing antimicrobial agents for purulent nasal discharge (2002 to 1999 ratio = 1.61, p = 0.005) and productive cough (2002 to 1999 ratio = 2.35, p = 0.001). They also improved in their responses to the influence of patient/parent statement that antimicrobial agents were prescribed for similar symptoms in the past (2002 to 1999 ratio = 1.36, p = 0.012). Wisconsin clinicians practicing >10 years also demonstrated significant improvements in the adult case scenarios (2002 to 1999 ratio = 2.00, p<0.001) and the pediatric case scenarios (2002 to 1999 ratio = 1.43, p = 0.002)] and in the questions concerning patient expectations and peer norms. The 2002 to 1999 ratio was 1.40 (p = 0.031) for patient expectations

Table 4. Perceptions and beliefs regarding patient expectations and peer-established norms, 2002 versus 1999

	Proportion giving desired response (%)†					
	Wisconsin (WI)		Minnesota (MN)		WI % 2002/ WI % 1999	MN % 2002/ MN % in 1999
Belief or attitude*	1999	2002	1999	2002	(adjusted)‡§	(adjusted)‡§
Most of my patients think I should prescribe for cough, cold, or flulike symptoms.	36	42	42	48	1.19	1.07
It is hard for me to withhold antibiotics because other clinicians in my community prescribe them for cough, cold, or flulike illness.	62	71	64	64	1.18 (p = 0.014)	1.00

*See Figure 1 for complete text of each statement.
†Desired response—disagree or strongly disagree.
‡Only significant p values presented; p values for baseline comparisons not presented.
§Ratios and corresponding p values adjusted for sex, years in practice, practice setting, and clinician type.

and 1.28 (p = 0.021) for peer norms. However, only the responses to the pediatric case scenarios improved significantly among physicians in practice >10 years (p = 0.027) after accounting for the secular trend in Minnesota.

Wisconsin clinicians in practice ≤10 years improved in fewer areas. They improved significantly in responses regarding the influence of purulent nasal discharge (p<0.001) and productive cough (p<0.001) on antimicrobial agent prescribing practices, and both factors remained significant after accounting for the secular trend. No significant change occurred in the other responses. A direct comparison between Wisconsin clinicians practicing ≤10 years to those practicing >10 years demonstrated no significant difference with regard to improvement in knowledge or the response to the clinical scenarios.

Familiarity with WARN and WARN Materials

Ninety percent of primary care clinicians in Wisconsin had heard of WARN. Of those, 70% had used WARN patient education materials. Of those using the materials, 41% reported that they were "very useful," and 59% said that they were "somewhat useful."

Discussion

The results of this study demonstrated significant improvement among primary care clinicians in multiple outcome measures after a multifaceted educational campaign to promote appropriate antimicrobial drug use in Wisconsin was implemented. From 1999 to 2002, clinicians perceived less patient or parent demand for antimicrobial agents and were less likely to report that antimicrobial agent prescribing was influenced by social and nonpredictive clinical factors. Clinicians demonstrated improved decision-making in adult and pediatric case scenarios for URIs and bronchitis and perceived less pressure from patients and peers to prescribe. Minnesota clinicians also demonstrated improvement in some of these factors, but the magnitude of improvement was consistently greater among Wisconsin clinicians, and the improvements in several of these factors in Wisconsin remained significant even after the secular trend in Minnesota was accounted for.

The greater improvements in responses from Wisconsin clinicians over time compared with Minnesota clinicians suggest that the WARN program had a positive effect on clinician knowledge and beliefs. This effect is supported by the observations that among Wisconsin clinicians, a high level of recognition and acceptance of WARN was achieved, and that from 2000 through 2002, the use of WARN educational materials was widespread.

The WARN campaign was initiated in 1999 as a large-scale demonstration project designed to promote appropriate antimicrobial agent use for outpatient respiratory illness. It was the largest of its kind in the United States and the first to evaluate whether prescribing practices could be improved for an entire state. At the time the project was initiated, clinicians perceived a high demand for antimicrobial agents and displayed prominent gaps in knowledge regarding outpatient antimicrobial agent use for URIs and bronchitis (4,25–28). At the same time, knowledge of appropriate antimicrobial agent use was limited among much of the general public (27–30).

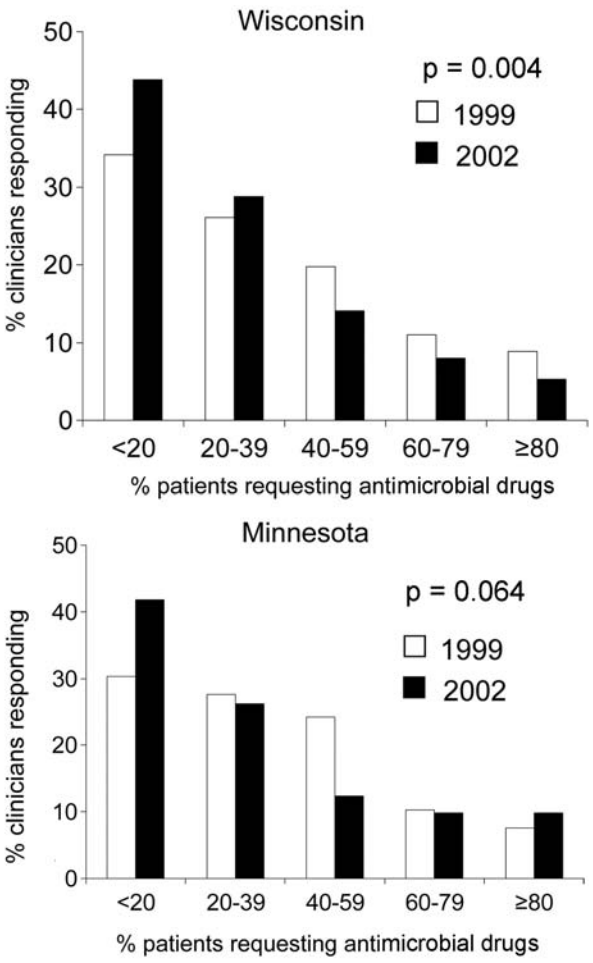

Figure 3. Proportion of clinicians reporting various estimates of the percentage of parents of their pediatric patients who requested antimicrobial drugs for their child's cough, cold, or flulike symptoms in 1999 and 2002.

The results of this study are consistent with those of other studies demonstrating the impact of multifaceted educational efforts that specifically focused on physicians, patients, or the general public. Campaigns that focused on parents, using videotaped presentations in pediatric waiting rooms, showed modest to significant improvement in parental knowledge and attitudes about appropriate antimicrobial agent use (31,32), but in-service reviews of judicious antimicrobial agent use guidelines for clinicians had no effect on antimicrobial agent prescribing rates (32).

Intensive education of both the clinicians and the community has led to significant decreases in antimicrobial agent prescription rates, as shown by studies in Knox County, Tennessee (33), eastern Massachusetts, northwest Washington state (34), the Denver metropolitan area (35), northern Wisconsin communities (36), and rural Alaskan villages (37). Only the Tennessee study addressed a large general population, whereas the other studies focused on rural communities or managed care populations.

In subgroup analysis, we found the greatest improvements among Wisconsin clinicians who had been in practice for >10 years. Although these improvements coincided with the secular trend observed in Minnesota, they demonstrate that this group of physicians should be targeted for further education. One potential explanation for why clinicians who were trained more recently showed fewer improvements is that they might already have a greater awareness of issues regarding increasing antimicrobial drug resistance and were trained more rigorously in the principles of judicious antimicrobial drug use. This hypothesis was supported by the baseline assessment, which showed that clinicians practicing ≤10 years performed better on the clinical case scenarios. These results parallel other findings that clinicians who are temporally further away from medical training programs prescribe antimicrobial drugs more frequently (38), although this finding has not been consistently demonstrated (4,26).

This study did not include objective measures of antimicrobial drug prescribing. Prior studies have shown that changes in knowledge and attitudes do not necessarily translate into changed in clinical practice (4,25). The medical culture surrounding antimicrobial drug prescribing in the United States is influenced by multiple external factors (e.g., peer practices, pharmaceutical detailing, geographic region, and managed care restrictions), and these may influence practice more than knowledge of current guidelines. The accompanying study by Belongia et al. addresses the impact of WARN on antimicrobial drug prescribing rates in Wisconsin relative to those of Minnesota (23).

A limitation of this study was the lack of statistical power to detect modest improvements after accounting for the secular trend in Minnesota. The magnitude of improvement in Wisconsin consistently exceeded that in Minnesota, but the difference was often deemed statistically insignificant. A larger sample size may have provided additional power to distinguish between these smaller differences. In addition, a higher proportion of Minnesota clinicians gave the correct or desired response to several of the baseline survey items compared with Wisconsin clinicians, although many of these differences were not statistically significant. Minnesota clinicians may have had less room to improve and might have already been more familiar with recommendations regarding judicious antimicrobial drugs use; therefore, Minnesota might not have been wholly optimal as a control state. Additionally, Minnesota clinicians and public along the Wisconsin-Minnesota border may have been exposed to WARN materials and advertisements. An added limitation is that this study included only 2 states. If substantially more resources had been available, a controlled, multistate intervention study would have provided more robust and generalizable results. A larger study may no longer be feasible, given the success of current campaigns in promoting awareness on a national level. The efficacy of large multifaceted interventional campaigns will be difficult to evaluate because unexposed populations no longer exist.

In conclusion, this study suggests that the WARN campaign had at least a modest positive effect on the knowledge and decision-making of primary care clinicians in Wisconsin. Clinicians in practice >10 years demonstrated the greatest improvements and may benefit most from educational interventions. Further research should include the development and evaluation of interventions to improve antimicrobial agent selection (narrow-spectrum versus broad-spectrum) and an assessment of new clinical strategies to optimize antimicrobial agent usage (e.g., a 72-hour waiting period for selected patients with mild acute otitis media) (39,40). The documented success of these smaller campaigns in changing the medical culture surrounding antimicrobial drug prescribing has prompted its expansion to the national level. A national public education campaign was launched by CDC in September 2003 to further generate provider and public awareness of these issues and to curb the inappropriate use of antimicrobial drugs.

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Impact of Statewide Program To Promote Appropriate Antimicrobial Drug Use

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The Wisconsin Antibiotic Resistance Network (WARN) was launched in 1999 to educate physicians and the public about judicious antimicrobial drug use. Public education included radio and television advertisements, posters, pamphlets, and presentations at childcare centers. Physician education included mailings, susceptibility reports, practice guidelines, satellite conferences, and presentations. We analyzed antimicrobial prescribing data for primary care physicians in Wisconsin and Minnesota (control state). Antimicrobial prescribing declined 19.8% in Minnesota and 20.4% in Wisconsin from 1998 to 2003. Prescribing by internists declined significantly more in Wisconsin than Minnesota, but the opposite was true for pediatricians. We conclude that the secular trend of declining antimicrobial drug use continued through 2003, but a large-scale educational program did not generate greater reductions in Wisconsin despite improved knowledge. State and local organizations should consider a balanced approach that includes limited statewide educational activities with increasing emphasis on local, provider-level interventions and policy development to promote careful antimicrobial drug use.

Antimicrobial drug–resistant strains of community-acquired pathogens, including *Streptococcus pneumoniae* and *Staphylococcus aureus*, have emerged as serious global health threats (1–3). Multiple studies have demonstrated a strong and consistent link between antimicrobial drug use and antimicrobial resistance at both individual and population levels (4–9). Despite this link, inappropriate and ineffective use of antimicrobial agents for viral respiratory infections is common (10–16). In 1998, the Institute of Medicine issued a workshop report that addressed the growing problem of antimicrobial drug resistance and potential strategies to prolong the effectiveness of existing drugs (17). The report found that physicians and patients have not received adequate information about the appropriate use of antimicrobial drugs and the short- and long-term risks of overuse. Several approaches were suggested, including multifaceted clinician education, clinical practice protocols, feedback on local resistance trends, patient-oriented educational materials, and use of popular media for public education.

We describe the activities and impact of a 5-year, multifaceted educational campaign to reduce outpatient antimicrobial drug prescribing in Wisconsin. The Wisconsin Antibiotic Resistance Network (WARN) was launched in 1999 as a federally funded demonstration project to educate primary care physicians and the public about drug resistance and judicious antimicrobial drug use. Antimicrobial drug prescribing rates were assessed annually and compared to Minnesota prescribing rates, where educational activities were limited before 2002.

Methods

WARN Organization

WARN was established as a collaborative project involving the Marshfield Clinic Research Foundation, Wisconsin Medical Society, and Wisconsin Division of Public Health. The Wisconsin Medical Society collaborated with the Marshfield Clinic Research Foundation to develop the public education campaign, and the latter organization was responsible for data collection and evaluation. The Division of Public Health assisted with program planning and collected invasive pneumococcal isolates for susceptibility testing.

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For most of the project period, WARN employed 2 program managers and 2 health educators. Representatives of each position were based in Madison and in Marshfield to cover the southern and northern parts of the state. An advisory board was established with representation from managed care organizations, employers, local public health agencies, primary care practices, infectious disease experts, childcare centers, pharmacists, and the Centers for Disease Control and Prevention. Campaign themes, characters, messages, and print materials were developed during the first year (1999). An expert panel was convened to develop local clinical practice guidelines for acute respiratory illness in Wisconsin. Educational needs were assessed through surveys of primary care clinicians and the general public (18). Limited public and physician educational activities were initiated in 2000, and the program was implemented in full from 2001 to 2003. Educational interventions were designed to be multifaceted and consistent with behavioral research that suggested a need for a variety of educational strategies with repetitive and reinforcing messages (19–21).

Public and Physician Education

The tag lines for the public education campaign were "There's no excuse for overuse!" and "Get smart about antibiotics!" The campaign mascots, Annie Biotic and Moxie Cillin, were cartoon characters designed to appeal largely to children and parents. Public education materials included posters, brochures, stickers, coloring sheets, magnets, and disease-specific parent handouts (Table 1). All materials were available free of charge to healthcare providers, clinics, and community organizations. Spanish translations were also available for many of the written materials. A Web site was established through the Wisconsin Medical Society to describe WARN activities and facilitate ordering materials. The distribution volume for WARN educational materials is shown in Table 2.

Outreach to childcare centers was a major focus of community education because children attending group child care have high rates of respiratory illness. Annual mailings were sent to >5,000 licensed childcare centers, and on-site presentations were given at 170 centers. Additional presentations were made at childcare conferences and in college classes on early childhood education. Physician-education activities began in 2000 and included direct mailings with samples of WARN materials, development and distribution of guidelines for judicious antibiotic use, satellite broadcasts, dissemination of pneumococcal antimicrobial resistance data, and professional presentations (Table 1).

Media Campaign

The media campaign included paid advertising and strategies to maximize coverage. The campaign was launched with a press conference in 1999, followed by

Table 1. Major WARN activities and initiatives, 2000–2003*

Activity	2000	2001	2002	2003
Public education				
Mailing to all licensed family and group childcare providers		X	X	X
Exhibits at health fairs and community events	X	X	X	X
Public appearances by costumed characters	X	X	X	X
Distribution of sample materials to pharmacies	X			
Slide presentations at childcare centers	X	X	X	X
Newsletter copy distributed to healthcare organizations	X	X		
WARN paycheck stuffers for state employees	X		X	X
Slide presentations for community and state organizations	X	X	X	X
Physician education				
Sample materials and order form mailed to >8,000 licensed primary care clinicians and pharmacists		X	X	X
Satellite broadcasts on management of respiratory illness	X	X		
Academic detailing packets distributed to health plans, clinics, public health staff	X	X	X	X
Narrated slide presentation on CD-ROM mailed to primary care clinicians		X		
Presentations at healthcare facilities, professional meetings, and conferences	X	X	X	X
Distributed invasive pneumococcal susceptibility report for Wisconsin	X	X	X	X
Distributed original (2000) and revised (2002) clinical practice fact sheets to ≈9,000 clinicians and pharmacists	X		X	
WARN resource binder distributed to 16 health plans		X		
Media campaign				
Advertisements on Wisconsin Radio Network or Wisconsin Public Radio	X	X	X	X
Guest editorials in newspapers	X	X	X	X
Governor declared "Get Smart About Antibiotics Month"	X			
Television advertisements—Dick Van Dyke and Bill Nye			X	X
Earned media—radio and/or television news coverage	X	X	X	X
Earned media—newspaper coverage	X	X	X	X

*WARN, Wisconsin Antibiotic Resistance Network.

Table 2. Distribution of WARN educational materials (2000–2003)*

Type of material	Approximate no. distributed
WARN parent brochures	700,000
WARN posters	26,000
CDC posters (new in 2003)	900
CDC adult brochures	400,000
Spanish-language posters	5,000
Viral illness card	18,000
CDC viral prescription pad (new in 2003)	300
Parent illness handouts	23,000
Coloring sheets	450,000
Stickers	620,000
Magnets	50,000
Clinical practice fact sheets for respiratory illness	20,000
Pneumococcal susceptibility reports	38,000

*WARN, Wisconsin Antibiotic Resistance Network; CDC, Centers for Disease Control and Prevention.

repeated news media coverage of WARN and antimicrobial resistance issues from 2000 through 2003 (Table 1). This coverage included newspaper reports in Milwaukee, Madison, and multiple smaller communities, as well as local television news stories in Milwaukee, Madison, and Wausau. Paid advertising was initiated on radio stations broadcasting throughout the state and on television stations. Because of the high cost, television advertisements were limited to periods of 2 to 4 weeks during the peak respiratory illness season.

Research Design and Outcome Measures

Multiple evaluation components were developed to measure the effect of the WARN educational campaign. The major outcome for this report was annual antimicrobial drug use, measured by the number of primary care prescriptions and the volume of retail antimicrobial drug sales.

During the late 1990s, a national secular trend of declining antimicrobial drug use occurred (22–24). To distinguish the impact of WARN from the secular trend, antimicrobial drug–prescribing measures were obtained for both Wisconsin and Minnesota. Although Minnesota is not representative of the entire country, the use of a comparison state provided the opportunity to distinguish intervention-related changes from regional trends in use that were unrelated to the intervention. Minnesota was chosen for geographic proximity and similarity in terms of population size and ethnic distribution. Before 2002, patient educational activities in Minnesota were limited. A group of 6 Minnesota managed care plans distributed ≈17,000 cough and cold kits to patients during the 2000–2001 respiratory illness season, and 31,000 kits during the 2001–2002 season. An article promoting appropriate antimicrobial drug use was published in the April 2001 issue of Minnesota Medicine (25), but no other formal programs to educate Minnesota physicians on appropriate antimicrobial drug use were implemented until late 2002, when sample materials (posters, buttons, "prescription pads" for symptomatic therapy) were mailed to managers at 377 Minnesota clinics and urgent care centers.

Measures of Antimicrobial Drug Use

Prescribing data and retail volume distribution data (measuring retail sales) were obtained from a commercial source (IMS Health, Inc., Plymouth Meeting, PA, USA) for the states of Wisconsin and Minnesota. Physician prescribing data were available for 1998 and 2000 through 2003. Prescribing and volume distribution data were not available for individual drugs within each class. The source data did not include any information regarding the specific diagnosis or patient characteristics.

The prescribing databases included only new outpatient prescriptions, and they were derived from transactional data provided by 59% of all retail pharmacies in Wisconsin and Minnesota. Approximately 65% of chain pharmacies and 51% of independent retail pharmacies contributed raw prescribing data. Prescriptions from unsampled stores were estimated on the basis of prescription totals from matched nearby stores, with weighting to adjust for differences in total retail sales volume, which was available for nearly all stores. Estimates were also weighted to account for the distance between sampled stores and matched unsampled stores, with closer stores contributing more to the estimated prescription volume. The proportion of all prescriptions in each state that were based on estimated data from unsampled stores was 33%–37%.

Physician-level prescribing data included data for all licensed physicians with any of the following primary specialty codes: family (and general) practice, internal medicine, pediatrics, or emergency medicine. Physicians were classified geographically as practicing within or outside of the largest metropolitan statistical area in each state. These 2 metropolitan statistical areas were Milwaukee-Waukesha (4 counties) and Minneapolis-St. Paul (11 counties).

Retail volume distribution was determined by the volume of antimicrobial drugs distributed to retail outlets on a monthly basis from 1999 to 2002. This distribution was derived from an independent data source relative to the physician-level prescribing data. Retail distribution data (measured in kilograms) were reported by wholesalers and distributors serving pharmacies in both states. Retail volume was not linked to specific prescriptions or providers and therefore represented a measure of total outpatient antimicrobial drug use in each state. Volume was based on distribution to retailers rather than actual sales to patients, and distributed drugs could be returned to wholesalers without being sold. In this situation, returned drugs were subtracted from the total distributed in a given month to

yield the net retail distribution for each drug class. Inpatient pharmacies, prisons, veterinary offices, nursing homes, dialysis clinics, and federal government sites were excluded from the volume distribution data. The retail volume sales database captured 93% of actual antimicrobial drug distribution in Wisconsin and Minnesota. Volume sales were divided by the annual population estimates in each state and reported as grams per capita.

The following product groups were included in the assessment of outpatient prescribing and retail volume sales: amoxicillin/penicillin, amoxicillin-clavulanate, cephalosporins, macrolides, extended-spectrum macrolides (azithromycin, clarithromycin), fluoroquinolones, tetracyclines, trimethoprim-sulfamethoxazole, and other sulfa drugs (including erythromycin/sulfisoxazole). Solid and liquid formulations were reported separately, and liquid formulations were used as surrogates for pediatric prescribing. Broad-spectrum antimicrobial drugs were also analyzed separately. Although no standard definition of broad spectrum antimicrobial drugs exists, a previous report on national trends in antimicrobial drug use classified the following groups as broad-spectrum: extended-spectrum macrolides, fluoroquinolones, second- and third-generation cephalosporins, and amoxicillin-clavulanate (23). We used the same classification with 1 exception. In this study we classified all cephalosporins as broad-spectrum because we were unable to distinguish first-, second-, and third-generation cephalosporins.

Prescriber Cohort

A cohort of primary care physicians was established to monitor and compare longitudinal trends in prescribing antimicrobial drugs. The cohort was defined as primary care physicians in Minnesota and Wisconsin who prescribed at least 1 antimicrobial drug in each month during the baseline year (1998) and each of the follow-up years (2000–2003). This criterion was used to avoid including residents and other physicians in temporary practice settings. It also avoided including nonpractice time in prescribing rate calculations, since information on individual practice patterns was not available. The annual antimicrobial prescribing rate was calculated by dividing the number of new, filled prescriptions by the number of physicians in the cohort.

The original 1998 prescriber database included 9,164 primary care physicians in Minnesota or Wisconsin who prescribed any antimicrobial drug. Of those, 4,115 (45%) prescribed ≥1 antimicrobial drug per month in 1998 and annually from 2000 through 2003. This group made up the final cohort for analysis of longitudinal prescribing trends. A secondary analysis of prescribing trends was performed based on the larger group of primary care physicians (n = 12,790) who prescribed ≥1 antimicrobial drugs during any of the follow-up years but who were not necessarily in continuous practice.

Statistical Analysis

Prescribing rates for the 4,115 physicians in practice throughout the study period were computed as the number of antimicrobial prescriptions in a given year divided by the number of physicians. Prescribing rates were therefore equivalent to the mean number of prescriptions for antimicrobial drugs per physician each year. Population-based or patient-based prescribing rates could not be calculated because the populations served by the prescribing cohort were undefined. To compare prescribing rates for each year of the intervention period (2000–2003) to baseline (1998), we fit Poisson regression models. Generalized estimating equations (GEE) were employed to account for within-physician correlation (26). An autoregressive working correlation structure was specified in the Poisson-GEE models, and appropriate steps were taken to accommodate the absence of data for calendar year 1999. Statistical comparisons of changes in rates in Wisconsin versus those in Minnesota were derived from these models.

Differences in baseline antimicrobial prescribing were addressed by fitting additional Poisson-GEE models using data from the intervention period with the natural log of the baseline number of antimicrobial drug prescriptions included as an independent variable. Prescribing rates per physician-month were computed when analyzing the 12,790 physicians who wrote such prescriptions in ≥1 month of the study period. Months where a physician wrote at least 1 prescription for an antimicrobial drug were included in the denominators of rates (i.e., a physician was assumed not to be in practice in months when he did not write any such prescriptions). Per capita antimicrobial drug sales were calculated based on annual population estimates (www.census.gov). Analyses were completed by using the SAS software (SAS Institute Inc., Cary, NC, USA).

Results

The 4,115 primary care physicians in long-term practice included 2,009 (49%) in Minnesota and 2,106 (51%) in Wisconsin. The proportion of physicians in family practice was higher in Wisconsin, and the proportion in internal medicine was higher in Minnesota (Table 3). During 1998, these physicians generated 1.5 million prescriptions for antimicrobial drugs in each state, and the crude antimicrobial prescribing rate was nearly identical across states. From 2000 through 2003, the prescribing rate for antimicrobial drugs in both states gradually declined (Figure 1). From 1998 to 2003, the antimicrobial prescribing rate was reduced by 19.8% in Minnesota and by 20.4% in Wisconsin. The percentage reduction was 19.5% and

Table 3. Characteristics of 4,115 primary care physicians in long-term practice, Minnesota and Wisconsin

Characteristic*	Minnesota, n (%)	Wisconsin, n (%)
Specialty		
Family practice†	1,245 (62.0)	1,043 (49.5)
Internal medicine	396 (19.7)	565 (26.8)
Pediatrics	256 (12.7)	309 (14.7)
Emergency medicine	112 (5.6)	189 (9.0)
Practice location‡		
Milwaukee-Waukesha MSA	–	726 (34.8)
Minneapolis-St. Paul MSA	1,216 (61.5)	–
Other counties	762 (38.5)	1,363 (65.2)

*MSA, metropolitan statistical area.
†Includes 67 physicians in general practice.
‡48 physicians excluded because practice location category changed during follow-up period.

18.6%, respectively, in the secondary analysis, which included the 12,790 primary care physicians who prescribed antimicrobial drugs at any time during the follow-up period.

Retail sales of antimicrobial drugs (grams per capita) declined by 27.4% in Minnesota and 17.3% in Wisconsin from 1999 through 2002 (Figure 1). Sales of amoxicillin and penicillin exceeded those of other product groups in each year and accounted for 37% of all retail antimicrobial distribution. In both states, retail sales of fluoroquinolones remained level, and sales of amoxicillin-clavulanate increased. Sales of most other product groups declined by 15% or more.

Stratification of antimicrobial prescribing rates by specialty showed that the reduction in prescribing differed only for physicians in internal medicine and pediatrics (Table 4). Antimicrobial prescribing declined more in Wisconsin than Minnesota among internal medicine physicians, but the opposite was true for pediatricians. In Minnesota, the reduction in antimicrobial use was similar for physicians practicing in the Minneapolis-St. Paul metropolitan statistical area and those practicing elsewhere in the state (Table 5). In Wisconsin, the reduction in antimicrobial prescribing was much less in the Milwaukee-Waukesha metropolitan statistical area than in the remainder of the state. Antimicrobial prescribing declined significantly more in the Minneapolis-St. Paul metropolitan statistical area than in the corresponding Milwaukee-Waukesha area.

Prescriptions for liquid antimicrobial drugs (a surrogate for pediatric use) declined 29% in Wisconsin and 32% in Minnesota from 1998 to 2003 ($p = 0.17$). Prescriptions for solid formulations declined 15%–17% in each state. The percentage of prescriptions for broad-spectrum agents was unchanged (within 2%) in each state from 1998 through 2003.

Regression models were fit to compare prescribing rates in Wisconsin and Minnesota during each year from 2000 to 2003, with adjustment for specialty and baseline prescribing in 1998. Two separate models were generated. The first included physicians practicing in the Minneapolis-St. Paul metropolitan statistical area or the Milwaukee-Waukesha metropolitan statistical area. The second included physicians practicing in other counties of Minnesota or Wisconsin. In the latter model, the prescribing rate ratio was <1 in 2002 and 2003, indicating that, when the 2 largest metropolitan areas were excluded, Wisconsin physicians had significantly lower prescribing rates than those in Minnesota (Figure 2). In contrast, the prescribing rate ratio was >1 in each year within the 2 largest metropolitan areas, indicating that physicians in the Milwaukee-Waukesha MSA had higher prescribing rates than those in the Minneapolis-St. Paul MSA, after adjusting for specialty and baseline prescribing.

Discussion

WARN represents the largest program on appropriate antimicrobial drug use that has been evaluated to measure the effect on prescribing. Previously published studies demonstrated that interventions at the level of the physician, clinic, or community had a modest effect on prescribing (20,27–32). These focused programs are useful in evaluating specific intervention strategies in a relatively controlled setting. However, adoption of new practices may be slow even when the intervention is proven to be effective, and generalizability to larger populations may be limited. In contrast, large-scale programs can reach physicians and the general public in an entire state or large metropolitan area. The development of WARN reflected a need to implement and evaluate a large-scale demonstration project that could influence antimicrobial prescribing throughout Wisconsin in a relatively short time. At the time WARN was conceived and funded, knowledge of national trends in antimicrobial prescribing was limited, but we now know that prescribing declined substantially

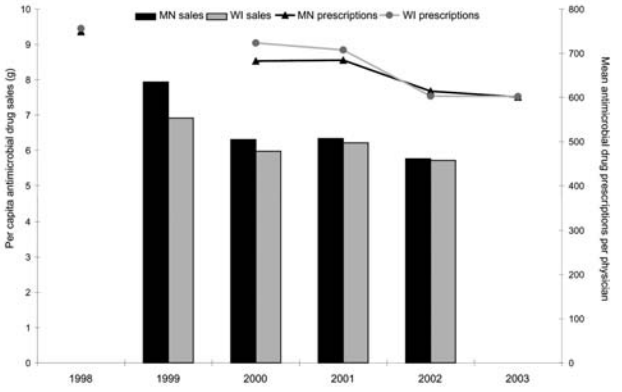

Figure 1. Temporal trends in per capita antimicrobial drug sales and the mean number of prescriptions per physician in Minnesota and Wisconsin.

Table 4. Changes in antimicrobial drug prescribing by specialty and state, 1998–2003*

	Wisconsin prescribing rate			Minnesota prescribing rate			
Specialty	1998	2003	% reduction	1998	2003	% reduction	p value†
Family practice	810	631	22	843	685	19	0.11
Internal medicine	540	447	17	366	329	10	0.03
Pediatrics	1,126	891	21	1,068	751	30	0.006
Emergency medicine	519	451	13	306	303	1	0.25

*The annual antimicrobial drug prescribing rate was calculated by dividing the number of new filled prescriptions by the number of prescribers in each specialty.
†p value for comparison of reduction in Wisconsin vs. Minnesota.

for both pediatric and adult populations in the 1990s (22–24,33).

Outpatient antimicrobial use declined substantially in both Wisconsin and Minnesota from 1998 to 2003, and no additional intervention-related effect was apparent in Wisconsin. Secondary analyses by specialty and practice location demonstrated variable reductions in prescribing of antimicrobial drugs, but to what extent these reductions were related to WARN interventions, as opposed to other factors that may be influencing secular trends, is unclear. The potential effect of these other factors is illustrated by the observation that Minnesota pediatricians improved their prescribing practices more than Wisconsin pediatricians, despite the absence of an organized, large-scale program to improve pediatric prescribing in Minnesota.

We found that changes in antimicrobial drug use were less pronounced in the Milwaukee-Waukesha metropolitan area than in other regions of Wisconsin. This finding contrasts with findings in Minnesota, where the decrease in antimicrobial drug use in the Minneapolis-St. Paul metropolitan area was similar to that in the rest of the state. When these large metropolitan areas were excluded from the analysis, prescribing of antimicrobial drugs decreased more in Wisconsin than in Minnesota. Several factors may have contributed to the relatively low impact of WARN in the Milwaukee area. These factors include the absence of paid staff working in Milwaukee, few connections with the Milwaukee medical community, and the large number of clinical practices and health plans.

Funding for WARN exceeded levels for other state-based programs on antimicrobial drug resistance, but it was far lower than the funding level for some other public education campaigns. Annual funding for WARN staffing, programs, and materials (excluding indirect costs and evaluation activities) was ≈$238,000–$342,000. By comparison, Wisconsin receives ≈$10 million in state funding and $1.1 million in federal funding each year for smoking prevention programs (Maureen Busalacchi, pers. comm.). WARN funding for education on antimicrobial resistance was not sufficient to conduct a widespread and sustained media campaign, although whether such a campaign would have led to further reductions in antimicrobial use is not known.

One other published study reported the effect of interventions promoting appropriate antimicrobial drug use in a large, highly populated area. In Knox County, Tennessee, a 12-month multifaceted campaign was conducted in 1997 and early 1998 (34). The clinician intervention included professional presentations, distribution of pediatric principles of judicious antimicrobial drug use (35), and newsletter articles. Patient and public education included distribution of print materials, news media coverage, and public service announcements. In the Medicaid managed care program, antimicrobial drug prescribing for respiratory illness declined 19% in Knox County and 8% in the control counties compared to the previous year (intervention attributable effect of 11%, $p<0.001$). The Knox County intervention was smaller in scale and shorter in duration than WARN, and the generalizability to non-Medicaid populations is unknown.

Evaluating large-scale, multifaceted educational programs such as WARN has several limitations. The control population in Minnesota was not isolated, and educational materials and messages may have diffused into the control area from a variety of sources. For example, during the WARN follow-up period, national guidelines on appropriate antimicrobial use were published and endorsed by the Centers for Disease Control and Prevention and major professional organizations (35–37). WARN also reached limited populations in Minnesota: radio advertisements

Table 5. Changes in prescribing rate for antimicrobial drugs, by practice location, 1998–2003*

	Wisconsin prescribing rate			Minnesota prescribing rate			
Practice location	1998	2003	% reduction	1998	2003	% reduction	p value†
Major metropolitan area‡	719	639	11	711	568	20	<0.001
Other areas of Minnesota and Wisconsin	778	583	25	814	657	19	0.005

*The annual prescribing rate for antimicrobial drugs was calculated by dividing the number of new filled prescriptions by the number of prescribers in each specialty.
†p value for comparison of reduction in Wisconsin vs. Minnesota.
‡Includes the Milwaukee-Waukesha metropolitan statistical area (4 counties) for Wisconsin and the Minneapolis-St. Paul metropolitan statistical area (11 counties) for Minnesota. Two Wisconsin counties were excluded from the latter area.

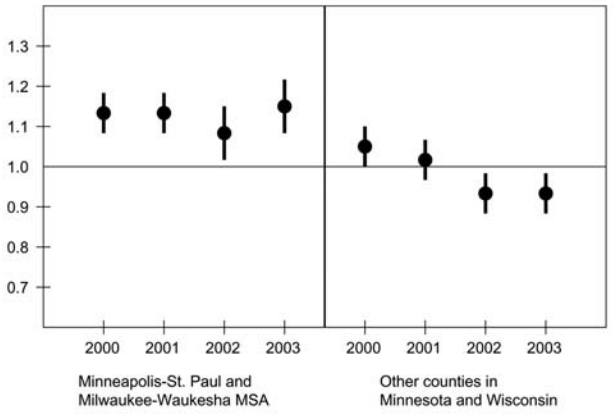

Figure 2. Antimicrobial prescribing rate ratios by year and practice location, adjusted for specialty and baseline (1998) prescribing rate. The vertical bars show 95% confidence intervals. Ratios <1 indicate lower antimicrobial prescribing by Wisconsin physicians relative to Minnesota physicians. MSA, metropolitan statistical area.

included areas of eastern Minnesota, and some Minnesota physicians received the WARN satellite broadcasts on appropriate antimicrobial drug use. In addition, efforts within Minnesota to improve prescribing of antimicrobial drugs increased during the follow-up period, particularly during 2002 and 2003. A single state may not be the optimal comparison population, and Minnesota in particular has a highly educated population and a proactive public health infrastructure with strong ties to the healthcare delivery system. As a result, the improvements in antimicrobial drug prescribing within Minnesota may have been greater than those in many other states. Finally, the commercial prescribing data used for the WARN evaluation did not include any information on visits or diagnoses. We therefore cannot determine if the declines in antimicrobial prescribing in Wisconsin and Minnesota were associated with a declining rate of visits for acute respiratory illness or if the rate of prescribing declined for specific diagnoses.

The centralized development of WARN programs and materials facilitated statewide distribution, but it also limited the level of clinician involvement at the local level. Direct, face-to-face communication with physicians was rarely possible. In contrast, practice-level interventions have shown modest success, and we speculate that these focused, participatory interventions may promote physician behavior change more directly than a mass education campaign such as WARN. However, WARN succeeded in changing physicians' knowledge and attitudes regarding appropriate antimicrobial drug use, and WARN materials were widely used by primary care clinicians throughout the state. In a survey of primary care clinicians, 90% of respondents had heard of WARN, and 70% of those had used WARN materials for patient education (38). Models of behavior change suggest that changes in prescribing behavior are preceded by important cognitive changes that proceed in stepwise fashion (39). Improvements in knowledge and beliefs among both physicians and patients may therefore be markers of progress, which may facilitate the future success of provider-level interventions developed by clinics and managed care organizations in Wisconsin.

Increased funding for state-level educational campaigns to promote appropriate antimicrobial drug use does not appear warranted by the results of this evaluation. However, the combined effect of national guidelines for appropriate use of such drugs, increasing attention by the media and professional organizations, and the Centers for Disease Control and Prevention national campaign may have contributed to the observed trend toward declining antimicrobial use. Progress toward decreasing inappropriate use is being made in many states, although antimicrobial prescribing rates remain excessive for bronchitis, and use of broad-spectrum antimicrobial drugs is increasing nationally (22,27,33). State and local organizations should consider a balanced approach that includes limited statewide educational activities with increasing emphasis on local, provider-level interventions and policy development. These activities might include academic detailing by physician opinion leaders, feedback on antimicrobial prescribing performance (including Health Plan Employer Data and Information Set measures), and economic incentives for careful antimicrobial use. These strategies may have the greatest effect if implemented as quality improvement initiatives in collaboration with the leadership of health plans and clinic organizations. Ongoing assessment of prescribing trends and rates of antimicrobial drug resistance will be needed to measure the ultimate effect of these efforts.

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Trypanosomiasis Relapse after Melarsoprol Therapy, Democratic Republic of Congo, 1982–2001

Jacques Pépin* and Bokelo Mpia†

Recently, a high proportion of patients with late-stage *Trypanosoma brucei gambiense* trypanosomiasis, who had been treated with melarsoprol in some disease-endemic areas, subsequently relapsed. To determine whether the frequency of postmelarsoprol relapses increased over time, we reviewed data from 2,221 trypanosomiasis patients treated with melarsoprol during this period in Nioki, Democratic Republic of Congo, from 1982 to 2001. The frequency of relapses was 5.6%(31/553), 6.8%(35/512), 4.5%(18/398), 11.4%(34/299), and 5.0%(17/343) for those treated from 1982 to 1985, 1986 to 1989, 1990 to 1993, 1994 to 1997, and 1998 to 2001, respectively. The higher frequency of relapses in 1994 to 1997 was associated with an incremental dosage regimen of melarsoprol. In multivariate analysis, after adjustment for treatment regimen, sex, residence, and trypanosomes in cerebrospinal fluid, postmelarsoprol relapses did not increase in Nioki, perhaps because 1) little drug pressure exists; 2) subtherapeutic doses have rarely been administered; 3) little potential exists for the preferential transmission of melarsoprol-resistant strains.

Recent reports suggest that the frequency of relapses after melarsoprol treatment of late-stage *Trypanosoma brucei gambiense* trypanosomiasis may be increasing, at least in 3 foci where the frequency of postmelarsoprol relapses is higher than the 5%–8% generally seen elsewhere (1). In Ibbe, southern Sudan, 18%–20% of patients treated with melarsoprol eventually relapsed (2,3), as did 27% of similar patients in the Arua focus of northwestern Uganda (4). In M'banza Congo, northern Angola, 25% of patients treated with melarsoprol had cerebrospinal fluid (CSF) positive for parasites during treatment or relapsed within 1 month (5). This development could have serious implications for countries highly endemic for *T.b. gambiense* trypanosomiasis (6,7). Eflornithine is the only alternative drug to melarsoprol for patients in late-stage trypanosomiasis, generally defined by either the presence of trypanosomes in the CSF, a CSF leukocyte count higher than 5/mm^3, or both, but this drug needs to be administered intravenously every 6 hours for 14 days, not an easy task for rural hospitals with limited human and financial resources.

An important question is whether rates of postmelarsoprol relapses are indeed rising in these foci or merely reflect a long-standing decrease in susceptibility to melarsoprol of local strains, which became more obvious when the incidence increased or when data collection and analysis improved. Anecdotal reports have indicated that the frequency of failures was 40% among patients treated with melarsoprol in the early 1970s in the Kimpangu hospital of Zaire (8). This hospital is located at the Angolan border, and many of its patients came from northern Angola. In Uganda and Sudan, no reports about rates of treatment failures have been published before these recent reports. In Nioki, Democratic Republic of Congo (DRC), data have been collected since 1982 on all patients in whom Gambian trypanosomiasis was diagnosed. We thus reviewed our database to determine whether secular changes had occurred in the frequency of postmelarsoprol relapses.

Methods

Nioki hospital, the only hospital of the district (population ≈110,000), is located in the Bandundu Province of DRC, at the confluence of the Mfimi and Molibampe Rivers. Several trypanosomiasis foci are located on the Mfimi River (inhabited by the Basakata) while others lie on the Molibampe River (populated by the Bampe) or on the road going north from Nioki (populated by the Baboma and the Basengele). These 3 areas, as well as Nioki town

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(population ≈35,000), represent natural subdivisions of the Nioki District. Patients from Nioki town can acquire their infection either in Nioki itself, where there are infective tsetse flies or when traveling in rural parts of the district.

All patients with *T.b. gambiense* trypanosomiasis treated in Nioki hospital from January 1, 1982, to December 31, 2001, were included in this observational study; follow-up data were accumulated until December 31, 2003. Most cases were diagnosed at Nioki hospital, while a minority were referred by case-finding mobile teams or rural health centers. Case-finding teams were active in the 1980s, collapsed around 1991 (9), and were reorganized on a more modest scale later on. The vast majority of patients lived within Nioki District, but case-patients from disease-endemic villages outside the district have also been included. The routine work-up of suspected case-patients included a lymph node aspirate when cervical lymphadenopathy was present and a lumbar puncture for CSF examination. Examinations of blood were performed only if other assays failed to show trypanosomes. Patients were considered to be in early stage if the CSF leukocyte count was 1–5/mm^3, or in late stage when CSF leukocyte count was >5/mm^3, and treatment was selected accordingly.

Melarsoprol was used throughout the study period for most adult patients with late-stage trypanosomiasis; a small number were treated with eflornithine during clinical trials of this drug. Until mid-1985, late-stage disease in children was treated with trimelarsan. Later, trimelarsan was no longer available, and melarsoprol was used for children as well. From 1984 to 1988, half of melarsoprol-treated patients also received prednisolone during a randomized controlled trial (10); thereafter, prednisolone was given to all melarsoprol-treated patients, except for brief periods of drug shortage. Details of treatment schemes are available in other publications (10–13), in which various parts of the cohort of patients reviewed here were reported. Most patients were given 2 or 3 series (separated by 1-week drug-free intervals) of 3 or 4 daily injections of intravenous melarsoprol at the full dosage (3.6 mg/kg, for a maximum of 180 mg), depending on the CSF leukocyte count. During shortages of pentamidine and suramin, melarsoprol (1 series of 3 injections) was sometimes used in the treatment of early-stage patients. From April 1996 to December 2001, a trial of 3 regimens of melarsoprol took place (14), and consenting patients were randomized to receive either the conventional dosage (3 series of 3 injections of 3.6 mg/kg), a new regimen of 10 consecutive daily injections of 2.16 mg/kg (15), or a regimen of 3 series of 3 injections in which an incremental dosage was used (1.8, 2.16, 2.52; then 2.52, 2.88, 3.24; then 3.6 mg/kg for the last 3 injections). Enrollment in the latter arm of the study was terminated prematurely in December 1998 when a high relapse rate became apparent (14).

After treatment, patients were followed up with lumbar punctures every 6 months for 2 years. Most of the follow-up lumbar punctures were performed at Nioki hospital; in the earlier years, some of these were performed by mobile teams during visits to disease-endemic villages. The patients were asked to come back sooner if they experienced symptoms compatible with a relapse (somnolence, constant headaches). The decision to administer a second treatment (and thus to consider this case as a relapse) was left to the discretion of the attending physician, but most fulfilled one of the following criteria: 1) trypanosomes found in the CSF (or rarely in the blood or lymph node aspirate); 2) CSF leukocyte count ≥50/mm^3 and higher than the previous determination; or 3) CSF leukocyte count of 20 to 49/mm^3, higher than the previous determination, with the presence of symptoms compatible with a relapse. When in doubt, the lumbar puncture was repeated 1–2 months later. In practice, distinguishing a genuine relapse from a reinfection is not possible, and we will use "relapse" to designate both. The primary analysis of risk of relapsing considered all relapses, regardless of the interval since melarsoprol treatment; in a secondary analysis, only relapses happening within 2 years of treatment were considered.

For each case, we collected data on age, sex, village of residence, date of diagnosis, mode of diagnosis (lymph node aspirate, blood examinations [wet smear, thick smear, hematocrit centrifugation technique, or any combination thereof], CSF examination), pretreatment CSF leukocyte count, treatment given, encephalopathy or death during treatment, and whether a diagnosis of relapse was made during posttreatment follow-up. Few data were missing with the exception of the village of residence (missing for 61 of 164 patients in 1982) and the precise age (unknown for 1 child and 152 adults). The database did not include information on each follow-up lumbar puncture, but only on those which led to a diagnosis of relapse. Thus, to estimate the frequency of relapses, we used as the denominators all patients who survived treatment.

Data were entered and verified on EpiInfo 6.04, and analyzed with Stata 8.0 (Stata Corporation, College Station, TX, USA). Proportions were compared with the χ^2 test. CSF leukocyte counts that had a non-normal distribution were compared with rank sum tests. Multivariate analysis was performed by logistic regression; variables that enhanced the fit of the model at the 0.05 level by using the likelihood ratio test were retained.

Results

Table 1 summarizes sociodemographic, clinical, and biologic characteristics of all patients during each 4-year period from 1982 until 2001. Several of the changes reflect the more systematic case-finding by mobile teams in the

Table 1. Demographic and clinical characteristics of all patients with *Trypanosoma brucei gambiense* trypanosomiasis treated in Nioki hospital, 1982–2001*

Characteristic	1982–1985 (%) (n = 1,074)	1986–1989 (%) (n = 889)	1990–1993 (%) (n = 535)	1994–1997 (%) (n = 447)	1998–2001 (%) (n = 374)	p value
Sex						<0.001
Females	669 (62)	472 (53)	283 (53)	217 (49)	161 (43)	
Males	405	417	252	230	213	
Age (y)						<0.001
≤14	239 (25)	178 (20)	91 (18)	69 (15)	63 (17)	
15–49	569 (60)	600 (68)	353 (69)	330 (74)	267 (71)	
≥50	146 (15)	105 (12)	66 (13%)	47 (11)	44 (12)	
Area of residence						<0.001
Nioki town	152 (15)	212 (24)	150 (28)	105 (23)	114 (30)	
Mfimi River	585 (58)	329 (37)	151 (28)	129 (29)	119 (32)	
Molibampe River	78 (8)	79 (9)	60 (11)	72 (16)	49 (13)	
Baboma/Basengele	137 (14)	146 (16)	58 (11)	62 (14)	43 (11)	
Out of district	61 (6)	123 (14)	116 (22)	79 (18)	49 (13)	
Trypanosomes in lymph node aspirate						<0.001
Yes	624 (58)	415 (47)	180 (34)	160 (36)	130 (35)	
No	450	474	355	287	244	
Trypanosomes in blood						<0.001
Yes	313 (29)	341 (38)	157 (29)	138 (31)	126 (34)	
No	761	548	378	309	248	
Trypanosomes in CSF						<0.001
Yes	135 (13)	278 (31)	246 (46)	239 (53)	187 (50)	
No	939	611	289	208	187	
CSF leukocyte count (per mm^3)						<0.001
1–5	407 (38)	286 (32)	75 (14)	32 (7)	26 (7)	
6–19	188 (18)	120 (13)	38 (7)	34 (8)	38 (10)	
20–99	189 (18)	144 (16)	122 (23)	118 (26)	109 (29)	
≥100	289 (27)	339 (38)	300 (56)	263 (59)	201 (54)	
Median CSF leukocyte count (per mm^3)	12	31	120	130	112	<0.001

*CSF, cerebrospinal fluid.

earlier half of this period: over time, the proportion of patients with a normal CSF leukocyte count decreased considerably, as did the proportion of patients with a positive lymph node aspirate, while the proportion of patients with CSF trypanosomes increased. The higher proportion of cases in women in the earlier years and subsequent decrease can be explained by this factor (women participate more in case-finding surveys) as well as by a shift in the distribution of cases, with a progressively higher proportion of cases in inhabitants of Nioki town (due to more men migrating out of villages, rural communities have more women then men, but Nioki town does not). Changes in the proportion of patients with trypanosomes in the blood were less striking; because examinations of blood were not conducted for all patients, these variations might reflect changes in the propensity of clinicians to order such assays.

Table 2 shows the same characteristics, but only for patients who were treated with melarsoprol. Similar changes in distribution of cases according to sex and area of residence were seen. Even though 96% of these patients were given melarsoprol because they had a CSF leukocyte >5/mm^3, a shift occurred over time towards more advanced disease upon diagnosis in recent years, as evidenced by the decrease in the proportion with a positive lymph node aspirate, an increase in the proportion with CSF trypanosomes, and a doubling of the median CSF leukocyte count. Treatment regimens varied considerably over time. The proportion of patients given prednisolone also increased after the randomized controlled trial was conducted (10), and its results generalized; the proportion of patients in whom a melarsoprol-induced encephalopathy developed decreased to 2% in the last 4-year period. The proportion of patients found to relapse, whether all relapses were considered or only those occurring within 2 years of diagnosis, increased significantly during 1994–1997, but decreased to its previous level from 1998 to 2001.

The risk factors for postmelarsoprol relapses during this 20-year period are shown in Table 3. Patients who died during treatment were excluded. In univariate analyses, relapses were significantly more common in male patients,

Table 2. Demographic and clinical characteristics of patients with *Trypanosoma brucei gambiense* trypanosomiasis treated with melarsoprol in Nioki hospital, 1982–2001*

	1982–1985 (%) (n = 587)	1986–1989 (%) (n = 543)	1990–1993 (%) (n = 417)	1994–1997 (%) (n = 320)	1998-2001 (%) (n = 354)	p value
Sex						<0.001
Females	368 (63)	269 (50)	214 (51)	143 (45)	151 (43)	
Males	219	274	203	177	203	
Age						0.003
≤14	84 (16)	115 (21)	74 (19)	62 (19)	62 (18)	
15–49	341 (66)	354 (66)	272 (69)	229 (72)	254 (72)	
≥50	95 (18)	71 (13)	48 (12)	28 (9)	38 (11)	
Area of residence						<0.001
Nioki town	94 (17)	117 (22)	116 (28)	79 (25)	104 (29)	
Mfimi River	304 (54)	201 (37)	112 (27)	88 (28)	113 (32)	
Molibampe River	25 (4)	45 (8)	48 (12)	47 (15)	48 (14)	
Baboma/Basengele	95 (17)	91 (17)	46 (11)	46 (14)	42 (12)	
Out of district	43 (8)	89 (16)	95 (23)	60 (19)	47 (13)	
Trypanosomes in lymph node aspirate						<0.001
Yes	323 (55)	232 (43)	134 (32)	112 (35)	125 (35)	
No	264	311	283	208	229	
Trypanosomes in blood						<0.001
Yes	151 (26)	197 (36)	106 (25)	88 (28)	114 (32)	
No	436	346	311	232	240	
Trypanosomes in CSF						<0.001
Yes	116 (20)	237 (44)	220 (53)	179 (56)	184 (52)	
No	471	306	197	141	170	
CSF leukocyte count (per mm^3)						<0.001
1–5	22 (4)	23 (4)	4 (6)	16 (5)	9 (3)	
6–19	158 (27)	114 (21)	30 (7)	18 (6)	38 (11)	
20–99	161 (27)	131 (24)	106 (25)	89 (28)	109 (31)	
≥100	246 (42)	275 (51)	257 (62)	197 (62)	198 (56)	
Median CSF leukocyte count (per mm^3)	65	101	135	140	116	<0.001
Melarsoprol regimens						<0.001
1 series of 3 injections	17 (3)	7 (1)	26 (7)	14 (5)	5 (1)	
2 series of 3 injections	163 (29)	123 (24)	31 (8)	11 (4)	3 (1)	
3 series of 3 injections	152 (27)	353 (69)	341 (86)	192 (64)	184 (54)	
3 series of 4 injections	221 (40)	29 (6)	0	0	0	
3 series of 3, incremental	0	0	0	44 (15)	24 (7)	
10 daily injections	0	0	0	38 (13)	127 (37)	
Prednisolone						
Yes	177 (30)	339 (62)	341 (82)	320 (100)	354 (100)	
No	410	204	76	0	0	
Melarsoprol-induced encephalopathy						0.002
Yes	31 (5.3)	35 (6.4)	9 (2.2)	11 (3.4)	8 (2.3)	
No	556	508	408	309	346	
Death during treatment						0.25
Yes	34 (5.8)	31 (5.7)	19 (4.6)	21 (6.6)	11 (3.1)	
No	553	512	398	299	343	
All relapses after treatment†						0.004
Yes	31 (5.6)	35 (6.8)	18 (4.5)	34 (11.4)	17 (5.0)	
No	522	477	380	265	326	
Relapses within 2 y of treatment†						<0.001
Yes	21 (3.8)	29 (5.7)	15 (3.8)	32 (10.7)	16 (4.7)	
No	532	483	383	267	327	

*CSF: cerebrospinal fluid.
†Excluding patients who died during treatment.

in patients with CSF trypanosomes, in patients treated from 1994 to 1997, and in patients treated with the incremental dosage regimen; relapses were much less common in patients from the Baboma/Basengele subdistrict. Table 3 also displays the results of the multivariate analysis. Associations between relapses and male sex, CSF trypanosomes, residence elsewhere than the Baboma/Basengele subdistrict, and treatment with the incremental dosage regimen were little altered by adjustment for confounding factors and remained statistically significant. When results were adjusted for these confounders (especially treatment regimens), diagnosis from 1994 to 1997 was no longer associated with a higher probability of relapse. The univariate association between CSF leukocyte count and relapses was also strongly confounded by treatment regimens. Removing from the analysis the small number of patients with a normal pretreatment CSF leukocyte count had no impact on any of the adjusted odds ratios (data not shown).

Discussion

In the Nioki focus of DRC, the frequency of failure of melarsoprol therapy among patients with late-stage *T. b. gambiense* trypanosomiasis increased in 1994 to 1997 but returned to its normal level in 1998 to 2001. This was driven essentially by a high risk for relapse among patients

Table 3. Risk factors for relapses following treatment with melarsoprol (excluding patients who died during treatment)*

	Relapses/total (%)	Odds ratios (95% CI)	Adjusted odds ratios (95% CI)
Sex			
Females	55/1,091 (5.0)	1.00	1.00
Males	80/1,014 (7.9)	1.61 (1.13–2.30)†	1.57 (1.08–2.27)†
Age (y)			
≤14	19/382 (5.0)	1.00	
15–49	97/1,380 (7.0)	1.44 (0.87–2.39)	
≥50	14/257 (5.4)	1.10 (0.54–2.24)	
Year of diagnosis			
1982–1985	31/553 (5.6)	1.00	1.00
1986–1989	35/512 (6.8)	1.24 (0.75–2.04)	0.83 (0.45–1.52)
1990–1993	18/398 (4.5)	0.80 (0.44–1.45)	0.47 (0.23–0.97)†
1994–1997	34/299 (11.4)	2.16 (1.30–3.59)†	0.82 (0.40–1.69)
1998–2001	17/343 (5.0)	0.88 (0.48–1.61)	0.31 (0.13–0.71)†
Area of residence			
Nioki town	40/486 (8.2)	1.00	1.00
Mfimi River	62/783 (7.9)	0.96 (0.63–1.45)	1.04 (0.67–1.61)
Molibampe River	13/197 (6.6)	0.79 (0.41–1.51)	0.76 (0.39–1.48)
Baboma/Basengele	4/302 (1.3)	0.15 (0.05–0.42)‡	0.14 (0.05–0.40)‡
Out of district	16/311 (5.1)	0.60 (0.33–1.10)	0.59 (0.32–1.10)
Trypanosomes in lymph node aspirate			
No	77/1,217 (6.3)	1.00	
Yes	58/888 (6.5)	1.03 (0.73–1.47)	
Trypanosomes in CSF			
No	62/1,233 (5.0)	1.00	1.00
Yes	73/872 (8.4)	1.73 (1.22–2.45)†	1.52 (1.02–2.27)†
CSF leukocyte count (per mm^3)			
1–5	1/94 (1.1)	0.23 (0.03–1.79)	
6–19	15/341 (4.4)	1.00	
20–99	39/572 (6.8)	1.59 (0.86–2.93)	
≥100	80/1,098 (7.3)	1.71 (0.97–3.01)	
Melarsoprol regimens			
1 series of 3 injections	1/69 (1.4)	0.21 (0.03–1.53)	0.29 (0.04–2.18)
2 series of 3 injections	11/331 (3.3)	0.49 (0.26–0.93)†	0.47 (0.23–0.95)†
3 series of 3 injections	80/1,222 (6.5)	1.00	1.00
3 series of 4 injections	15/250 (6.0)	0.91 (0.52–1.61)	0.62 (0.30–1.26)
3 series of 3, incremental	16/68 (23.5)	4.39 (2.40–8.04)	4.68 (2.26–9.69)
10 daily injections	12/165 (7.3)	1.12 (0.60–2.10)	1.58 (0.74–3.38)
Prednisolone			
No	33/643 (5.1)	1.00	
Yes	102/1,462 (7.0)	1.39 (0.93–2.08)	

*CI, confidence interval; CSF, cerebrospinal fluid.
†$p<0.05$.
‡$p<0.001$.

participating in a randomized trial who were treated with a regimen of incremental dosage of melarsoprol (14). The risk for relapse decreased as soon as enrollment of patients in this arm of the trial was terminated. This overall stability in the frequency of postmelarsoprol relapses is remarkable considering that over time the pretreatment characteristics of patients changed in ways (a higher proportion of cases among men, more patients with CSF trypanosomes) that should normally have led to an increase in the risk for relapse. These confounding factors and the otherwise stable crude risk for relapse resulted in significantly lower odds of relapse in patients treated in 1990 to 1993 and 1998 to 2001 compared to those treated at the beginning of the study period. The crude risk for relapse among patients treated in Nioki in 1998 to 2001 was similar to that reported among patients treated in Léopoldville (Kinshasa) 50 years ago (16).

We did not have information on the completeness of follow-up for each patient in the cohort. However, previous studies in Nioki in which this was measured have consistently shown that >80% of patients were followed up for ≥2 years after initial treatment (14,17). During the study period, Nioki hospital was the only one in that part of DRC to offer treatment with eflornithine to patients who relapsed after treatment with melarsoprol. It thus seems unlikely that relapses among our patients would have been identified and managed by other healthcare providers. Some relapsing patients might have died at home without a diagnosis being made, but given the slow course of the disease and the short distances to Nioki hospital, this situation probably did not occur very often. Thus, although using as denominators all patients who survived melarsoprol treatment might have somewhat underestimated the true frequency of relapses, we think that this bias was not substantial and probably did not change over time.

At least 3 factors that generally contribute to the emergence of resistance to antimicrobial agents are not found in the context of the treatment of African trypanosomiasis, which probably explains the stable frequency of postmelarsoprol relapses in Nioki over 2 decades. First, little drug pressure exists: melarsoprol is not used to treat any other condition, and supplies are controlled by a national organization, according to the number of cases reported by each health facility. Second, subtherapeutic doses have not been administered on any sizeable scale; on the contrary, most patients have probably been overtreated ever since the drug became available (15,18). Third, little potential exists for the preferential transmission of melarsoprol-resistant strains because patients with relapses rarely harbor trypanosomes in the bloodstream or lymph nodes, only in the CSF. Such patients are likely not very infectious.

As in Uganda and Angola (4,19), we found an association between the presence of CSF trypanosomes and failure of melarsoprol. Given the limited CSF penetration of melarsoprol, modest geographic variations in in vitro susceptibility might have an impact on the frequency of postmelarsoprol relapses. Twenty-four hours after the administration of melarsoprol, plasma levels are in the range of 2-4 μg/mL, while CSF levels are much lower, from 0.02 to 0.07 μg/mL (18,20,21). For 12 isolates of *T.b. gambiense* from northwestern Uganda, the MIC of melarsoprol was higher (0.009–0.072 μg/mL) than for 2 isolates from Côte d'Ivoire (0.001–0.018 μg/mL); the higher MIC of 0.072 μg/mL was superior to levels that can be expected in CSF (22). Fifty percent inhibitory concentrations measured in *T.b. gambiense* isolates from northwestern Uganda were also higher than in isolates from Côte d'Ivoire (23). In such circumstances, CSF concentrations of melarsoprol might be insufficient to eliminate all parasites. So far, no evidence that genetically determined variations in drug pharmacokinetics might explain this heterogeneity in the frequency of postmelarsoprol failures (21,23). Within the Nioki focus, a lower risk for melarsoprol failure in the Baboma/Basengele subdistrict has been consistently noted for more than a decade (11); we speculate that this might reflect a limited exchange of trypanosomes between the various foci, in which case the in vitro susceptibility of the initial strain(s) in a given focus would be maintained over the years.

Finally, our results suggest that variations in the regimens of melarsoprol used in each country may also explain some, but not all, of the high rates of postmelarsoprol failures in specific locations. A regimen of incremental dosage was indeed used in Uganda (4) and in some patients at Kimpangu hospital, Zaire (24). Whether graded dosing was used in Sudan is unclear. In Angola, the same regimen (3 series of 4 injections: 1.2, 2.4, 3.6, 3.6 mg/kg for each series) was used in M'banza Congo, where 25% of patients relapsed (5), and in Dondo, where only 5%–6% of patients relapsed (15,19). Unfortunately, melarsoprol MICs for strains from the M'banza Congo focus have not yet been measured, but trypanosomes from this focus are likely intrinsically more resistant to melarsoprol than elsewhere.

These findings, although reassuring, underline the necessity of a strengthened surveillance system for African trypanosomiasis, through which secular trends in the frequency of treatment failures among patients with *T.b. gambiense* trypanosomiasis would be monitored in a small number of sentinel centers. The World Health Organization is developing such a system (25). Its measures will need to take into consideration modifications in the baseline characteristics of patients (most of which will be a result of changes in the intensity of case finding), changes in therapeutic regimens, and the completeness of follow-up by using Kaplan-Meier analyses.

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Methicillin-resistant *Staphylococcus aureus* in Community-acquired Skin Infections

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Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common pathogen among patients with skin and soft tissue infections seeking treatment at a Los Angeles (USA) area emergency department. The proportion caused by MRSA increased from 29% in 2001 to 2002 to 64% in 2003 to 2004. No clinical or historical features reliably predict MRSA etiology.

Historically, methicillin-resistant *Staphylococcus aureus* (MRSA) infection was associated with patients in hospitals and skilled nursing facilities. In recent years, reports of community-associated MRSA infections (CA-MRSA) have been increasing (1,2). Such outbreaks have been associated with prisons, intravenous drug use, athletic teams, and men who have sex with men (1,2). CA-MRSA has primarily been described in skin and soft tissue infections (SSTIs), but the agent has also been associated with severe sepsis and pneumonia, primarily in pediatric patients (3,4). Recent studies have described an increasing proportion of MRSA isolates that are community-associated compared to hospital-associated isolates (5), but we are not aware of any published studies reporting the prevalence of CA-MRSA among patients with sporadic SSTI. The proportion of SSTIs that are caused by CA-MRSA has important implications for empiric antimicrobial therapy.

The Study

We participated in several clinical trials of antimicrobial drugs for SSTI for which cultures were obtained from all enrolled patients. This opportunity made it possible for us to determine the prevalence of CA-MRSA among a group of emergency department patients with SSTIs. This report describes the proportion of emergency department patients with community-acquired SSTIs due to MRSA.

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The study was performed in a county-owned hospital in the Los Angeles, California (USA) area, which serves a largely uninsured, low-income population. More than 43,000 persons are treated in the emergency department each year. At the hospital, we have participated in a number of clinical trials of various antimicrobial agents for treating SSTIs. All patients enrolled in these studies had cultures obtained from the infected site. Eligibility criteria for the studies included age ≥18 and an SSTI with purulent material available for culture. One study included patients with uncomplicated infections that were suitable for outpatient treatment with oral agents. Patients were also enrolled in 3 studies of complicated infections for which the treating physicians believed admission for intravenous antimicrobial drugs was indicated. Patients were excluded if they had previously received antimicrobial drugs for the infection, unless antimicrobial drugs had been taken for >72 hours with treatment failure. Patients were also excluded if they had simple abscesses that did not require antimicrobial agents, if they had severe infections involving bone or joint, or if they required amputation of an affected limb.

Specimens were obtained from the site of infection and transported by using sterile Dacron swabs. Specimens were processed and cultured with standard techniques (6). *S. aureus* was identified by colony morphologic features, coagulase tests, and catalase tests. MICs were determined by VITEK, GPS 106 or 109 card (bioMérieux, Durham, NC, USA), according to manufacturer's instructions. MIC breakpoints and quality control protocols were used according to standards established by NCCLS (7).

Clinical data were prospectively collected as part of the clinical trials. In mid-2002, we began prospectively collecting information on recent jail exposure. Those patients enrolled previously were contacted by telephone, if possible, to obtain information on jail exposure. This study was approved by the Olive View–UCLA institutional review board.

From January 2002 through December 2002, a total of 24 patients were enrolled in an outpatient antimicrobial drug study. From August 2001 through March 2004, we enrolled 72 patients in 3 inpatient studies, and each had only 1 site of infection. Patients were 20–60 years of age, with a median age of 42. Men made up 77% of the study group. None of the patients resided in long-term care facilities, and none of the infections was believed to be hospital-acquired.

MRSA was isolated from 44 (46%) of 96 patients (8 outpatients, 36 admitted). The proportion of infections yielding MRSA increased from 14 (29%) of 49 during 2001 to 2002 to 30 (64%) of 47 from January 2003 through March 2004. Other pathogens isolated included the following: 15 methicillin-susceptible *S. aureus*, 19 *Streptococcus* spp., 4 coagulase-negative staphylococci, 2 diphtheroids;

2 *Citrobacter* spp., 2 *Escherichia coli*; and 1 *Enterococcus* sp. No organism was isolated from 7 patients.

Among 44 MRSA patients, 6 had been hospitalized within the last year. None had indwelling catheters or other recognized risk factors for MRSA. Five had diabetes; otherwise, none had a notable associated coexisting illness. Fifteen had previously received oral antimicrobial drugs for the current infection, but treatment was unsuccessful. Nine had recently used injected illegal drugs. Nine were homeless. Of 36 MRSA patients for whom the information was available, 3 had been in Los Angeles County Jail within the last year, where an MRSA outbreak was recently described (2). Most patients had no apparent epidemiologic risk factors associated with recent CA-MRSA outbreaks. No clinical or epidemiologic features were predictive of an MRSA cause (Table).

Antimicrobial susceptibilities of the 44 MRSA isolates were as follows: clindamycin 98%, erythromycin 2%, levofloxacin 16% (64% had intermediate susceptibility to levofloxacin), rifampin 98%, tetracycline 82%, trimethoprim/sulfamethoxazole 100%. Fourteen of our MRSA isolates from early 2003 were tested in the laboratory at the Los Angeles County Department of Health Services. The isolates were found by pulsed-field gel electrophoresis to be identical to the strain associated with the outbreak at the Los Angeles County Jail, which belongs to the USA 300 ST:8 group (8,9). None of the 14 isolates tested had inducible clindamycin resistance by the D test.

Conclusions

Our report demonstrates that the proportion of patients with community-acquired SSTI caused by MRSA is increasing, and CA-MRSA is now the most common cause of community-acquired SSTIs at our center. Other reports have suggested that CA-MRSA is becoming more common in other geographic areas in the United States and Europe (10,11). A high proportion of CA-MRSA strains (such as the USA 300 ST:8 strain) have been found to carry the Panton-Valentine leukocidin gene, which has been associated with SSTI and necrotizing pneumonia (9,12). We have noted anecdotally that many patients with CA-MRSA exhibit a spontaneous abscess or furunculosis that the patient thinks was caused by a spider bite.

The bacterial causes of common community-acquired SSTIs are generally gram-positive organisms such as *S. aureus* and *Streptococcus pyogenes*. Because of the predictable etiology of these infections, most physicians do not routinely obtain cultures from these patients. Obtaining cultures of SSTIs is now of greater importance to monitor the extent of CA-MRSA infections in one's community and guide therapy in areas in which CA-MRSA is already prevalent.

Most community-acquired SSTIs are treated with antimicrobial drugs such as cephalexin and dicloxacillin. Patients requiring intravenous therapy are most commonly given agents such as cefazolin or oxacillin. In areas with a high prevalence of CA-MRSA, empiric treatment for SSTIs with β-lactam agents such as cephalexin or dicloxacillin may no longer be appropriate. Oral agents such as clindamycin or trimethoprim/sulfamethoxazole and rifampin should be considered for CA-MRSA. Although inducible clindamycin resistance was not found in the few patients we tested, clinical failure due to inducible clindamycin resistance among CA-MRSA has been reported (13). Whether the addition of rifampin to trimethoprim/sulfamethoxazole improves outcomes in SSTI is not clear, but this combination appears to be more effective in eradicating MRSA colonization (14). Macrolides, tetracycline, and fluoroquinolones have inconsistent activity against the MRSA isolates identified in our study and other reports of CA-MRSA (11). For severe infections treated in the inpatient setting, clindamycin or vancomycin should be included as part of empiric therapy.

Adequate drainage and débridement of SSTIs are important in treatment. We did not find a higher rate of MRSA among those patients in whom previous antimicrobial drug treatment had been unsuccessful and believe inadequate drainage was the reason.

Whether additional measures to eliminate carriage of MRSA in these patients or their close household contacts would be of any benefit is not known. Chlorhexidine body washes and nasal mupirocin would be reasonable measures for those with recurrent SSTI or close contacts with similar infections (15).

Our report has several limitations. One of the criteria for study enrollment was availability of purulent material

Table. Clinical features and epidemiologic characteristics of patients with skin and soft tissue infections*

Feature/characteristic	MRSA, n = 44 (%)	Other pathogens, n = 52 (%)
Hospitalized in last year	6 (14)	6 (12)
Prior, unsuccessful antimicrobial drug treatment	15 (34)	15 (29)
Injection drug use	9 (20)	16 (31)
Jail in last year†	3/36 (8)	3/48 (6)
Homeless	9 (20)	10 (19)
Abscess present	41 (93)	44 (85)

*MRSA, methicillin-resistant *Staphylococcus aureus*.
†This information was not available for 8 MRSA patients and 4 patients with other pathogens.

for culture. Most patients had skin abscesses. Patients with cellulitis without a purulent exudate are not represented in our study sample. We did not culture every possible SSTI seen at the emergency department, but we believe the patients enrolled in these studies reflect the general population with culturable SSTIs. All samples cultured during 2003 to 2004 were from patients with infections that required hospital admission, so these results may not reflect those patients with minor infections suitable for outpatient treatment. Prevalence of CA-MRSA can vary considerably between geographic areas, and our facility may not be typical of southern California or other areas.

MRSA may now be the most common pathogen among patients with community-associated SSTIs in some areas. Physicians should consider obtaining cultures in these patients. In areas with a high prevalence of CA-MRSA, empiric therapy for SSTIs with agents such as clindamycin or trimethoprim/sulfamethoxazole and rifampin would be appropriate.

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Rifampicin Resistance in Tuberculosis Outbreak, London, England

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Mycobacterium tuberculosis isolates cultured from 6 patients associated with an isoniazid-resistant *M. tuberculosis* outbreak acquired rifampicin resistance. The *rpoB* gene sequence showed that resistance was associated with rare mutations in each isolate. Three isolates had a mutation outside the rifampicin resistance–determining region.

In January 2000, 4 cases of smear-positive pulmonary tuberculosis (TB) caused by *Mycobacterium tuberculosis* in young men from the local community were identified during a 1-week period at a hospital in north London, United Kingdom (1). Three isolates were shown to be isoniazid-monoresistant TB. Further investigation showed 155 confirmed or probable cases, 132 in London and 23 outside London, which suggests a large outbreak of a unique strain in the London area (2). Confirmed case-patients were defined as patients with isolates of *M. tuberculosis* resistant to isoniazid that had the same band pattern on restriction length polymorphism typing (RFLP); these patients were residents of London at the time of their diagnosis, which had been made since January 1995 (2). Probable cases were defined as for confirmed cases except that isolates underwent rapid epidemiologic typing [RAPET] but are awaiting RFLP typing (2). RAPET is a rapid screening molecular typing method developed at the Mycobacterium Reference Unit (MRU) (3). In October 2002, the TB isolate from 1 patient associated with the outbreak had developed resistance to rifampicin. Initially, the isolate was tested with a commercial line probe rifampicin resistance–determining hybridization assay, Inno-LiPA (Innogenetics Belgium, Gent, Belgium). However, the line probe assay failed to identify rifampicin resistance in this isolate, and rifampicin resistance was only detected on phenotypic antimicrobial sensitivity testing at MRU. Subsequently, 5 additional rifampicin-resistant isolates from different patients associated with this outbreak were identified at MRU. The aim of this study was to determine the basis for the rifampicin resistance in these 6 isolates by sequencing the entire *rpoB* gene.

Rifampicin resistance can occur as a result of mutations on the *rpoB* gene that encodes the β-subunit of RNA polymerase (4). More than 95% of these mutations occur on an 81-bp fragment of the gene between bases 1276 and 1356 (432–458 in the *rpoB* gene of *M. tuberculosis* and codon 507–534 in the *Escherichia coli rpoB* gene) (5,6). This region is known as the rifampicin resistance–determining region (RRDR), or "hotspot," and is used as a target for direct sequencing and commercial line probe assays.

The Study

The isoniazid-monoresistant and multidrug-resistant tuberculosis (MDR-TB) isolates were obtained from the patients' source hospital or MRU. All isolates were identified as belonging to the same strain of *M. tuberculosis* (RAPET or IS*6110* typing), and all drug-susceptibility testing was carried out at MRU according to standard procedures. The wildtype control isolate used for these studies was *M. tuberculosis*, H37Rv (ATCC, 9360 National Collection of Type Culture, London, UK). Three isolates, 018, 483, and 915, were from patients in whom MDR-TB developed as a result of poor compliance with therapy, whereas isolates 604, T7, and 371 were from patients who contracted primary MDR-TB.

DNA was prepared from the 6 isolates of *M. tuberculosis* by emulsifying 2–3 colonies in 400 μL Tris-EDTA buffer and heating the suspension in a water bath for 40 min at 80°C. Polymerase chain reaction (PCR) was performed on the extracted DNA with 6 sets of primers designed to amplify 6 overlapping fragments of the *rpoB* gene from the 6 *M. tuberculosis* isolates (Table). Ten microliters of DNA was added to the PCR mix containing 81.4 μL PCR-quality water, 10 μL potassium chloride buffer (Bioline Ltd., London, UK), 0.4 μL of each primer (100 mmol) (Sigma-Genosys Ltd., Haverhill, UK), 3 μL deoxynucleoside triphosphates (5 mmol), and 1 μL Taq (Bioline). The amplification was performed on a Techgene thermal cycler (Techne, Princeton, NJ, USA). PCR products were separated by gel electrophoresis on a 1.5% agarose gel, and DNA bands were stained with ethidium bromide. Primers and excess nucleotides were removed from the amplified DNA with a PCR clean-up kit (Qiagen, Inc., Valencia, CA, USA). The amount of DNA in the cleaned-up product was quantified by comparing the intensity of the band to bands of known intensity in a HyperLadder marker (Bioline).

Forward and reverse cycle sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction DNA sequencing kit

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Table. Sequences of 6 sets of primers designed to amplify 6 overlapping fragments of the *rpoB* gene from *Mycobacterium tuberculosis* isolates

Fragment	Amplicon size	Primer position	Primers
1	697 bp	–135 to –112*	Forward 5′ GTT TAG TTG CGT GCG TGC
		541–562	Reverse 5′ CTTGTCAAT GGT CTC GTC GAA
2	688 bp	451–472	Forward 5′ TTC CCG ATG ATG ACC GAG AAG
		1119–1139	Reverse 5′ GGA TCA GCT CGC CGA CCG TA
3	706 bp	1029–1048	Forward 5′ CGA GGG TCA GAC CAC GAT G
		1714–1735	Reverse 5′ GCG GGG CGA GAC GTC CAT GTA
4	681 bp	1624–1645	Forward 5′ ATC GAT GCG GAC GGT CGC TTC
		2284–2305	Reverse 5′ CGG GAT GTC GCG GGT GAT CTC
5	811 bp	2194–2215	Forward 5′ TCC AAC CGC CTG GTC GAA GAG
		2887–3005	Reverse 5′ CGT CGA CAC AAT GGC GTT
6	847 bp	2797–2815	Forward 5′ TGT GCC CAC AGC GGC TGG
		+113 to +135*	Reverse 5′ CTT TTT GAC CTC GCC ATA GGA C

*Denotes a primer position in the sequence before the start (–) or after the end (+) of the *rpoB* gene sequence.

(Applied Biosystems, Inc., Foster City, CA, USA). Briefly, 40 ng of cleaned-up DNA was added to 10.8 μL PCR-quality water, 3 μL buffer, 3.2 μL of forward or reverse 1 mmol primer, and 1 μL of cycle sequencing ready reaction mix. The labeled DNA was precipitated by adding 14.5 μL PCR-quality water, 62.5 μL 95% ethanol, and 3 μL sodium acetate solution (2.3 mol/L) and centrifuging (13,000 × *g*, 15 min, 4°C). The supernatant was removed with a fine-tipped pipette, and the pellet was cleaned with 200 μL 70% ethanol and then recentrifuged (13,000 × *g*, 15 min, 4°C). Again the supernatant was removed, and the pellet was dried at 37°C for 30 min. Four microliters of formamide and 1 μL of dextran loading buffer were added to each pellet, and 1.5 μL of sample was added to each well of the sequencing gel. The 6 fragments of the *rpoB* gene from each of the 6 isolates were then sequenced with an ABI 377 Applied Biosystems sequencer. The sequences obtained from each isolate were joined together to form a continuous whole gene sequence, aligned with ClustalW (http://www.ebi.ac.uk/clustalw/) and compared to the wildtype to identify base-pair mismatches.

In addition, all 6 isolates were tested with a line probe resistance–determining hybridization assay, Inno-LiPA (Innogenetics Belgium), as described in the manufacturer's instructions. Briefly, an 81-bp region of the *rpoB* gene was amplified with biotinylated primers, which yielded a biotinylated target sequence, and hybridized with specific oligonucleotide probes immobilized on a parallel strip. After hybridization, streptavidin labeled with alkaline phosphatase was used to detect any hybrids. Inno-LiPA consists of 10 oligonucleotide probes (19–23 bases in length), encompassing the 81-bp region (RRDR) of the *rpoB* gene. One is specific for *M. tuberculosis* complex, whereas the other 5 partially overlapping wildtype probes (S1–S5) cover the region from positions 507 to 534 of the *rpoB* gene. These S-probes hybridize to the wildtype (rifampicin-sensitive) DNA sequence. Failure of any of these S-probes to hybridize indicates that a mutation has occurred. Four other probes (R2, R4a, R4b, and R5) are specific for amplicons carrying the most common *rpoB* mutation that confers rifampicin resistance.

The results of the Inno-LiPA assay showed 5 of the 6 isolates, which were phenotypically rifampicin-resistant, were negative. The target DNA hybridized to all 5 wildtype S-probes and none of the R-probes, which demonstrated that the assay failed to detect rifampicin resistance in these 5 isolates. The Inno-LiPA assay cannot detect mutations outside the RRDR. Failure to detect rare mutations within the RRDR may be caused by nonspecific hybridization of the wildtype S-probes because of slight fluctuations in temperature during the hybridization process. Isolate 483 showed a weak DNA hybridization reaction with the S4 probe, indicating that a mutation was present on codon 451 (codon 526 in *Escherichia coli*). The exact nature of the mutation could not be determined with this assay.

Analysis of the sequence data identified specific mutations in *rpoB* in all 6 strains studied (Figure 1). Three had mutations within the RRDR, and of these, 2 were C-to-G mutations at codon 456, inducing a serine to tryptophan amino acid conversion (S456W). The third was an A-to-G mutation at codon 451, resulting in a change from histidine to arginine (H451R) (Figure 1). No other mutations were identified in the DNA sequences outside the RRDR in these 3 isolates. The 3 other isolates had the mutation G to T at codon 176, outside the RRDR, which caused a change from valine to phenylalanine (V176F). Neither RRDR mutations nor any other mutations were found in the *rpoB* gene sequences of these 3 isolates.

Of the mutations found within the RRDR, H526R occurs in <4%, and S531W occurs in ≈1.4% of all rifampicin-resistant isolates, respectively (7). Mutations outside the RRDR account for <4% of rifampicin resistance, and few have been described (7–9). In a previous study (7), V176F was found in 5 of 18 isolates with no mutations in the RRDR from Asia (9,10) and Africa

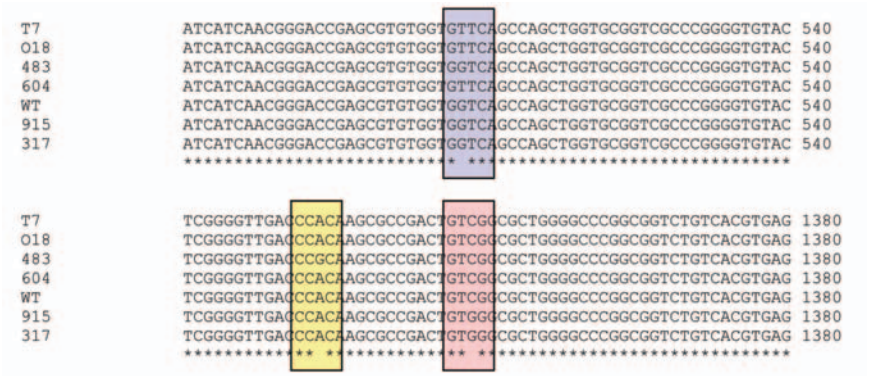

Figure 1. Alignment of *rpoB* gene sequences showing the mutations detected in this case study. WT, wild-type; T7, 018, 604 - V176F (G to T, highlighted in blue); 483, H526R (A to G, highlighted in yellow); 915, 317, S531W (C to G, highlighted in pink).

(11,12). To our knowledge, this is the first time this type of mutation has been detected in strains isolated in the United Kingdom.

Three different mutations in the *rpoB* gene, V176F, H526R and S531W, were detected in the isolates associated with the isoniazid-resistant outbreak. Given that all the mutations found in this study are rare and that MDR-TB developed during treatment in 3 patients (018, 483, and 915) (Figure 2), the rifampicin-resistance mutations observed in these 3 patients likely occurred on 3 independent occasions, as a result of poor compliance with therapy. Two of these patients, 018 and 915, were contacts of the 3 patients who subsequently had primary MDR-TB (patients T7 and 604 [V176F] and 317 [S531W]) (Figure 2).

Conclusions

Our study highlights problems associated with using a line probe hybridization assay to detect rifampicin resistance in *M. tuberculosis*. The Inno-LiPA assay cannot detect mutations outside the RRDR, and failure to detect rare mutations within the RRDR may be caused by non-specific hybridization of the wildtype S-probes because of slight fluctuations in temperature during the hybridization process. In this study, all the rare mutations, inside and outside the RRDR, were detected by sequencing the entire *rpoB* gene. The inability to identify MDR-TB isolates results in treatment failure and increased risk for transmission of resistant disease in the community. We recommend that, when the index of suspicion for MDR-TB is high and the line probe assays fail to detect mutations conferring rifampicin resistance, the entire *rpoB* gene should be sequenced to prevent unnecessary delay in diagnosing MDR-TB.

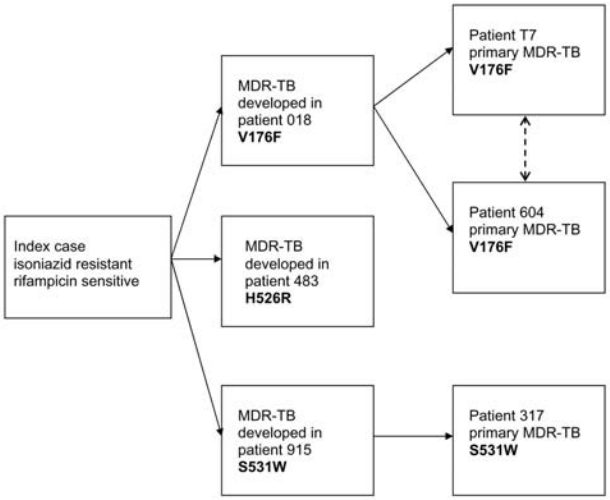

Figure 2. Suggested epidemiologic relationship between 6 cases of multidrug-resistant tuberculosis (MDR-TB). Resistance to rifampicin developed in patients 018, 483, and 915 while on therapy, whereas patients T7, 604, and 317 contracted primary MDR-TB. The type of mutation present in each patient's strain of MDR-TB is highlighted in bold.

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Dr. Jenkins works as a registered clinical scientist in the Department of Microbiology at the Royal Free Hospital in London. Her research involves molecular typing and identification of pathogenic bacteria in general, with a specific interest in *Mycobacterium* species.

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Free-living Canada Geese and Antimicrobial Resistance

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We describe antimicrobial resistance among *Escherichia coli* isolated from free-living Canada Geese in Georgia and North Carolina (USA). Resistance patterns are compared to those reported by the National Antimicrobial Resistance Monitoring System. Canada Geese may be vectors of antimicrobial resistance and resistance genes in agricultural environments.

The epidemiology of zoonotic diseases is growing in scope and importance in public health as the interface between animal and human habitats narrows and new diseases emerge. Historically, zoonotic disease research has emphasized occupational or animal-origin foodborne exposures. However, environmental exposure pathways to zoonotic pathogens are increasingly documented as foodborne disease surveillance and control efforts prove successful. Nonanimal-origin sources of zoonotic infection, such as raw fruits and vegetables, nuts, and water, are reported more often (1–3). Although fecal contamination of raw food products in fields is an important source of zoonotic infection (1), the source of contamination is usually not determined. Consequently, environmental reservoirs of microbes of public health importance need to be investigated.

Canada Geese (*Branta canadensis*)(Figure) populations have steadily increased in the past 50 years and have become a nuisance in some areas (4,5). The large amount of feces produced by geese congregating around surface water bodies is a source of environmental contamination and, potentially, zoonotic pathogens (4–7). Feces from large flocks are major contributors to fecal coliform levels in reservoirs that supply drinking water for some cities (5,6), and free-living bird populations can serve as reservoirs for pathogenic bacteria such as *Salmonella* (8,9), *Escherichia coli* (10), *Campylobacter* (10,11), *Listeria* (11), and *Chlamydia* (9). Thus, wild bird populations can amplify and eventually transmit infectious microbes to humans by directly contaminating agricultural fields or surface waters used for drinking, recreation, or crop irrigation.

Free-living and domestic bird populations can also be reservoirs of drug-resistant bacterial pathogens or resistant genetic elements. Antimicrobial-resistant organisms in domestic animals such as poultry, beef, and swine are well documented (12,13) and have been implicated as reservoirs for multidrug-resistant foodborne pathogens. Interaction with waste materials from these livestock species may confer resistant pathogens and genetic elements to free-ranging wildlife, potentially creating an additional environmental reservoir of resistant organisms (8). We examine the impact of habitat on antimicrobial susceptibilities of *E. coli* isolates recovered from different flocks of resident, free-living Canada Geese to determine the potential for these animals to be additional sources of antimicrobial resistance through exposure pathways that originate in the environment.

The Study

Collaborators from separate regions collected cloacal swabs or fresh guano from Canada Geese at 4 geographically diverse surface water bodies in Georgia (n = 72) and North Carolina (n = 90). Cloacal swabs were taken from each of 24 Canada Geese captured at each Georgia site. At the North Carolina site, groups of resident geese were followed by a study investigator on 8 occasions, and fresh guano was collected from 7 to 8 birds. Geographic locations represented the following land uses: recreational (Stone Mountain Park, GA), agricultural (Craven County, NC; Griffin, GA), and industrial (Lake Juliette, GA). The Craven County site was near a swine housing facility, and geese were observed using swine waste lagoons and adjacent surface waters and farm fields. The Griffin site was not near food animal facilities but was adjacent to test plots used by crops and soils scientists at the University of Georgia Experiment Station. Neither the Griffin Lake nor the lake at Stone Mountain Park is downstream from a sewage treatment plant; however, Lake Juliette, a reservoir for an electrical generation plant, is formed by Rum Creek and water pumped from the Ocmulgee River, on which several sewage treatment facilities reside.

Isolation and biochemical identification of *E. coli* from free-ranging geese were performed as follows. In Georgia, cloacal swabs were transported to the investigators' laboratory and stored at 4°C. The following day, swabs were used to inoculate brain heart infusion broth (BHIB) and incubated overnight at 37°C. BHIB cultures were subsequently streaked for isolated colonies on MacConkey agar plates; 1 lactose-fermenting colony was selected from each goose sample that exhibited growth on agar. In North

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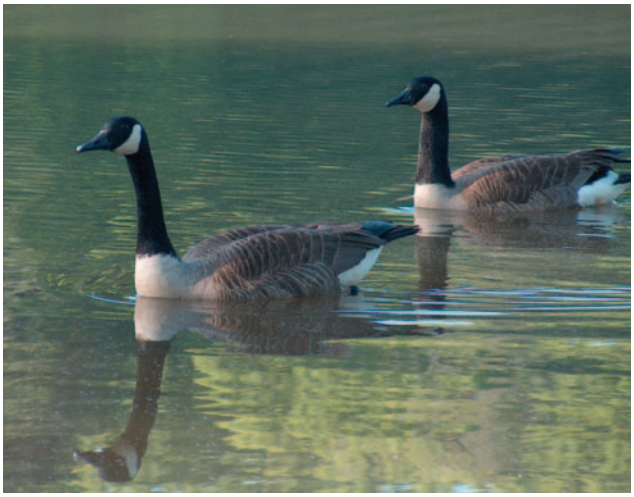

Figure. Free-living populations of Canada Geese (*Branta canadensis*) can serve as reservoirs of antimicrobial-resistant bacteria such as *Escherichia coli*.

Carolina, bird samples were pooled and transported to the laboratory on ice in sterile, plastic bottles and stored at 4°C overnight. A 1-g sample of guano was diluted in sterile phosphate-buffered saline, and 3 separate dilutions were filtered through 47-mm, 0.45-μm pore-size, gridded cellulose ester filters. After overnight culture on mFC agar plates at 37°C, filters containing a countable number of fecal coliform colonies (20–100 CFUs) were transferred to plates containing nutrient agar and 4-methylumbelliferyl-β-D-glucuronide (MUG) and incubated for an additional 3–4 h. Colonies fluorescing blue under long wavelength UV light were selected for biochemical confirmation. Up to 5 presumptive *E. coli* colonies were selected from each sampling round. All isolates were identified to genus and species level with the Vitek System (bioMériux Vitek, Hazelwood, MO, USA).

Antimicrobial-susceptibility patterns for each confirmed bacterial isolate were determined by broth microdilution with the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, OH, USA) and interpreted according to NCCLS criteria for dilution susceptibility testing methods when applicable (14). Antimicrobial susceptibilities were assessed for amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim/sulfamethoxazole. *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were quality-control organisms. Differences in the proportion of resistant isolates were analyzed by chi-square test (SAS, ver. 8.01, SAS Institute Inc., Cary, NC, USA).

Isolates were also screened for class 1 integrase gene *intI*1 and integron-associated antimicrobial resistance genes *sul1* and *aadA1* by polymerase chain reaction (PCR). Isolates that exhibited resistance to β-lactam antimicrobial agents were screened by PCR for TEM β-lactamase gene, bla_{TEM}, with appropriate positive and negative control strains. DNA template for PCR was prepared as described by Hilton et al. (15).

The Table describes antimicrobial resistance phenotypes and associated resistance determinants in *E. coli* isolates recovered from Canada Geese stratified by geographic site. No gram-negative enteric bacteria were isolated from geese at Stone Mountain Park, although *E. coli* isolates were recovered from the bird waste from Griffin, Lake Juliette, and Craven County. The number of isolates recovered was much higher among geese in agricultural areas compared to other land usages (e.g., industrial or recreational). The proportion of isolates resistant to antimicrobial agents was significantly greater ($p = 0.0004$) among *E. coli* isolates from Craven County geese, where interaction with swine waste lagoons was observed. Antimicrobial resistance patterns in this population matched those most commonly reported for swine *Enterobacteriaceae* from the National Antimicrobial Resistance Monitoring System (NARMS) studies (e.g., tetracycline, streptomycin, sulfamethoxazole, and ampicillin resistance) (12,13). Most *E. coli* isolates (72%) recovered from Craven County geese exhibited resistance to ≥1 antimicrobial agent. In contrast, resistant *E. coli* recovered from agricultural geese in Georgia (Griffin) with no apparent contact with livestock wastes had a lower proportion of resistance (19%) and only exhibited resistance to β-lactam antimicrobial agents (cefoxitin-amoxicillin/clavulanic acid-cephalothin).

All *E. coli* isolates, except those from Craven County, were negative for class 1 integrons. Forty-four percent of *E. coli* isolates (n = 25) from Craven County Canada Geese possessed ≥1 antimicrobial-resistant determinant. Nine *E. coli* isolates were positive for class 1 integrase gene *intI*1; 6 isolates possessed a TEM β-lactamase.

Conclusions

Outbreaks of illness associated with raw food products have been increasing, in part because of increased human consumption of fresh produce (1). However, several sources of preharvest contamination have been identified, including fecal material, contaminated irrigation water, and wild fowl (1). In a previous study, 32% of the *Salmonella* isolates from wild birds submitted to the Southeastern Cooperative Wildlife Disease Study were resistant to sulfamethoxazole, and 18.1% were resistant to both sulfamethoxazole and streptomycin (8). These findings are likely a result of interaction of these populations

Table. Antimicrobial resistance phenotypes and genotypes of *Escherichia coli* isolated from Canada Geese sampled in Georgia and North Carolina

	Site†			
	Lake Julliette	Craven County	Griffin	Stone Mountain
Antimicrobial resistance phenotype* and genotype	n = 2	n = 25 (%)	n = 21 (%)	n = 0
Pansusceptible‡	2	7 (28)	17 (81)	
Ampicillin		5 (20)	1 (5)	
Amoxicillin/clavulanic acid		0	2 (10)	
Cefoxitin		0	2 (10)	
Cephalothin		1 (4)	4 (19)	
Tetracycline		16 (64)	0	
Sulfamethoxazole		6 (24)	0	
Gentamicin		2 (8)	0	
Kanamycin		2 (8)	0	
Streptomycin		14 (56)	0	
Nalidixic acid		1 (4)	0	
Resistance to ≥3 antimicrobial agents		12 (48)	2 (10)	
Integron and antimicrobial resistance genes				
*intI*1		9 (36)	0	
sul1		3 (12)	0	
aadA1		3 (12)	0	
bla_{TEM}		6 (24)	0	

*All isolates, regardless of geographic locale, were susceptible to ceftiofur, ceftriaxone, imipenem, amikacin, apramycin, chloramphenicol, ciprofloxacin, and trimethoprim/sulfamethoxazole.
†No gram-negative bacteria were isolated from geese captured at Stone Mountain.
‡Isolates were susceptible to all 18 antimicrobial agents tested.

with environmental sources of enteric bacteria. In our study, the spectrum of *E. coli* resistance was very different among agricultural habitat geese, depending upon their exposure to livestock wastes. With growing populations of Canada Geese and associated evidence that they contribute to microbial water contamination (5,6), we hypothesized that observed resistance patterns might be related to the anthropogenic land usage of the bird habitats and that Canada Geese could serve as a vector of antimicrobial resistance genes between sources of fecal wastes and other environmental media. Little or no resistance was observed among the *E. coli* isolates recovered from Canada Geese in regions with no known direct contact with liquid wastes. However, geese in direct contact with liquid swine wastes had a significantly higher prevalence of antimicrobial resistance. Comparing these data with those reported recently by NARMS shows similar resistance profiles between *E. coli* isolates recovered from Canada Geese in contact with livestock wastes (Craven County) and those recovered from both food animals and fresh fruits and vegetables (12,13). In addition, a substantial number of isolates from several Canada Geese that had direct contact with lagoons containing liquid swine waste carried integrons and their associated resistance genes.

This and other studies suggest that resident, free-living, and migratory birds can be potential vectors of zoonotic pathogens, including antimicrobial-resistant variants, between waste-handling facilities and other agricultural resources, such as crops and water. Although all of our study populations of Canada Geese were nonmigratory, this species could serve to disperse bacteria between widely separated locations. In addition, since these birds use farm ponds and waste lagoons and graze on pastures inhabited by cattle and other livestock, the opportunities exist for new health problems in wildlife populations to emerge as well as new reservoirs of zoonotic disease to form. This work is the basis of continuing efforts to examine the potential role of wildlife in agricultural habitats as vectors of antimicrobial resistance in the environment.

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Macrolide- and Telithromycin-resistant *Streptococcus pyogenes*, Belgium, 1999–2003[1]

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We found a 13% macrolide resistance in 3,866 *Streptococcus pyogenes* isolated from tonsillopharyngitis patients; 59% macrolide-resistant isolates were distributed in 5 clones, suggesting the importance of both resistance gene transfer and clonal dissemination in the spread of these organisms. We also report one of the largest collections of telithromycin-resistant isolates.

Streptococcus pyogenes causes several million cases of upper respiratory tract infection each year. The problem of these infections is growing as resistance increases among *S. pyogenes* to the macrolide group of antimicrobial drugs commonly used to treat such infections (1–4). *S. pyogenes* acquires resistance by 2 main mechanisms. The first is active drug efflux mediated by an ATP-binding cassette transporter wherein *mef*(A) encodes the transmembrane domains and *msr*(D) encodes the ATP-binding domains (5). This pattern of resistance is demonstrated by an M phenotype. In the second mechanism, gene products of *erm*(B) or *erm*(A) methylate the macrolide-binding site on 23S rRNA and stall bacterial protein synthesis. This pattern of resistance is demonstrated by either a constitutive (cMLS) or an inducible (iMLS) phenotype. A third, rare, mechanism is modification of the drug binding site on rRNA by mutation that is expressed as an M or a cMLS phenotype. The newest generation of macrolides, the ketolides, are also active against macrolide-resistant strains; however, few *S. pyogenes* strains of the cMLS phenotype have been found to be ketolide resistant (6).

In Belgium, the first ketolide to be used clinically, telithromycin, was approved in October 2002 to treat community-acquired respiratory infections in patients >12 years of age. We investigated the temporal trends in resistance and clonality among macrolide (including telithromycin)-resistant *S. pyogenes* recovered from patients with tonsillopharyngitis during surveillance studies conducted in Belgium.

The Study

During 1999–2003, a total of 4,031 nonduplicate, putative *S. pyogenes* isolates were collected from 10 Belgian provinces at the reference center with the date of isolation, specimen source, and patient's age and residential address. By using a battery of tests, for example, β-hemolysis on blood agar, Gram stain, catalase production, pyrrolidonyl arylamidase, presence of Group A antigen, and bacitracin susceptibility, 3,866 isolates were confirmed to be *S. pyogenes*. The age of the patient was known in 3,654 cases. Population statistics are detailed in the first half of Table 1.

By using erythromycin (78 μg) and clindamycin (25 μg) double-disk diffusion (Neo-Sensitab discs; Rosco, Taastrup, Denmark), all 3,866 *S. pyogenes* isolates were further screened for a phenotypic expression of macrolide resistance, which was identified in 506 (13%) isolates. The proportion of macrolide-resistant isolates among the total *S. pyogenes* isolated from each of the 10 Belgian provinces fluctuated from 0% to 40% over the 5 years studied. The 3 known phenotypes, cMLS, iMLS, and M, were identified in 209 (41%), 18 (4%), and 279 (55%) isolates, respectively. Changes in prevalence of the 3 phenotypes among macrolide-resistant *S. pyogenes* over 5 years are presented in the second half of Table 1.

MICs of erythromycin, clindamycin, tetracycline (Sigma-Aldrich, St. Louis, MO, USA), clarithromycin (Abbott, Louvain-la-Neuve, Belgium), azithromycin (Pfizer, Groton, CT, USA), and telithromycin (Aventis, Romainville, France) were further determined by agar dilution (7). Susceptible and resistance breakpoints for telithromycin were ≤1 μg/mL and ≥4 μg/mL, respectively. Briefly, a 10^4 CFU/spot inoculum was incubated at 37°C for 18–24 h in ambient air. The MIC profiles of the 3 macrolide-resistant phenotypes to various antimicrobial drugs are presented in the online Appendix Table 1 (available at http://www.cdc.gov/ncidod/eid/vol11no06/04-1247_ app1.htm). The yearly prevalence (1999–2003) of telithromycin resistance (MIC ≥4 μg/mL) among macrolide-resistant *S. pyogenes* was 2%, 7%, 11%, 13%, and 10%, respectively. Thus, the total telithromycin-resistant isolates (N = 50) identified here constitute the largest collection reported. Of the 50 telithromycin-resistant *S. pyogenes*, 49 belonged to the cMLS and 1 to the iMLS phenotype. These isolates exhibited erythromycin MICs of 128–>512 μg/mL. Thirty (60%) telithromycin-resistant

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Table 1. Yearly prevalence of *Streptococcus pyogenes* isolates screened and of macrolide-resistant *S. pyogenes* distributed by patient age group and phenotype, 1999–2003

	1999	2000	2001	2002	2003
Total *S. pyogenes* screened	598	336	633	1,226	1,073
Isolated from adults (mean age, 34.7 y; SD, 11.1 y; range, 17 to 91 y)	220 (36.7%)	144 (43.1%)	245 (38.7%)	469 (38.2%)	453 (42.0%)
Isolated from children (mean age, 7.2 y; SD, 3.5 y; range, 3 mo to 16.9 y)	357 (59.6%)	172 (51.2%)	367 (58.0%)	675 (55.0%)	552 (51.0%)
Macrolide-resistant *S. pyogenes*	81 (14%)	41 (12%)	73 (12%)	215* (18%)	96* (9%)
Isolated from adults	23 (4%)	16 (5%)	29 (5%)	82 (7%)	38† (4%)
Isolated from children	56 (9%)	22 (7%)	44 (7%)	126‡ (10%)	50† (5%)
Constitutive phenotype	49/81 (8%)	10/41§ (3%)	28/73 (4%)	68/215 (6%)	54/96 (5%)
M phenotype	32/81 (5%)	29/41 (9%)	39/73 (6%)	141/215 (12%)	38/96§ (4%)
Inducible phenotype	–	2/41 (1%)	6/73 (1%)	7/215 (1%)	4/96 (0.4%)

*Increase and decrease in macrolide resistance from 2001 to 2002 and from 2002 to 2003, respectively, was significant ($p<0.001$).
†Prevalence of macrolide-resistant *S. pyogenes* decreased significantly among both children and adults from 2002 to 2003 ($p<0.0001$).
‡Prevalence of macrolide-resistant *S. pyogenes* increased significantly among children from 2001 to 2002 ($p = 0.005$).
§Decrease in prevalence of cMLS isolates from 1999 to 2000 ($p = 0.005$) and of M phenotype isolates from 2002 to 2003 ($p<0.0001$) was significant.
Pearson's χ^2-test with Bonferonni post-hoc adjustments was used for all multiple comparisons. $p<0.05$ (2-sided) was significant.

S. pyogenes were isolated from children, of which 28 (56%) were ≤12 years of age.

We further investigated clonality in all macrolide-resistant isolates and in a random selection of 331 macrolide-susceptible isolates by pulsed-field gel electrophoresis (PFGE) and *emm* typing on reverse line blotting as described previously (1). PFGE was performed by using *Sma*I; however, for most *mef*(A)-positive isolates that proved refractory to *SmaI* restriction, *SfiI* restriction was utilized. PFGE patterns were analyzed by using GelCompar software 4.0 (Applied Maths, Kortrijk, Belgium). The 506 macrolide-resistant *S. pyogenes* were typed into 17 *emm* types and 76 PFGE types, of which 53 (70%) types were distributed among M phenotype isolates (Appendix Table 2 available at http://www.cdc.gov/ncidod/eid/vol11no06/04-1247_app2.htm). Ratios of PFGE types to number of *S. pyogenes* isolates were 0.18 and 0.09 for the M and cMLS phenotypes, respectively. Table 2 shows the temporal evolution over 5 years of the 5 major cMLS and M phenotype clones. Clones 1, 4, and 23 constituted 99%, 98%, and 100% of all the macrolide-resistant *emm*22, *emm*28, and *emm*11, respectively, isolated during the course of this study, while clones 1,001 and 1,002 constituted 97% and 39% of the *emm*1 and *emm*4 macrolide-resistant *S. pyogenes* serotypes, respectively. Serotypes *emm*1, *emm*4, *emm*11, *emm*22 and *emm*28 formed 70% of the total macrolide-resistant *S. pyogenes*. Among the 331 macrolide-susceptible *S. pyogenes* analyzed, the prevalence of clones 1, 4, 23, 1,001, and 1,002 was 5% (n = 18), 1% (n = 3), 0.3% (n = 1), 2% (n = 5), and 0.3% (n = 1), respectively (data not shown). Telithromycin resistance was distributed among 7 cMLS serotypes (*emm*22, *emm*28, *emm*11, *emm*12, *emm*77, *emm*6, and, *emm*2).

We next studied the genotype for the 3 macrolide-resistant phenotypes. Polymerase chain reaction was performed for *erm*(A), *erm*(B), and *mef*(A) (1,9,10). Isolates negative for all 3 genes were screened for ribosomal mutations in L4, L22, and portions of 23S RNA genes by using published primers (11). Amplimers were analyzed by direct double-strand sequencing (3730 DNA Analyzer, Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator Version 3.1 Kit (Applied Biosystems). Nucleotide sequence alignment was done with SeqMan (DNASTAR Inc., Madison, WI, USA). Phenotypic and genotypic profiles of the macrolide-resistant *S. pyogenes* were generally consistent; however, 3% of the resistant iso-

Table 2. Temporal changes in the distribution of major pulsed-field gel electrophoresis and *emm* types among the 3 macrolide-resistant *Streptococcus pyogenes* phenotypes*

Macrolide-resistant phenotype	Pulsed-field gel electrophoresis cluster (*emm* type)	Frequency (n = 506)	No. (%) of macrolide-resistant *S. pyogenes*				
			1999 (n = 81)	2000 (n = 41)	2001 (n = 73)	2002 (n = 215)	2003 (n = 96)
Constitutive	1 (*emm*22)	70	45 (56%)	7 (17%)†	9 (12%)	7 (3%)	2 (2%)
	4 (*emm*28)	45	–	–	4 (5%)	15 (7%)	26 (27%)
	23 (*emm*11)	28	–	–	1 (1%)	6 (3%)	21 (22%)
M	1001 (*emm*1)	128	7 (9%)	12 (29%)	23 (32%)	80 (37%)‡	6 (6%)‡
	1002 (*emm*4)	28	2 (2.5%)	2 (5%)	7 (10%)	7 (3%)	10 (10%)

*A ≤6-band difference was employed to assign isolates to a clone according to Tenover et al. (8). PFGE clusters up to 100 designate restriction with *Sma*I and clusters ≥1,000 designate restriction with *Sfi*I.
†Decrease in prevalence of the 1/*emm*22 clone from 1999 to 2000 was highly significant ($p<0.001$).
‡Both the increase and decrease in prevalence of the 1001/*emm*1 clone from 2001 to 2002 and from 2002 to 2003, respectively, were significant ($p<0.01$).

lates carried 2 resistance genes. Of the 209 cMLS isolates, 199 carried *erm*(B), 9 carried *erm*(B)+*mef*(A), and 1 carried *erm*(B)+*erm*(A). Of the 279 M phenotype isolates, 273 carried *mef*(A), 1 carried *erm*(B)+*mef*(A), and 4 carried *mef*(A)+*erm*(A). The 1 isolate that was negative for all 3 genes carried a single A2059G (*Escherichia coli* numbering system) mutation in the 23S rRNA gene. The A2059G mutation, although quite frequent in *S. pneumoniae*, has been rarely observed in *S. pyogenes*. Finally, of the 18 iMLS isolates, 5 carried *erm*(B) and 13 carried *erm*(A).

Ten percent of the macrolide-resistant strains harboring *erm*(B) alone or with *mef*(A) were also telithromycin-resistant, and telithromycin has additional binding sites on 23S rRNA. Therefore, we hypothesized that either mutations in the *erm* gene promoter region have upregulated methylase expression or that mutations in the coding region have changed the methylase specificity to include the additional binding sites of telithromycin. Alternatively, mutations at the additional binding sites on the 23S rRNA genes might also disable the binding arm; however, a recent study described only a low level of telithromycin resistance in the presence of these mutations (12). Utilizing 3 pairs of overlapping primers (primer sequences available on request), DNASTAR software, and sequence data of *Tn1545* (National Center for Biotechnology Information, Rockville, MD, USA, accession no. X52632), the entire *erm*(B) gene, including the promoter and control peptides, were sequenced from 10 telithromycin-resistant isolates. In addition, L4, L22, and portions of 23S rRNA genes were also amplified as above. Analysis of the sequencing data showed a single H118R (A677G) substitution in the *erm*(B) coding region of all 10 telithromycin-resistant isolates. While our study was ongoing, the H118R substitution in *erm*(B) was also confirmed independently for 2 telithromycin-resistant isolates (6).

Conclusions

We demonstrated in this study that overall macrolide resistance in Belgium is driven by an epidemic spread of a few major clones as well as by resistance gene transfer among genetically diverse *S. pyogenes*. On average, we demonstrated a 2-fold (13%) increase in macrolide resistance in Belgium from 1999 to 2003, compared to that observed from 1995 to 1997 (6.5%) (1). Although, a general increase in macrolide-resistance in Europe has been observed during the last few years, resistance levels tend to differ considerably between countries. For instance, while resistance rates in Germany (6) and Poland (4) were similar to those observed in Belgium, considerably higher resistance levels were observed in Spain and Portugal (2), as well as in Italy (3). Provincial variations in macrolide-resistance observed in Belgium have also been reported in other countries (3); however, the precise causes underlying such variations within or between countries are not fully understood. Macrolide consumption might be one factor that explains the regional variations in macrolide-resistant *S. pyogenes* in Spain and Finland (13,14), especially when consumption surpasses a critical threshold (15). However, in France, one of the highest macrolide consumption within Europe is not paralleled by an equally high resistance in *S. pyogenes* (16,17). The identification of telithromycin-resistant *S. pyogenes* in our study, many of which were already present in the community before the introduction of telithromycin in Belgium, also suggest that antimicrobial drug use and development of resistance might be dissociated to some extent. Clearly, other factors like natural fluctuations in prevalence of clones (18), patient compliance with antimicrobial drug regimens, fitness costs of drug resistance, or even tetracycline consumption (tetracycline and macrolide-resistance genes cosegregate) (19) might be important determinants for the development and spread of macrolide-resistant *S. pyogenes*. Thus, any direct link between macrolide use and resistance in *S. pyogenes* should be interpreted cautiously.

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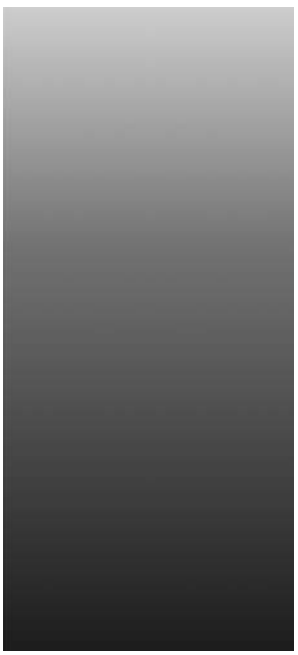

Hospitalization and Antimicrobial Resistance in *Salmonella* Outbreaks, 1984–2002

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Few studies have evaluated the health consequences of antimicrobial-resistant *Salmonella* strains associated with outbreaks. Among 32 outbreaks occurring in the United States from 1984 to 2002, 22% of 13,286 persons in 10 *Salmonella*-resistant outbreaks were hospitalized, compared with 8% of 2,194 persons in 22 outbreaks caused by pansusceptible *Salmonella* strains (p<0.01).

Nontyphoidal *Salmonella* strains are a frequent cause of foodborne disease outbreaks in the United States; they account for ≈13% of outbreaks reported to the Centers for Disease Control and Prevention (CDC) from 1993 to 1997 (1). Antimicrobial resistance is common among salmonellae and has been increasing, particularly in *Salmonella enterica* serotype Typhimurium, the most common *Salmonella* serotype (2,3). In the 1990s, a strain of *S.* Typhimurium resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT) emerged in the United States and Europe; most of these isolates were phage definitive type 104 (DT104) (2).

Antimicrobial therapy is not required for most *Salmonella* infections, but it may be lifesaving in patients with or at risk for extraintestinal infection (4). Increasing levels of antimicrobial resistance are concerning because treatment may fail if the infecting strain of *Salmonella* is resistant to the prescribed agent (5). Also, when the proportion of *Salmonella* strains that are resistant increases, the total prevalence of human *Salmonella* infections increases (6). Resistant salmonellae preferentially cause illness in persons who take antimicrobial drugs for medical conditions unrelated to *Salmonella* infection (7–9).

One previous study has formally evaluated the human health consequences of antimicrobial-resistant *Salmonella* strains associated with outbreaks. In 1984, Holmberg et al. reviewed CDC investigations conducted from 1973 to 1983 to determine the rate of hospitalization and death in outbreaks caused by antimicrobial-resistant salmonellae compared with outbreaks caused by pansusceptible salmonellae (10). Because the epidemiology of both *Salmonella* infections and antimicrobial resistance has changed in the past 20 years, we repeated this analysis for outbreaks investigated from 1984 to 2002.

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The Study

We reviewed final reports of nontyphoidal *Salmonella* outbreaks investigated by CDC from 1984 to 2002. We excluded outbreaks that occurred in a healthcare setting or outside the United States, including on cruise ships. When antimicrobial susceptibility was not recorded in the final report, we searched CDC microbiology records for susceptibility test results on isolates collected as part of the outbreak. Outbreaks were only included if susceptibility data were available for >1 isolate. Because different laboratories performed susceptibility testing, the antimicrobial agents tested and the methods used varied between outbreaks. We classified outbreaks as resistant when the outbreak strain was resistant to ≥1 antimicrobial agent; other outbreaks were considered pansusceptible. Outbreaks caused by resistant strains were additionally classified as R-type AC/KSSuT when the outbreak strain was at least resistant to ampicillin, chloramphenicol or kanamycin, streptomycin, sulfamethoxazole, and tetracycline. Outbreaks were additionally classified as resistant to a clinically important agent when the outbreak strain was at least resistant to ampicillin, trimethoprim-sulfamethoxazole, aminoglycosides, fluoroquinolones, or a third-generation cephalosporin.

Data from the final investigative reports were used for the analysis. When analyzing data according to outbreaks, we calculated the medians for percentage hospitalized and percentage who died and compared medians with the Wilcoxon rank-sum test. When analyzing data according to ill persons, we pooled data from the reports, calculated proportions, and compared proportions with chi-square or, when appropriate, Fisher exact test. The denominators for percentage hospitalized and died varied depending on the number of persons in whom outcome data were ascertained. All p values were 2-tailed. Data were analyzed by using SAS v.9 (SAS Institute, Cary, NC, USA).

From 1984 to 2002, CDC investigated 48 community outbreaks of nontyphoidal *Salmonella* strains in the United States. Of these, 47 (98%) had a final report available for review (online Appendix Table, available at http://www.cdc.gov/ncidod/eid/vol11no06/04-1231_app.htm). We restricted our analyses to the 39 (83%) outbreaks in which data about antimicrobial susceptibility were available. These 39 outbreaks affected 23,206 persons. The largest

outbreak occurred in 1985, in which culture-confirmed *S.* Typhimurium infection associated with milk consumption developed in 16,659 persons (11).

Strains from 11 (28%) outbreaks were resistant, and 28 (72%) were pansusceptible. The 11 outbreaks caused by resistant strains involved 18,698 persons. Of these 11 outbreaks, 7 (64%), involving 17,182 persons, had strains that were at least R-type AC/KSSuT, and 9 (82%), involving 17,919 persons, had strains that were resistant to a clinically important agent.

Hospitalization data were available for 32 outbreaks involving 21,702 ill persons. The hospitalization rates were higher for each type of outbreak caused by resistant salmonellae compared with outbreaks caused by susceptible salmonellae ($p<0.01$ for each comparison) (Table). To account for differences in the size of outbreaks, we compared the median proportion of persons hospitalized and compared hospitalization rates after excluding a large *Salmonella* outbreak that occurred in 1985. The median proportion hospitalized for each type of outbreak caused by resistant strains (26%) was >2.5 times higher than the median proportion hospitalized for outbreaks caused by pansusceptible strains (10%, $p<0.05$ for all resistance patterns). The difference in hospitalization rates between outbreaks caused by resistant and susceptible strains was similar after we excluded the large 1985 outbreak of resistant *S.* Typhimurium, in which the percentage hospitalized was 22%. The results also remained similar after excluding *S.* Enteritidis outbreaks, in which rates of hospitalization and isolate resistance were low.

Mortality data were available for 24 outbreaks involving 21,927 persons. A greater proportion of persons died in resistant outbreaks than in pansusceptible outbreaks, but the difference was not significant (0.1% in outbreaks caused by resistant strains vs. 0.06% in outbreaks caused by pansusceptible strains, $p = 0.57$) (Table).

The 8 outbreaks in which no susceptibility data were available involved 1,914 ill persons. Three (38%) outbreaks were due to *S.* Enteritidis and 2 (25%) to *S.* Typhimurium. In the 6 outbreaks for which hospitalization data were available, 70 (20%) of 353 persons were hospitalized. In the 4 outbreaks for which mortality data were available, 7 (0.4%) of 1,708 persons died.

Conclusions

Outbreaks caused by antimicrobial-resistant, nontyphoidal salmonellae were associated with an increased rate of hospitalization compared with outbreaks caused by pansusceptible salmonellae. The results were similar regardless of the definition of resistance used. This association has been found previously in studies of sporadic illness (12,13).

Several possible mechanisms may explain the higher hospitalization rate. Persons who take antimicrobial drugs for reasons unrelated to gastroenteritis have an increased risk of developing antimicrobial-resistant *Salmonella* infections; such patients may be taking antimicrobial drugs because they have medical conditions that increase their risk for hospitalization (7–9). We doubt that this explains the differential hospitalization rate observed in our study, because the outbreaks we studied occurred in diverse community settings. A second explanation for the higher hospitalization rate is that persons with resistant *Salmonella* infections may fail empiric antimicrobial treatment, and their physicians subsequently hospitalize them for inpatient therapy. A third explanation is that resistant salmonellae may be more virulent because of some unknown factor. In the United States, resistant salmonellae are more often associated with hospitalization and bloodstream infection compared to pansusceptible salmonellae (13). In Canada and Denmark, studies have also found excess death rates associated with resistant *Salmonella* infection (14,15). In England and Wales, a study found no association between resistance and bloodstream infection, but that study had substantial limitations, including the failure to use pansusceptible salmonellae as a referent group and the failure to adjust for confounders, such as age (16).

Since the review of *Salmonella* outbreaks published in 1984, several changes have occurred in the epidemiology

Table. Hospitalization and death rates among nontyphoidal *Salmonella* outbreaks by resistance pattern, 1984–2002*

Resistance pattern	Outbreaks		Patients	
	n (median % [range])	p value	No./total (%)	p value
Hospitalization				
Pansusceptible	22 (9.7 [0–37.5])	Referent	164/2,194 (7.5)	Referent
Resistant >1	10 (26.2 [9.3–49.3])	<0.01	2,913/13,286 (21.9)	<0.01
R-type AC/KSSuT	7 (26.1 [9.3–48.9])	0.02	2,827/12,806 (22.1)	<0.01
Clinically important agent	9 (26.1 [9.3–48.9])	0.04	2,877/13,213 (21.8)	<0.01
Death				
Pansusceptible	16 (0 [0–0.6])	Referent	2/3,283 (0.06)	Referent
Resistant >1	8 (0.1 [0–1.4])	0.05	23/18,644 (0.1)	0.57
R-type AC/KSSuT	5 (0 [0–0.7])	0.21	20/17,150 (0.1)	0.56
Clinically important agent	6 (0 [0–0.7])	0.69	20/17,865 (0.1)	0.80

*AC/KSSuT, ampicillin, chloramphenicol, kanamycin, streptomycin, sulfamethoxazole, tetracycline.

of antimicrobial-resistant salmonellae. First, *S.* Typhimurium DT104 has emerged as a cause of antimicrobial-resistant *Salmonella* infections. Five outbreaks in this study were caused by *S.* Typhimurium with R-type AC/KSSuT; isolates with this resistance pattern are often DT104 (2,17). The hospitalization rate was higher in these 5 outbreaks (median proportion hospitalized: 17%) than in 3 outbreaks caused by pansusceptible *S.* Typhimurium, which suggests that resistance or related factors, rather than just serotype, contribute to the differential rates of hospitalization. Second, a strain of *S.* Newport resistant to at least 9 agents and with diminished susceptibility to ceftriaxone recently emerged in the United States; 1 outbreak in this review was caused by this strain and had a hospitalization rate greater than that seen in most other resistant outbreaks (18). Third, *S.* Enteritidis has emerged as a frequent cause of foodborne outbreaks (19). *S.* Enteritidis strains are infrequently resistant to antimicrobial agents, and in this review, *S.* Enteritidis outbreaks were associated with low hospitalization rates. Excluding *S.* Enteritidis outbreaks did not change the results of our analysis.

The previous analysis by Holmberg et al. found that the rate of death was significantly greater in outbreaks caused by resistant strains (4%) compared with outbreaks caused by pansusceptible strains (0.2%) (10). Rates of death in our study were lower. Because this study covered a more recent period, improvements in medical care may explain the overall lower death rates. Studies with larger sample sizes are needed to determine whether antimicrobial resistance is associated with increased fatality rates.

Our study has some limitations, the most important of which is selection bias. State health departments investigate hundreds of *Salmonella* outbreaks annually. Occasionally, state health departments request assistance from CDC in investigating these outbreaks. The reasons for such requests vary but may be related to size, severity, setting, or other features of the outbreak. While concern about a high hospitalization rate might encourage a state health department to request assistance from CDC in a *Salmonella* outbreak, susceptibility results are usually not known initially and are unlikely by themselves to influence the request for assistance.

Another limitation is that our study was based on final reports, not raw data. As a result, we were unable to adjust for the different patient populations affected, including age, medical condition, and other factors that could affect hospitalization. Excluding outbreaks that occurred in healthcare settings may have reduced some of this bias. Adjustment for serotype, which could also affect hospitalization rates, was also not possible because of the small sample size.

Despite these limitations, we believe this study adds to the weight of evidence about the health effects of antimicrobial-resistant salmonellae. The evidence now includes data from outbreaks and sporadic illness in the United States from 1970 to 2002. Data from sporadic illness and 1 outbreak is also available from Denmark (14,20). Across these diverse studies, higher hospitalization rates are consistently found in patients infected with resistant salmonellae. Such data about the adverse human health effects of resistant salmonellae should be incorporated into programs that promote appropriate antimicrobial use in humans and animals.

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Cephalosporin and Ciprofloxacin Resistance in *Salmonella*, Taiwan

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We report the prevalence and characteristics of *Salmonella* strains resistant to ciprofloxacin and extended-spectrum cephalosporins in Taiwan from January to May 2004. All isolates resistant to extended-spectrum cephalosporins carried bla_{CMY-2}, and all ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis isolates were genetically related.

Resistance to extended-spectrum cephalosporins (ESCs) or fluoroquinolones in *Salmonella enterica* has become a global concern (1). ESC resistance in *Salmonella* strains is usually due to the production of plasmid-mediated extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases, and among these β-lactamases, the CMY-2 AmpC enzyme has been reported most often (1–3). Resistance to fluoroquinolones in *Salmonella* strains is usually due to the accumulation of mutations in the quinolone resistance–determining regions (QRDRs) of DNA gyrase genes (1,4,5). Resistance to both ESCs and fluoroquinolones remains extremely rare in salmonellae.

In Taiwan, increasing resistance to fluoroquinolones and the emergence of CMY-2–producing ESC-resistant strains in salmonellae have been noted (3–6). The emergence of *Salmonella* strains resistant to both ceftriaxone and ciprofloxacin was reported more recently in Taiwan and may pose a serious therapeutic problem (7,8). We conducted the present study to investigate the prevalence and characteristics of *Salmonella* strains resistant to ciprofloxacin and ESCs in Taiwan.

The Study

From January to May 2004, a total of 600 *Salmonella* isolates from 585 patients were obtained from 5 medical centers and 14 district hospitals throughout Taiwan; these isolates were serotyped with commercial antisera (Difco, Detroit, MI, USA). The 4 most common serotypes of *Salmonella enterica* (Enteritidis, Typhimurium, Stanley, and Choleraesuis) accounted for 66.8% of all isolates. Two isolates were untypeable, and the remainder were typed into 42 serotypes (data not shown), which were each represented by 1 to 23 isolates.

MICs of antimicrobial agents were determined by the agar dilution method (9). Resistance to ciprofloxacin (MIC ≥4 μg/mL) was seen in 50 (8.3%) isolates (Table l); 20 (3.3%) were resistant (MICs ranging from 8 to >64 μg/mL) to ceftazidime, ceftriaxone, cefotaxime, or aztreonam (Table 2); 6 isolates showed decreased susceptibilities to 1 or 2 of the 4 ESCs (MICs 0.5–2 μg/mL); 10 (1.7%) isolates were resistant to both ciprofloxacin and ESCs. *S.* Choleraesuis had high rates of resistance to ciprofloxacin (84.4%), ESCs (17.8%), and both (17.8%). None of the 26 *Salmonella* isolates with resistance or decreased susceptibility to ESCs produced ESBL, according to the double-disk synergy method (10). Among the 20 ESC-resistant isolates, 10 isolates were ciprofloxacin-resistant, 4 isolates showed decreased susceptibility to ciprofloxacin (MIC 0.25–1 μg/mL) and resistance to nalidixic acid, and 6 isolates were susceptible to ciprofloxacin and nalidixic acid (Table 2). All 20 ESC-resistant isolates were susceptible to cefepime (MIC <0.03 μg/mL) and imipenem (MIC <1 μg/mL), and 17 isolates were resistant to >1 non–β-lactam agent.

All 20 ESC-resistant isolates expressed a β-lactamase of pI 9.0 by isoelectric focusing (3,11); 11 of these isolates expressed an additional pI 5.4 β-lactamase (Table 2). bla_{CMY-2} was detected in all ESC-resistant isolates. bla_{TEM-1} was detected in the 11 isolates with the pI 5.4 β-lactamase by polymerase chain reaction (PCR) and sequence analyses with the primers for the entire bla_{TEM}-related and bla_{CMY-2}-related structural genes (2,3).

The QRDR sequences of *gyrA*, *gyrB*, *parC*, and *parE* of the 20 ESC-resistant *Salmonella* isolates were determined by PCR and sequence analyses (5). All 10 ciprofloxacin-resistant isolates showed 2 mutations at the Ser-83 and Asp-87 codons in *gyrA* and a single mutation at the Ser-80 codon in *parC* (Table 2). Four isolates with decreased susceptibility to ciprofloxacin had a single mutation at either the Ser-83 or the Asp-87 codon in *gyrA*. All 20 ESC-resistant isolates showed no mutations in the QRDRs of *gyrB* and *parE*.

ESC resistance was transferred from 18 of the 20 ESC-resistant *Salmonella* isolates to *Escherichia coli* C600 in the liquid mating-out assay (3,12). All transconjugants showed decreased susceptibilities to the 4 ESCs tested (MICs 16–64 μg/mL) and cefoxitin (MIC 64–128 μg/mL) and were susceptible to all non–β-lactam agents tested. A pI 9.0 vz β-lactamase and bla_{CMY-2} were detected by isoelectric focusing and PCR assays, respectively, in all transconjugants. Restricted by the endonuclease *Eco*RI, the 18 transferred plasmids produced 9 major restriction

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Table 1. Resistance to ciprofloxacin, extended-spectrum cephalosporins, and both in *Salmonella enterica* serotypes, by region and pulsotype, Taiwan, January–May 2004

Resistance and region*	No. of resistant *S. enterica* isolates/no. of total isolates (%)						Pulsotypes of Choleraesuis isolates (no. of isolates)
	Enteritidis	Typhimurium	Stanley	Choleraesuis	Uncommon serotypes†	All serotypes	
Ciprofloxacin resistance	1/161 (0.6)	0/142 (0)	0/53 (0)	38/45 (84.4)	11/199 (5.5)	50/600 (8.3)	
Northern	0/96 (0)	0/38 (0)	0/14 (0)	13/16 (81.3)	6/88 (6.8)	19/252 (7.5)	A (11), D (1), E (1)
Central	1/32 (3.1)	0/34 (0)	0/14 (0)	6/8 (75.0)	4/37 (10.8)	11/125 (8.8)	A (5), C (1)
Southern	0/25 (0)	0/60 (0)	0/24 (0)	18/20 (90.0)	1/65 (1.5)	19/194 (9.8)	A (14), B (1), C (1), F (1), G (1)
Eastern	0/8 (0)	0/10 (0)	0/1 (0)	1/1 (100)	0/9 (0)	1/29 (3.4)	A (1)
ESC resistance	0/161 (0)	0/142 (0)	3/53 (5.7)	8/45 (17.8)	9/199 (4.5)	20/600 (3.3)	
Northern	0/96 (0)	0/38 (0)	1/14 (7.1)	1/16 (6.3)	7/88 (8.0)	9/252 (3.6)	
Central	0/32 (0)	0/34 (0)	1/14 (7.1)	0/8 (0)	1/37 (2.7)	2/125 (0.8)	
Southern	0/25 (0)	0/60 (0)	1/24 (4.2)	7/20 (35.0)	1/65 (1.5)	9/194 (4.6)	
Eastern	0/8 (0)	0/10 (0)	0/1 (0)	0/1 (0)	0/9 (0)	0/29 (0)	
Ciprofloxacin and ESC resistance	0/161 (0)	0/142 (0)	0/53 (0)	8/45 (17.8)	2/199 (1.0)	10/600 (1.7)	
Northern	0/96 (0)	0/38 (0)	0/14 (0)	1/16 (6.3)	1/88 (1.1)	2/252 (0.8)	E (1)
Central	0/32 (0)	0/34 (0)	0/14 (0)	0/8 (0)	1/37 (2.7)	1/125 (0.8)	
Southern	0/25 (0)	0/60 (0)	0/24 (0)	7/20 (35.0)	0/65 (0)	7/194 (3.6)	A (4), C (1), F (1), G (1)
Eastern	0/8 (0)	0/10 (0)	0/1 (0)	0/1 (0)	0/9 (0)	0/29 (0)	

*ESC, extended-spectrum cephalosporin.
†Includes 197 isolates of 42 uncommon serotypes and 2 untypeable isolates.

patterns (Figure 1 and Table 2). Patterns E and I were further divided into 4 and 2 subtypes, respectively. bla_{CMY-2} on the transferred plasmids was demonstrated by Southern hybridization with the bla_{CMY-2} probe.

The 38 ciprofloxacin-resistant *S.* Choleraesuis isolates were genotyped by pulsed-field gel electrophoresis on a CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the PulseNet protocol (13). Banding patterns generated by *Xba*I restriction were compared with BioNumerics software (Applied Maths, Kortrijk, Belgium). The 38 isolates showed a close relationship (Dice correlation coefficient of 90%) and had only 1 pulsotype, based on Tenover criteria (Figure 2) (14). The pulsotype was divided into 7 pulsosubtypes, among which

Table 2. Characteristics of 20 *Salmonella* isolates resistant to extended-spectrum cephalosporins

Serotype	Specimen type	pI (s)	Resistance pattern*	*gyrA* at position†		*parC* at position 80 (AGC [Ser])‡	Isolate (restriction pattern of transferred bla_{CMY-2}+ plasmid)§
				83 (TCC [Ser])	87 (GAC [Asp])		
Albany	Urine	9.0	Am ESC Fx Cm Na Sxt Tc†	–	AAC (Asn)	–	SA04.028 (C)
Cairo	Stool	9.0, 5.4	Am ESC Fx Cm Na Gm Km Sxt Tc†	TTC (Phe)	–	–	NC04.001 (H1), NC04.002 (H1), NC04.003 (H1)
	Urine	9.0	Am ESC Fx Cm Cp Na Sxt Tc	TTC (Phe)	GGC (Gly)	AAC (Arg)	NC04.004 (H2)
Chester	Stool	9.0	Am ESC Fx	–	–	–	NG04.016 (G)
Choleraesuis	Wound	9.0, 5.4	Am ESC Fx Cm Cp Na Gm Km Sxt Tc	TTC (Phe)	AAC (Asn)	ATC (Ile)	NL04.050 (B)
	Blood	9.0, 5.4	Am ESC Fx Cm Cp Na Gm Tc	TTC (Phe)	AAC (Asn)	ATC (Ile)	SB04.003 (A)
	Blood	9.0, 5.4	Am ESC Fx Cm Cp Na Gm Km Sxt Tc	TTC (Phe)	AAC (Asn)	ATC (Ile)	SE04.005 (F), SG04.060
	Blood	9.0, 5.4	Am ESC Fx Cm Cp Na Gm Km Sxt	TTC (Phe)	AAC (Asn)	ATC (Ile)	SG04.039 (E1), SG04.086
	Joint fluid	9.0, 5.4	Am ESC Fx Cm Cp Na Gm Km Sxt Tc	TTC (Phe)	AAC (Asn)	ATC (Ile)	SG04.042 (E2), SG04.047 (E4)
Kaduna	Tissue	9.0, 5.4	Am ESC Fx Cm Cp Na Sxt Tc	TTC (Phe)	AAC (Asn)	ATC (Ile)	CE04.015 (I)
Saintpaul	Stool	9.0	Am ESC Fx Gm	–	–	–	NG04.011 (G), NG04.018 (G)
Stanley	Stool	9.0, 5.4	Am ESC Fx Cm Sxt Tc	–	–	–	CG04.039 (D)
	Stool	9.0	Am ESC Fx Cm Sxt Tc	–	–	–	NB04.022 (A), SE04.006 (E3)

*Am, ampicillin; ESC, extended-spectrum cephalosporins; Fx, cefoxitin; Cm, chloramphenicol, Cp, ciprofloxacin; Na, nalidixic acid; Gm, gentamicin; Km, kanamycin; Sxt, trimethoprim-sulfamethoxazole; Tc, tetracycline.
†The *S.* Albany isolate and the 3 *S.* Cairo isolates showed decreased susceptibilities to ciprofloxacin (MIC 0.25–1 μg/mL).
‡Nucleotide and amino acid changes at the QRDRs of *gyrA* and *parC*. –, no alterations in the genes.
§For each isolate, the first letter indicates region (C, central region; N, northern region; S, southern region), and the second letter represents hospital. Isolates NC04.001, NC04.002, and NC04.003 were from the same patients; all other isolates were from different patients.

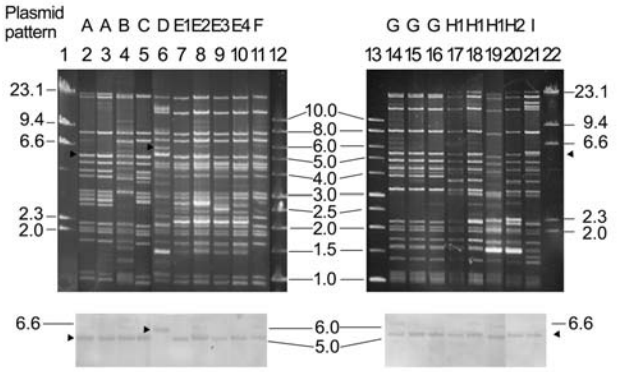

Figure 1. *Eco*RI restriction patterns of transferred CMY-2-encoding plasmids of 18 *Salmonella* isolates. The result of the hybridization assay with the bla_{CMY-2} probe labeled with digoxigenin (Roche Molecular Biochemicals, Mannheim, Germany) is shown below the gel, and arrowheads indicate the locations of the restriction fragments that were hybridized. Lanes 2-21, plasmids from transconjugants of *Salmonella* isolates NB04.022, SB04.003, NL04.050, SA04.028, CG04.039, SG04.039, SG04.042, SE04.006, SG04.047, SE04.005, NG04.011, NG04.016, NG04.018, NC04.001, NC04.002, NC04.003, NC04.004, and CE04.015; lanes 1 and 22, molecular marker II (Roche Molecular Biochemicals); lanes 12 and 13, a 1-kb molecular marker (Promega Co., Madison, WI, USA)

were 1–4 band differences. Five ESC-resistant isolates displayed the same pulsosubtypes (IA or IC) as ESC-susceptible isolates (Table 1 and Figure 2).

Conclusions

We describe the prevalence of resistance to ciprofloxacin and ESCs among salmonellae isolated from January to May 2004 in Taiwan. We found widespread resistance of *Salmonella* isolates to both ESCs and ciprofloxacin; high prevalence of resistance to ciprofloxacin, ESCs, and both in *S.* Choleraesuis; and widespread prevalence of CMY-2–producing *Salmonella* isolates of various serotypes in Taiwan.

The prevalence of *Salmonella* isolates resistant to both ceftriaxone and ciprofloxacin may pose a therapeutic problem. CMY-2 is one of the AmpC enzymes, which are usually less active against cefepime and cefpirome than ESBLs (15). Accordingly, we have used cefepime to successfully treat several patients infected with CMY-2–producing and ciprofloxacin-resistant *S.* Choleraesuis (8). Therefore, AmpC-producing strains should be differentiated from ESBL-producing strains by phenotypic or genotypic methods when ESC-resistant *Salmonella* strains are isolated in the clinical microbiology laboratory (15).

The ciprofloxacin-resistant rate in *S.* Choleraesuis in Taiwan has been >60% since 2001; the high prevalence was mainly due to clonal spread of resistant strains (4–6). The ciprofloxacin-resistant rate in *S.* Choleraesuis in this report (84.4%) was higher than those reported previously (≤70%) (4–6). bla_{CMY-2} in *Salmonella* in Taiwan was first reported in 2 *S.* Typhimurium strains isolated in 2000 (3). The first reported *S.* Choleraesuis strain with bla_{CMY-2} was a ciprofloxacin-resistant strain isolated in 2002 (7). All our 38 ciprofloxacin-resistant *S.* Choleraesuis isolates, including 8 ESC-resistant isolates, were genetically related. Moreover, we found possibly unrelated bla_{CMY-2}-positive plasmids (lanes 3, 4, 7, 8, 10, and 11 in Figure 1) among closely related isolates (Figure 2). These data together suggest that the development and rapidly increasing prevalence of ESC and ciprofloxacin resistance in *S.* Choleraesuis in Taiwan might result from the extremely high prevalence of ciprofloxacin resistance followed by

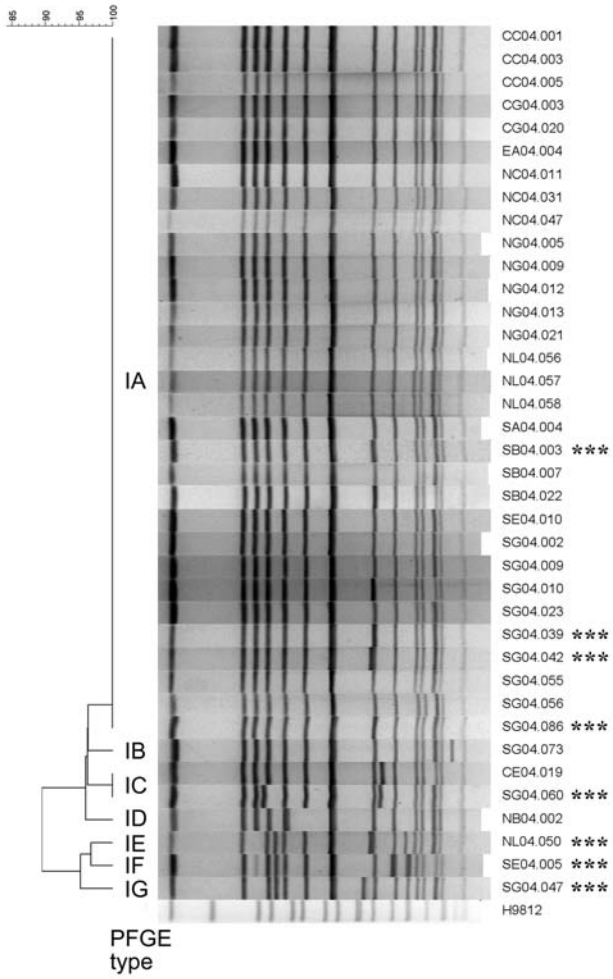

Figure 2. Dendrogram (left) obtained from cluster analysis of *Xba*I-generated macrorestriction patterns of 38 ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis (right). Asterisks indicate extended-spectrum cephalosporin-resistant isolates. A percent scale of similarity is shown above the dendrogram. Pulsed-field gel electrophoresis (PFGE) types are shown between the gel and the dendrogram. H9812, *S. enterica* serotype Braenderup strain H9812, which was used as reference size marker.

the horizontal transfer of bla_{CMY-2} into ciprofloxacin-resistant epidemic strains rather than from the spread of a clone that had been resistant to ciprofloxacin and ESCs.

All our ciprofloxacin-resistant S*almonella* isolates tested had mutations in the QRDRs of *gyrA* and *par*, a finding consistent with previously reported results (1,4,5). The rates of ciprofloxacin resistance in the 3 most common serotypes, Enteritidis, Typhimurium, and Stanley, remained very low (0%–0.6%). Six of 11 ciprofloxacin-resistant isolates in the group of uncommon serotypes belonged to serotype Schwarzengrund and accounted for 42.9% of all serotype Schwarzengrund isolates. Thus, the high rate (5.5%) of ciprofloxacin resistance in this group was in part due to the high prevalence of ciprofloxacin resistance in serotype Schwarzengrund.

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Global Spread of Multiple Aminoglycoside Resistance Genes

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Emergence of the newly identified 16S rRNA methylases RmtA, RmtB, and ArmA in pathogenic gram-negative bacilli has been a growing concern. ArmA, which had been identified exclusively in Europe, was also found in several gram-negative pathogenic bacilli isolated in Japan, suggesting global dissemination of hazardous multiple aminoglycoside resistance genes.

Multidrug-resistant gram-negative super microbes have been emerging worldwide. Since carbapenems and fluoroquinolones are the last resort against infections caused by gram-negative bacilli (1,2), the proliferation and dissemination of such clinical isolates that produce metallo-β-lactamases and acquire mutations in *gyrA* and *parC* genes have become a global threat (3,4). Aminoglycosides, including amikacin and tobramycin, are still potent agents for use against resistant bacilli. One of the most common resistance mechanisms against aminoglycosides is the production of aminoglycoside-modifying enzymes, such as aminoglycoside acetyltransferases, aminoglycoside phosphorylases, and aminoglycoside adenyltransferases (5), which are mainly mediated by transferable large plasmids.

Recently, a series of special methylases that protect microbial 16S rRNA, the main target of aminoglycosides, was identified in several nosocomial pathogens, including *Pseudomonas aeruginosa* (6), *Serratia marcescens* (7), and *Klebsiella pneumoniae* (8). The newly identified 16S rRNA methylases RmtA and RmtB were reported from Japan in 2003 and 2004, respectively (6,7). The gene for ArmA was initially sequenced in *Citrobacter freundii* isolated in Poland (GenBank accession no. AF550415) and later characterized in *K. pneumoniae* isolated in France in 2003 (8). In 2004, nosocomial spread of ArmA- or RmtB-producing *Escherichia coli* and *K. pneumoniae* was reported from Taiwan (9).

These enzymes are capable of conferring an extraordinary high level of resistance (MIC >512 mg/L) against most clinically important aminoglycosides as was observed among aminoglycoside-producing actinomycetes, suggesting their probable phylogenic relationship with the intrinsic 16S rRNA methylases of actinomycetes (Figure). RmtA shared 82% amino acid identity with RmtB, but the amino acid sequence similarities between 16S rRNA methylases isolated from pathogenic gram-negative microbes and those from aminoglycoside-producing actinomycetes were relatively low (≤33%). From analyses of the genetic environments of genes encoding 16S rRNA methylases, the *rmtA* gene is likely associated with the mercury-resistant transposon Tn*5041* (10); the *rmtB* gene was found in the flanking region of Tn*3*-like structure (7). The *armA* gene was found on a large plasmid which carries a type 1 integron (8) that mediates various gene cassettes responsible for multiple antimicrobial resistance. The structure of these genetic environments implied that the genes for these 16S rRNA methylases are mediated by mobile genetic elements carried by transferable large plasmids (7,8,10). In fact, the *rmtA* gene was transferred from *P. aeruginosa* strain AR-2 to an aminoglycoside-susceptible *P. aeruginosa* strain 105 by conjugation in vitro (6). The *rmtB* gene was also transferred from *S. marcescens* S-95 to *E. coli* by transformation (7). The *armA* gene was located on a composite transposon Tn*1548* (11).

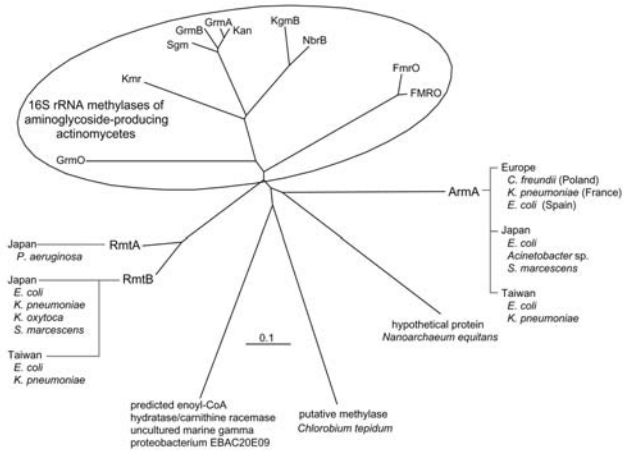

Figure. Phylogenic relationship among the 16S rRNA methylases. Each amino acid sequence was subjected to the analysis referred to the following sources: FmrO, accession no. JN0651; Kmr, accession no. AB164642; GrmA, accession no. M55520; GrmB, accession no. M55521; GrmO, accession no. AY524043; Kan, accession no. AJ414669; Sgm, accession no. A45282; KgmB, accession no. S60108; NbrB, accession no. AF038408; FMRO, Q08325; RmtA, (6); RmtB, (7); ArmA, (8); predicted enoyl-CoA hydratase/carnithine racemase of uncultured marine gamma proteobacterium EBAC20E09, accession no. AAS73112; putative methylase of *Chlorobium tepidum*, accession no. AAM72273; hypothetical protein of *Nanoarchaeum equitans*, accession no. AAR39385. The ClustalW program provided by the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/search/clustalw-e.html) was used in this study.

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Thus, the growing concern was that these newly identified aminoglycoside-resistance genes could easily spread and be further disseminated among the glucose-nonfermentative gram-negative bacilli, including *P. aeruginosa* and *Acinetobacter* spp. and the genera belonging to the family *Enterobacteriaceae*.

The Study

We conducted a preliminary screening of the 16S rRNA methylase-producing bacilli on our gram-negative microbial stock of 2,877 strains isolated from Japanese hospitals within the past several years. Arbekacin, a semisynthetic aminoglycoside belonging to the kanamycin group, requires 2 modifications at the (6′) aminogroup and the (2′′) hydroxyl group for inactivation, so this agent is not inactivated by known plasmid-mediated aminoglycoside-modifying enzymes. Therefore, a high-level arbekacin resistance (MIC >512 mg/L) was used as a marker for screening the 16S rRNA methylase-producing strains. All arbekacin-resistant strains were subjected to polymerase chain reaction (PCR) analysis to detect *rmtA*, *rmtB*, or *armA*, and all strains were PCR positive, except for a strain of *Acinetobacter* demonstrating a very high level of resistance to arbekacin (MIC 1,024 mg/L). This strain was later shown to produce both aminoglycoside 6′-acetyltransferase and 2′′-adenyltransferase (12), so arbekacin was inactivated in this strain by both 6′-acetylation and 2′′-adenylation. Each PCR primer set was used to detect *rmtA* and *rmtB* genes as in our previous reports (6,7). The PCR primers for amplification of *armA* were newly designed (forward: 5′-AGG TTG TTT CCA TTT CTG AG-3′, reverse: 5′-TCT CTT CCA TTC CCT TCT CC-3′), and the predicted size of the amplicon was 590 bp. These 3 sets of PCR primers were very reliable in detecting *rmtA*, *rmtB*, and *armA* genes, respectively. Each PCR amplicon was then subjected to sequencing analyses on both strands to confirm its nucleotide sequences for detecting mutations in the methylase genes.

As reported in our previous study, *rmtA* and *rmtB* genes had been found in *P. aeruginosa* isolates (6,10) and in 1 strain of *S. marcescens* (7), respectively. As shown in the Table, 5 *P. aeruginosa* strains isolated after our previous report (6) were *rmtA* positive. The *rmtB* gene was additionally identified in 4 *K. pneumoniae*, 2 *E. coli*, and 1 *K. oxytoca* strains in Japan. To our surprise, the *armA* gene, which had been found in various gram-negative microbial species belonging to the family *Enterobacteriaceae* exclusively in Europe as reported by Galimand et al. (13), was also identified in Japan in 1 strain each of *E. coli*, *S. marcescens*, and *Acinetobacter* sp. Notably, the *armA* and *rmtB* genes were also recently identified in *K. pneumoniae* and *E. coli* in Taiwan (9). Furthermore, the genetic environment of the *armA* gene found in *C. freundii* isolated in Poland was similar to that of *K. pneumoniae* isolated in France. The genetic environments of the *armA* gene found in the 3 Japanese microbial species, *E. coli*, *S. marcescens*, and *Acinetobacter* sp. (GenBank accession nos. AB116388 and AB117519), were also similar to those found in Europe (GenBank accession nos. AF550415 and AY220558). These findings suggest that the ArmA-producing gram-negative nosocomial microbes that harbor a very similar genetic environment carrying the *armA* gene have spread globally.

Conclusions

As described previously, arbekacin still shows a very broad antimicrobial spectrum from gram-positive to gram-negative nosocomial microbes and has been approved solely to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections in Japan since 1990 to ensure the prudent use of this agent. The emergence and presence of the 16S rRNA methylase-producing gram-negative bacilli,

Table. Methylase-producing strains of 16S rRNA identified after previous study (6)

Species and strain	Type	Year of isolation	Hospital	Prefecture
Pseudomonas aeruginosa P122	RmtA	2002	A	Aichi
P. aeruginosa P340	RmtA	2002	B	Gifu
P. aeruginosa 02-386	RmtA	2002	C	Saitama
P. aeruginosa 03-29	RmtA	2003	D	Aichi
P. aeruginosa 03-230	RmtA	2003	E	Shizuoka
Escherichia coli 01-139	RmtB	2001	H	Yamanashi
Klebsiella pneumoniae 01-140	RmtB	2001	H	Yamanashi
Klebsiella oxytoca 01-141	RmtB	2001	H	Yamanashi
K. pneumoniae 01-142	RmtB	2001	H	Yamanashi
E. coli C316	RmtB	2002	F	Hyogo
Serratia marcescens S95	RmtB	2002	G	Kohchi
K. pneumoniae 03-252	RmtB	2003	H	Yamanashi
K. pneumoniae 03-518	RmtB	2003	H	Yamanashi
E. coli C316-2	ArmA	2003	F	Hyogo
S. marcescens ARS8	ArmA	2003	I	Tochigi
Acinetobacter sp. ARS6	ArmA	2003	J	Kanagawa

however, has not been well recognized in Japan to date; arbekacin has not been listed among the antimicrobial agents for daily antimicrobial susceptibility testing of gram-negative microbes.

The use of semisynthetic aminoglycosides, including arbekacin, in Japanese clinical settings for >10 years may have promoted the emergence and dissemination of the 16S rRNA methylase-producing gram-negative microbes in Japan. The large amount of various aminoglycosides used in livestock-farming environments could have also been a selective pressure for the emergence and spread of pathogenic microbes that harbor genetic determinants for the newly identified 16S rRNA methylases, as exemplified by recent isolation of ArmA-producing *E. coli* from swine in Spain (GenBank accession no. AY522431).

Since acquisition of multidrug resistance against clinically important antimicrobial agents such as carbapenems and fluoroquinolones has been developing rapidly worldwide, the acceleration of even greater aminoglycoside resistance among gram-negative bacilli promises to become an actual clinical concern in the near future, just as vancomycin-resistant enterococci (VRE) did in the 1990s (14). The emergence of gram-positive cocci including MRSA and VRE that acquire the 16S rRNA methylase could also be a grave clinical matter, although fortunately no such hazardous microbes have been identified. Thus, steps must be taken to block further proliferation of these multidrug-resistant gram-negative super microbes, including *P. aeruginosa*, *K. pneumoniae*, and *Acinetobacter* spp., as well as multidrug-resistant cocci such as MRSA and VRE, which have acquired an extraordinarily high level of resistance to various aminoglycosides through production of 16S rRNA methylases, especially in clinical environments.

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armA and Aminoglycoside Resistance in *Escherichia coli*

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We report *armA* in an *Escherichia coli* pig isolate from Spain. The resistance gene was borne by self-transferable IncN plasmid pMUR050. Molecular analysis of the plasmid and of the *armA* locus confirmed the spread of this resistance determinant.

Aminoglycosides are used to treat a broad range of life-threatening infections in humans and animals (1,2). Thus, surveillance of resistance to these antimicrobial agents and study of the corresponding mechanisms in pathogenic bacteria remain ongoing challenges in microbial research (3). Since 1996, the Spanish Veterinary Antimicrobial Resistance Surveillance Network has monitored antimicrobial resistance in both healthy and ill animals, with the aim of assessing the state of veterinary antimicrobial resistance in Spain (4–6). *Escherichia coli* is used as a model bacterium for the analysis of isolates from sick animals. Veterinary diagnostic laboratories from official and private sectors provide the isolates, following a classic passive monitoring system with a centralized analytic approach. To avoid bias, a single isolate per farm per year (or animal in the case of pets) is studied. The identity of the farm (or owner) is kept confidential to facilitate participation. In addition, isolates are included only if their antimicrobial susceptibility has not been determined before being sent to the network. Although formal sampling is not performed, involvement of several laboratories throughout the country elicits an accurate estimate of antimicrobial susceptibility in Spain (4–6). From 1996 to 2003, the number of isolates tested was >2,300 for *E. coli* and 700 for *Staphylococcus aureus*.

The Study

E. coli MUR050 from the feces of a diarrheic pig was isolated in 2002. Routine antimicrobial susceptibility testing showed unusual resistance to amikacin. The isolate was also resistant to other aminoglycosides (streptomycin, gentamicin, and neomycin, but not apramycin), as well as to members of other classes (sulfonamides, trimethoprim, quinolones). Because of this unusual phenotype, the standard identification procedure (Gram staining, oxidase, indol, methyl red, citrate, and Vogues-Proskauer) (6) was confirmed by partial sequencing of the ribosomal 16S rRNA gene (data not shown) (7). Additional study of antimicrobial susceptibility by Etest (AB Biodisk, Solna, Sweden) indicated that the strain was also highly resistant to the 4,6-disubstituted deoxystreptamines netilmicin and tobramycin (Table). We initiated an investigation to find the molecular determinants of this pattern. Plasmid DNA purified from MUR050 (Qiagen, Inc., Chatworth, California, USA) was used to transform *E. coli* INVαF′ (Invitrogen, Paisley, United Kingdom) (8) to yield strain INVαF′ (pMUR050), which was highly resistant to aminoglycosides and sulfonamides. Resistance to aminoglycosides and the sulfonamides could be transferred from MUR050 to other *E. coli* by conjugation at a frequency of $\approx 1 \times 10^{-4}$ per donor colony-forming unit (9).

Search for antimicrobial resistance determinants by polymerase chain reaction (PCR) using plasmid pMUR050 DNA as template indicated the presence of genes *sul1* for resistance to sulfonamides, *ant3″*9 for resistance to streptomycin-spectinomycin, and *aph3′*-I for resistance to kanamycin and neomycin. Plasmid pMUR050 DNA was digested with PstI and ligated with pUC18 DNA; the ligation mixture was used to transform INVαF′ cells that were plated on agar containing ampicillin (50 μg/mL) and tobramycin (10 μg/mL). The resulting colonies harbored plasmid p18MUR050 with an insert of ≈9 kb, which when sequenced showed *armA*, the structural gene for a 16S rRNA methylase (10). To confirm that *armA* was responsible for aminoglycoside resistance in this strain, 2 oligonucleotides, armF (5′-GGTGC-GAAAACAGTCGTAGT-3′) and armR (5′-TCCT-CAAAATATCCTCTATGT-3′), were used to amplify *armA*, which was purified, ligated into pUC18, and transformed into *E. coli* INVαF′ with selection on ampicillin and tobramycin. Transformant INVαF′ (p*armA*) was highly resistant to 4,6-disubstituted deoxystreptamines (Table), which demonstrated that *armA* was responsible for high-level aminoglycoside resistance in MUR050.

Until now, the *armA* gene has been found only on conjugative plasmids of the IncL/M incompatibility group and associated with the $bla_{CTX\text{-}M3}$ gene coding for a broad-spectrum β-lactamase (11). Plasmid pMUR050, however, did not confer resistance to the cephalosporins and did not belong to the IncL/M family, as tested by PCR (data not shown), which indicated that *armA* was carried by a different replicon. Plasmid pMUR050 DNA was digested with *Sau3*A, and the resulting fragments were ligated with plasmid pBluescript KS+ DNA (Stratagene, La Jolla, CA,

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Table. Minimum inhibitory concentrations (MICs) of various antimicrobial agents for *Escherichia coli* MUR050 and *E. coli* with and without *armA*

	MIC (μg/mL)*				
E. coli	Amikacin	Tobramycin	Netilmicin	Gentamicin	Ciprofloxacin
MUR050	>256	>256	>256	>256	>32
INVαF′	0.5	0.5	0.5	0.75	0.002
INVαF′ (pMUR050)	>256	>256	>256	>256	0.002
INVαF′ (p18MUR050)	>256	>256	>256	>256	0.002
INVαF′ (p*armA*)	>256	>256	>256	>256	0.002

*MICs were determined by Etest (AB Biodisk).

USA). The ligation mixture was transformed into *E. coli* INVαF′ and plated on agar containing ampicillin (50 μg/mL). The inserts of plasmids from 96 random clones were sequenced with standard oligonucleotides with an ABI Prism DNA Sequencer apparatus (Perkin-Elmer, Foster City, CA, USA). Approximately half of the sequence had a high degree of identity (80%–100%) with genes of conjugative plasmid R46, which belongs to the IncN incompatibility group (12). Among them, sequences involved in plasmid replication and mobilization, such as *repA* and *oriT*, were identified (data not shown), which confirmed that *armA* was carried by an R46-like replicon. This finding suggests that *armA* could be part of a mobile genetic element that has translocated between plasmids of diverse evolutionary origins. Upstream from *armA* an open reading frame (ORF) has 69% identity with that of a transposase from *Listonella anguillarum* (GenBank accession no. AAO92373); this ORF and *armA* are flanked by 16-bp directly repeated sequences with a single mismatch (5′-aggtttccactacagt-3′) (GenBank accession no. AY522431). However, since aminoglycosides are rarely used in porcine production in Spain (13), and no veterinary drugs containing amikacin are registered, the *sul1* and *armA* genes were conceivably co-selected by the use of sulfonamides. These antimicrobial agents are not among the 4 antimicrobial agents authorized in Europe for growth promotion (14) but are used orally to treat bacterial infections such as group E streptococcal pneumonia or atrophic rhinitis and diarrhea (2).

Conclusions

These data support the notion that *armA* is disseminated both by conjugation and transposition, which makes further spread of *armA* likely. The complete sequence of plasmid pMUR050 is being determined and should help in elucidating the genetic basis for dissemination of *armA*.

The origin of the *armA* gene is still unknown. Aminoglycoside-producing environmental bacteria possess 16S rRNA methylases to protect themselves from the antimicrobial agent that they produce (15). In addition to ArmA (11), 2 other 16S rRNA methylases have been reported recently in human pathogens from Japan: RmtA in *Pseudomonas aeruginosa* (16) and RmtB in *Serratia marcescens* (17). One could speculate that actinomycetes have transferred the methylase genes to pathogenic bacteria in the environment that further spread on diverse replicons in various hosts. However, the degree of identity of *armA* with the structural genes for the 16S rRNA methylases in actinomycetes is <30%, which is not compatible with a recent transfer event. Alternatively, *armA* could have originated from a yet unknown aminoglycoside-producing actinomycetes (10).

In 2003, *armA* was identified as a new determinant that conferred high-level resistance to aminoglycosides in a French clinical isolate of *Klebsiella pneumoniae* (10) and was subsequently detected in human isolates of *Enterobacteriaceae* from various countries in Europe (11). The finding of *armA* in animal isolates shows that veterinary monitoring networks should include last-resort antimicrobial agents of high therapeutic value for humans, which could help in detecting early subtle shifts in antimicrobial resistance. In fact, *armA* was detected in an animal bacterium because amikacin was included in the standard antimicrobial panel in the Spanish Veterinary Antimicrobial Resistance Surveillance Network. Our finding of *armA* in a bacterium of animal origin highlights the importance of coordinated surveillance of human and animal isolates (18–20).

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Tuberculosis due to Resistant Haarlem Strain, Tunisia

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Multidrug-resistant tuberculosis was diagnosed in 21 HIV-negative, nonhospitalized male patients residing in northern Tunisia. A detailed investigation showed accelerated transmission of a *Mycobacterium tuberculosis* clone of the Haarlem type in 90% of all patients. This finding highlights the epidemic potential of this prevalent genotype.

The ability of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* to cause epidemics and spread globally contrasts with the initial perception that MDR tuberculosis (MDR-TB) has a reduced potential for transmission (1,2). In this respect, the W/Beijing type appears to be most common in humans and accounts for most reported MDR-TB outbreaks (3).

In this report, we provide evidence for the epidemic potential of another worldwide prevalent *M. tuberculosis* genotype, namely, the Haarlem family (4,5). The identified strain is MDR and has rapidly expanded within immunocompetent and nonhospitalized patients.

The Study

M. tuberculosis isolates were obtained from the Laboratory of Mycobacteriology of the Institut Pasteur de Tunis as part of the National Tuberculosis Surveillance Program. All samples (884 specimens) from patients with suspected TB residing in northern Tunisia (Bizerte) from August 2001 to October 2003 were forwarded to us by the referral regional hospital. This hospital serves a region with 483,086 people and an area of 3,501 km^2. The incidence of TB in this area from 2001 to 2002 was 29/100,000 male patients and 11/100,000 female patients. All patients received the standard chemotherapy regimen of the Tunisian National Tuberculosis Program, i.e., 2 months of rifampicin, isoniazid, pyrazinamide, and streptomycin, followed by 4 months of rifampicin and isoniazid (2RHZS/4RH). This regimen was introduced into the region in 1995. Of the 193 *M. tuberculosis* isolates recovered, 20 were MDR. The corresponding patients were interviewed, and detailed epidemiologic investigations were conducted according to described protocols (6). In April 2004, while the study was in progress, a new MDR case was diagnosed.

Analyses by IS*6110* restriction fragment length polymorphism (IS*6110* RFLP), ligation-mediated polymerase chain reaction (PCR), and spoligotyping were carried out by using standard protocols (7–9). Typing of the polymorphic GC-rich repetitive sequence (PGRS) with probe MTB484 (1) was conducted according to a previously reported protocol (10), with the exception that DNA was digested with *Alu*I. Isolates were assigned to principal genetic groups according to the polymorphisms in the *katG* and *gyrA* genes (11). The following primer pairs were used to sequence *rpoB*, *katG*, and *pncA* gene mutations that confer resistance to rifampicin, isoniazid, and pyrazinamide, respectively: *rpoB* (5′-ATCACACCGCAGACGTTG-3′, 5′-TGCATCACAGTGATGTAGTCG-3′); *katG* (5′- CGTCGAAACAGCGGCGCTGA-3′, 5′-CAAGCGCCAGCAGGGCTCTT-3′); and *pncA* (5′-GGCGCACACAATGATCGGTG-3′, 5′-GCTTTGCGGCGAGCGCTCCA-3′). The recently described single nucleotide polymorphisms in putative *M. tuberculosis* mutator genes *mutT1*, *mutT2*, *mutT3*, and *ogt* were investigated with the same protocol reported by Rad et al. (12). DNA sequencing was conducted directly on the purified PCR products by using the Prism Ready Reaction Dye Deoxy Terminator Cycle sequencing kit on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Epidemiologic and clinical data indicated that all patients with MDR-TB were male with a mean age of 31 years at diagnosis (Table 1). All were Tunisians and permanently resided in the northern part of the country (Bizerte). All patients were seronegative for HIV with no documented history of travel abroad, and none had a history of immunosuppression, diabetes, or respiratory diseases other than TB. Mapping of the 21 patients with MDR-TB according to their residence sites showed that they were mostly scattered over the northeastern part of the region (surface area ≈1,000 km^2) with no concentration in a particular locality (data not shown). Resistance to 5 first-line drugs was observed for most isolates (Table 2).

As indicated in Table 1, with the exception of patient P20, the DNA samples subjected to molecular typing were obtained from the initial isolate of all new patients. RFLP showed that 18 patients had nearly identical IS*6110* profiles (Figures 1 and 2). The predominant profile (occurring in 13 patients) showed 11 bands, while the remaining

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Table 1. Clinical characteristics of 21 patients with multidrug-resistant tuberculosis (MDR-TB), Bizerte, Tunisia, 2001–2004*

Patient	Age (y)	Sex	Case history	Isolate used for molecular typing	Initial diagnosis of MDR-TB	Epidemiologic characteristic	Chest radiography
P1	24	M	PT	Follow-up, Oct 2001	Sep 2001	Brother of patient 14	Right apical cavity nodular lesion
P2	26	M	NC	Initial	Oct 2001	Same penitentiary as patients 7 and 9	Left mid-lung nodular opacity with excavation
P3	25	M	NC	Initial	Oct 2001	None apparent	Bi-apical nodular opacity
P4	62	M	NC	Initial	Nov 2001	None apparent	Right apical nodular opacity
P5	26	M	PT	Follow-up, Feb 2002	Sep 2000	Brother of patient 18	Diffuse nodular lesions and multiple cavities
P6	23	M	PT	Follow-up, Feb 2002	Feb 2001	None apparent	Right apical and median bilateral nodular opacity
P7	24	M	PT	Follow-up, Mar 2002	Sep 2000	Same penitentiary as patients 2 and 9	Right lobe apical nodular opacity
P8	27	M	NC	Initial	Jun 2002	None apparent	Right apical nodular opacity
P9	34	M	PT	Follow-up, Jun 2002	Aug 2001	Same penitentiary as patients 2 and 7	Right apical nodular opacity and left diffuse nodular opacity
P10	21	M	NC	Initial	Jun 2002	None apparent	Right lobe apical nodular opacity and cavity
P11	42	M	NC	Initial	Jul 2002	None apparent	Basal nodular opacity of the right and left lung
P12	23	M	NC	Initial	Jul 2002	None apparent	Left apical cavity and nodular opacity
P13	29	M	PT	Follow-up, Aug 2002	Sep 2001	None apparent	Bilateral apical and diffuse opacity
P14	34	M	NC	Initial	Nov.2002	Brother of patient 1	Left apical cavity and nodular lesion
P15	51	M	PT	Follow-up, Nov 2002	ND	None apparent	Bilateral cavity
P16	17	M	NC	Initial	Mar 2002	None apparent	Bilateral nodular infiltration and cavity in the left lung
P17	17	M	NC	Initial	May 2003	Nephew of patient 14	Wright apical cavity and left lung nodular opacity
P18	21	M	NC	Initial	Jun 2003	Brother of patient 5	Right apical cavity and nodular opacity
P19	42	M	NC	Initial	Jun 2003	No interview (lost case)	Diffuse nodular opacity and multiple cavities
P20	53	M	NC	Follow-up, Oct 2003	Oct 2002	None apparent	Right apical cavities and left lobe infiltrate
P21	ND	M	NC	Initial	Apr 2004	Cousin of patient 14	ND

*PT, previously treated; NC, new case; ND, not determined.

5 patients had an additional IS*6110* band. The presence or absence of the additional IS*6110* band was not restricted to new or previously treated patients. The RFLP pattern of patient P11, a new patient, clearly showed a mixture of the 12-band profile and some additional IS*6110* bands (Figure 2A). Typing of his follow-up culture, which was obtained after 6 months of directly observed short-course therapy, as recommended by the World Health Organization, yielded only the 12-band profile (Figure 2A). Laboratory records and epidemiologic data indicate that this patient likely had a dual infection.

The isolate from patient P13 was typed by ligation-mediated PCR. Its profile was identical to the 18 other MDR isolates. Thus 19 patients with MDR-TB could be clustered according to IS*6110*-based typing. Effective epidemiologic links were identified for 9 (47%) patients (Table 1). Another similar RFLP pattern was observed for patient P20. It shows 10 IS*6110* bands (Figure 2B), 9 of which are common to the 12-band RFLP pattern described for the other isolates. The isolate from patient P19 displayed a 9-band IS*6110* profile that was clearly distinct from all the other patients with MDR-TB (data not shown).

With the exception of patient P19, the MDR isolates were identical in their PGRS profile (Figure 2) and spoligotype patterns (Table 2), which is characteristic of the Haarlem3 type (4). Sequence analysis of mutator and drug resistance genes conclusively confirmed that the 19 MDR isolates with nearly identical IS*6110* (both 12- and 11-band profiles) are genetically closely related. They all harbor the L209L, T15S, S531L, and S315T mutations in *mutT3*, *ogt*,

Table 2. Laboratory findings and genotyping of multidrug-resistant isolates from 21 tuberculosis patients, Bizerte, Tunisia, 2001–2004*

Patient	Smear result	Resistance pattern†	RFLP‡	Spoligotype	PGG§	Mutational analysis				
						rpoB	*katG*	*pncA*	*mutT3*	*Ogt*
P1	+++	HSREZ	11	Haarlem3¶	2	S531L+V610M	S315T	A-11C	L209L	T15S
P2	+	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P3	++	HSREZ	12	Haarlem3	2	S531L+V610M	S315T	T11G (L4W)	L209L	T15S
P4	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P5	-	HSREZ	12	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P6	+	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P7	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P8	-	HSRE	11	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P9	+	HSRE	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P10	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P11	-	HSREZ	12	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P12	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P13	-	HSRE	ND#	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S
P14	-	HRZ	11	Haarlem3	2	S531L+V610M	S315T	G insertion (391-392)	L209L	T15S
P15	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	A-11C	L209L	T15S
P16	++	HSR	12	Haarlem3	2	S531L+V610M	S315T	T11G (L4W)	L209L	T15S
P17	-	HSREZ	11	Haarlem3	2	S531L+V610M	S315T	G insertion (296-297)	L209L	T15S
P18	-	HSREZ	12	Haarlem3	2	S531L+V610M	S315T	T11G (L4W)	L209L	T15S
P19	-	HSRE	9	Other**	2	ΔN (AAC)519	S315T	G insertion (296-297)	WT	WT
P20	++	HSR	10	Haarlem3	2	S531L	S315	WT	L209L	T15S
P21	++	HR	11	Haarlem3	2	S531L+V610M	S315T	WT	L209L	T15S

*RFLP, restriction fragment length polymorphism; PGG, principal genetic grouping; WT, wild type (identical to strain H37Rv); ND, not determined.
†H, isoniazid; S, streptomycin; R, rifampicin; E, ethambutol; Z, pyrazinamide.
‡Number of IS*6110* bands.
§Principal genetic grouping according to *gyr*A and *kat*G polymorphisms (11).
¶Absence of spacers 31 and 33–36.
#IS*6110* typing was determined by ligation-mediated polymerase chain reaction and the profile was identical to the other outbreak-associated strains.
**Absence of spacers 15, 21–24, and 33–36.

rpoB, and *katG* genes, respectively (Table 2), whereas *mutT1* and *MutT2* showed a wild type genotype (data not shown). The occurrence of an additional uncommon mutation in the *rpoB* gene (V610M) confirmed the clonality of this MDR Haarlem strain since it was present only in 19 patients with MDR-TB. The variability of resistance to pyrazinamide and the mutational profile within the *pncA* gene (Table 2) strongly suggest that primary transmission from person to person occurred mainly with a strain that was simultaneously resistant to isoniazid and rifampicin.

To extend our analysis of the situation that prevailed in this region, samples from 143 (83%) of 172 patients without MDR strains were spoligotyped. Of these 143 patients, 41 (29%) were female. Overall, 31 (22%) of the 143 patients had Haarlem3 genotype TB. In contrast to the MDR-TB outbreak that involved only men, 6 women had a non-MDR Haarlem3 strain. Aside from the absence of clustering, ligation-mediated PCR typing showed that none of these non-MDR Haarlem3 isolates displayed a profile similar to the 19 MDR isolates involved in the transmission chain. Sequencing of the *rpoB* gene of 10 iso-

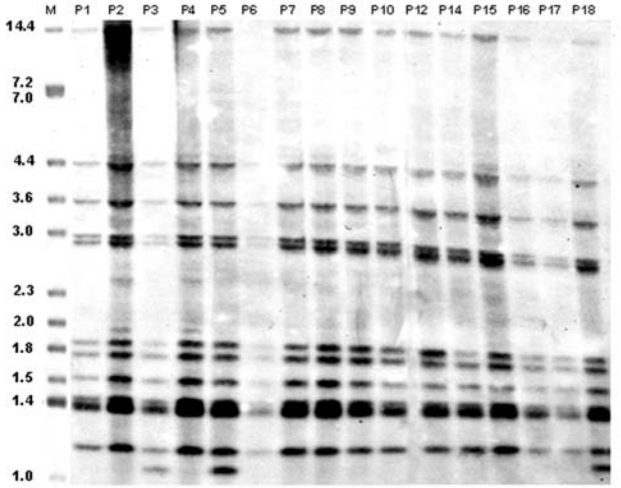

Figure 1. IS*6110* restriction fragment length polymorphism (RFLP) analysis of *Mycobacterium tuberculosis* isolates from 16 patients associated with the multidrug-resistant tuberculosis outbreak, Bizerte, Tunisia, 2001–2004. Lane M, reference strain MTB14323. Values above each well correspond to each patient as identified in Table 1. Values on the left are in kilobases.

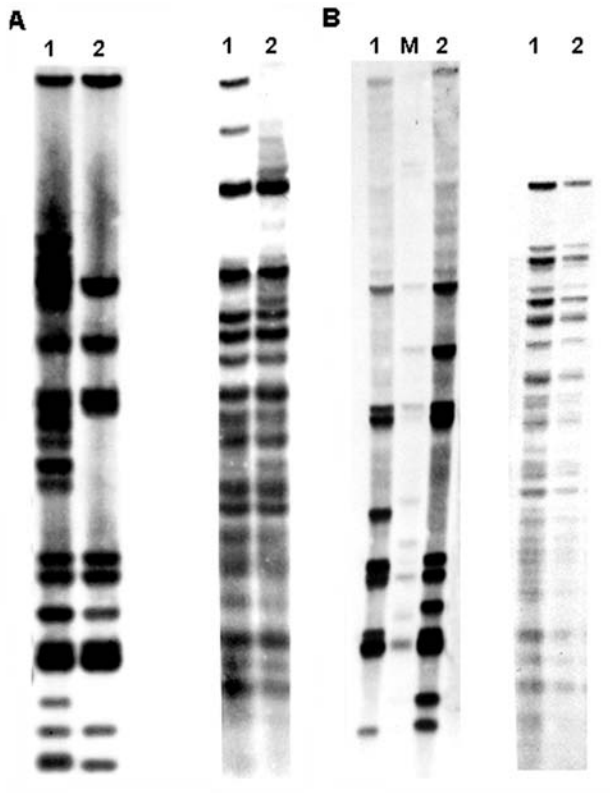

Figure 2. A) IS*6110* restriction fragment length polymorphism (RFLP) analysis (left) and polymorphic GC-rich repetitive sequence (PGRS) typing (right) of patient P11. Lane 1, initial isolate; lane 2, follow-up isolate. B) IS*6110* RFLP (left) and PGRS typing (right) of patient P20 (lane 1) compared with patient P3 (lane 2), a typical outbreak-associated patient. Lane M, reference strain MTB14323.

lates randomly selected from the 31 non-MDR Haarlem isolates showed the absence of the outbreak-associated mutation V610M. This finding is strongly indicative of a true clonal expansion and a typical MDR-TB outbreak. The W/Beijing type was absent in the analyzed pool of isolates.

Conclusions

The results indicate that an MDR strain of *M. tuberculosis* has been actively transmitted among 19 HIV-negative male patients in Tunisia. Several observations indicate that this particular Haarlem strain displays increased transmissibility, virulence, or both. First, the outbreak peaked suddenly within a relatively short period of 21 months; 17 new cases (89%) were reported from September 2001 to June 2003. Inspection of the hospital register for 2000 showed only 3 new patients with MDR isolates, including outbreak-associated patients P5 and P7 (Table 1). Second, no epidemiologic links or contact points could be traced for several patients, which suggests that brief exposure would have been sufficient for effective transmission. Because patients with MDR-TB do not respond to treatment, they may serve as constant sources of transmission. Such a situation is likely to have occurred for the patients with established epidemiologic links. Third, the incidence of TB in the region in which the outbreak occurred is not particularly high. Fourth, patients were seronegative for HIV with no history of treatment causing immunosuppression. Fifth, no AIDS-associated TB outbreak that might have increased the adaptability of the strain within the indigenous population had occurred in the region. Sixth, although the Haarlem strain was MDR, it was able to cause an outbreak in those vaccinated with bacille Calmette-Guérin and in persons who were not hospitalized.

Among the identified *M. tuberculosis* strain families (4,5), the W/Beijing type has been associated with outbreaks or microepidemics worldwide (3). The Haarlem strain family appears to be widespread (4), but its ability to cause outbreaks has been reported only twice, once in Argentina (13) and once in the Czech Republic (14). The distinctive feature of the present Haarlem MDR-TB outbreak is its accelerated transmission compared with the first 2 MDR-TB outbreaks.

Alterations within DNA repair genes (mutator genes) are thought to favor the emergence of MDR strains with an increased adaptability (12). In this respect, both W/Beijing and Haarlem strains accumulated mutations within their putative mutator genes. Widespread MDR strains might also benefit from their intrinsic adaptability (15). From an epidemiologic point of view, TB programs must conduct extensive surveillance of MDR strains of *M. tuberculosis* strain families because they might cause serious outbreaks.

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Community-associated Methicillin-resistant *Staphylococcus aureus*, Switzerland

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Two case-control studies evaluated the prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) carriage at hospital admission and characteristics of patients with CA-MRSA. Among 14,253 patients, CA-MRSA prevalence was 0.9/1,000 admissions. Although 5 CA-MRSA isolates contained Panton-Valentine leukocidin, only 1 patient had a previous skin infection. No easily modifiable risk factor for CA-MRSA was identified.

Recently, new strains of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), which cause soft tissue infections in healthy people, have been detected worldwide (1). The unique molecular feature of these CA-MRSA strains consists of 2 particular genetic elements, the type IV staphylococcal cassette chromosome (SCC) *mec* element and a virulence gene encoding a leukocyte-killing toxin called Panton-Valentine leukocidin (PVL), not found in hospital-acquired MRSA isolates (1).

Risk factors for CA-MRSA carriage are incompletely understood. Although antimicrobial drug use is well recognized as risk factor for hospital-acquired MRSA (2), results of previous investigations have been inconsistent regarding the association between previous antimicrobial drug use and acquisition of CA-MRSA (3,4). Recently, 2 studies from North America have suggested that recent antimicrobial drug use plays a role in CA-MRSA colonization (5,6).

Few systematic studies have assessed the epidemiology of CA-MRSA in Europe (7). Determining the epidemiology of CA-MRSA could help develop control measures and guide clinicians in identifying patients at high risk for CA-MRSA. Therefore, our prospective investigation sought to 1) determine the prevalence of CA-MRSA on hospital admission, 2) examine characteristics of patients carrying CA-MRSA, 3) test the hypothesis that previous antimicrobial drug exposure is associated with CA-MRSA carriage, and 4) evaluate the genetic diversity of CA-MRSA strains.

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The Study

Details of this prospective, observational study have been presented elsewhere (8). In brief, the study population consisted of 14,253 patients who were screened for MRSA carriage on admission to the Geneva University Hospitals between January 20, 2003, and August 31, 2003. Of these patients, 12,072 (85%) were hospitalized in the adult wards, 102 (1%) in pediatric wards, and 361 (2%) in psychiatric wards; 1,718 (12%) were seen in the emergency room and were discharged within 24 hours. MRSA screening was performed by nasal and inguinal swab samples, and cultures of specimens from other sites were performed when clinically indicated. A person fulfilled the CA-MRSA case definition if 1) the person had an MRSA isolate that yielded a SCC*mec* type different from the prevailing hospital-associated strain in the Geneva region (SCC*mec* type I [9]) and 2) the person had not been hospitalized within the last 3 years (3).

We performed 2 case-control studies. The first control group comprised all patients with MRSA carriage identified on admission who did not fulfill our case-definition of CA-MRSA. If a patient was admitted more than once during the study period, only the first admission was included in this analysis. The second control group consisted of a group of randomly selected MRSA-negative patients. The following potential risk factors for CA-MRSA carriage were documented: age, sex, origin of patient, coexisting conditions, severity of underlying illness, functional status, patient's prior location, presence of skin lesions, and antimicrobial drug use within the past 6 months.

MRSA was identified according to NCCLS guidelines (10). Typing of SCC*mec* elements and detection of PVL genes were carried out as described (9). A novel multiplex polymerase chain reaction (PCR) based assay was used for rapid genotyping of *S. aureus* isolates (11). This assay is based on variable-number tandem repeat typing (12) and has been modified to allow high throughput and automated analysis. In addition, 13 CA-MRSA isolates, 2 hospital-acquired MRSA isolates, and 2 references strains from the United States were genotyped by multilocus sequence typing (MLST) (13). PCR products were sequenced with an ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Allele numbers were assigned according to the program available from the MLST Web site (http://www.mlst.net).

We used the Student *t* test to compare continuous variables and the chi-square test or Fisher exact test to

compare proportions. Univariate comparisons were performed to determine characteristics of CA-MRSA patients. Data were analyzed with STATA, version 8.0 (Stata Corp, College Station, TX, USA).

During January through August 2003, 428 of 14,253 screened patients were discovered to be MRSA carriers on admission (prevalence 3.0%). Most MRSA isolates belonged to the type I cassette (n = 371, 26/1,000 admissions). MRSA SCC*mec* type IV was recovered in 46 patients (3.2/1,000 admissions), whereas types II, III and V were only rarely identified (n = 11). Thirty-seven of 46 patients (80%) with SCC*mec* type IV isolates had previous contact with the healthcare system, in particular with hospitals in neighboring France.

Thirteen patients fulfilled our case definition for having CA-MRSA (prevalence 0.9/1,000 admissions). The prevalence of CA-MRSA varied according to the hospital sector: it was highest in pediatric patients (9.8/1,000), followed by adult outpatients staying <24 hours (1.7/1,000) and adult inpatients (0.7/1,000). Important features of the 13 CA-MRSA cases are shown in the Table. Six CA-MRSA patients lived outside Switzerland: Kosovo (n = 2), France (n = 1), Senegal (n = 1), Madagascar (n = 1), and Libya (n = 1).

Ten CA-MRSA isolates harbored the SCC*mec* type IV, 2 the type V, and 1 the type II element (Figure). All CA-MRSA isolates were susceptible to trimethoprim-sulfamethoxazole, clindamycin, and vancomycin. Two isolates (SCC*mec* type V) were resistant to gentamicin; 2 other isolates (SCC*mec* type IV) showed resistance to fluoroquinolones; and 1 (SCC*mec* type II) was resistant to macrolides. Although 5 (38%) of 13 CA-MRSA isolates possessed the *pvl* gene, only 1 patient had a skin infection on admission. The other 4 patients had no history of infection.

Genetic analysis combining genotyping and MLST showed substantial diversity among CA-MRSA isolates (Figure). In particular, they were not related to the nosocomial strain endemic in the Geneva hospital setting (strains B5-63 and B5-64). Dendrogram analysis identified 2 closely related CA-MRSA isolates from patients living in Geneva (strains B2-55 and B3-11) who had never been hospitalized and had no apparent epidemiologic link. The pattern of 1 CA-MRSA isolate (B2-19), from a 38-year-old Geneva woman who used injection drugs, was related to the MW2 strain from North Dakota.

MLST of CA-MRSA isolates identified 7 sequence types (ST-1, -5, -8, -45, -80, -88, and -152), belonging to patients from different geographic origins (Figure). Five strains represented 2 distinct ST clones (ST-45 and ST-152) previously described in northern Europe and Israel; 1 isolate was related to the prototype CA-MRSA strain MW2 (ST-1); and 2 isolates corresponded to a clone reported from Asia (ST-88). The largest cluster contained strains previously described from several continents, including French clone ST-8, Mediterranean clone ST-80, and the international, so-called pediatric clone ST-5 (SCC*mec* type IV).

Patients with CA-MRSA differed in several ways from those who carried healthcare-associated MRSA (n = 346) and those who were free of MRSA on admission (n = 1,542, Table). Patients with CA-MRSA were younger and

Table. Characteristics of patients with community-associated, methicillin-resistant *Staphylococcus aureus* (CA-MRSA), compared to those of patients without MRSA and patients with healthcare-associated MRSA

Characteristic	Patients with CA-MRSA (n = 13) (%)	Patients without MRSA (n = 1,542) (%)	p value*	Patients with healthcare-associated MRSA (n = 346) (%)	p value†
Mean age (y ± SD)	45 ± 25	70 ± 18	<0.001	75 ± 15	<0.001
Male sex	6 (46)	682 (44)	1.0	199 (58)	0.57
Residency outside Switzerland	6 (46)	10 (1)	<0.001	8 (2)	<0.001
Healthcare worker	0 (0)	31 (2)	1.0	3 (1)	1.0
Emergency admission	9 (69)	724 (48)	0.17	166 (48)	0.16
Severity of disease					
Presence of ≥1 coexisting condition	8 (62)	1,082 (70)	0.55	289 (84)	0.05
Rapidly or ultimately fatal disease	0	277 (18)	0.11	95 (27)	0.02
Complete dependence for daily activities	0	114 (7)	0.62	57 (16)	0.24
Antimicrobial drug exposure					
Previous exposure (<6 mo)	1 (8)	318 (21)	0.49	197 (57)	<0.001
Current use at admission	3 (23)	148 (10)	0.13	41 (12)	0.21
Presence at admission of indwelling urinary catheter	0	73 (5)	1.0	64 (19)	0.14
Open skin lesions	2 (15)	100 (6)	0.21	65 (19)	1.0

*Community-associated MRSA case-patients versus non-MRSA controls.
†Community-associated MRSA case-patients versus controls with healthcare-associated MRSA strains (SCC*mec* type I isolates).

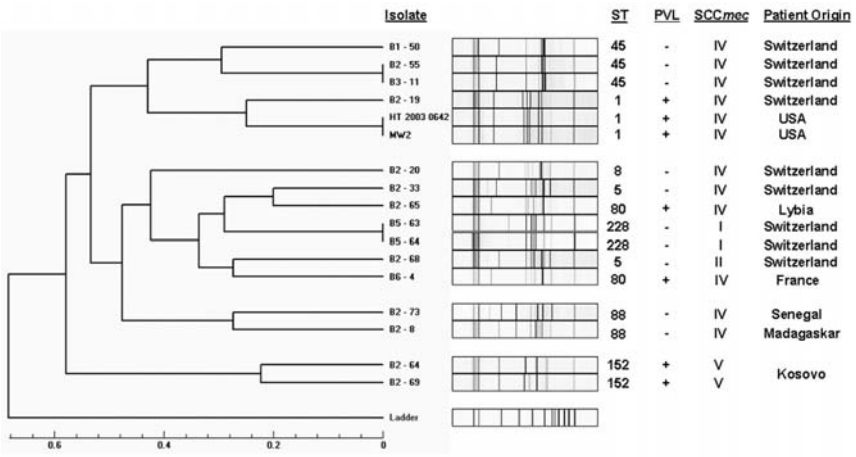

Figure. Analysis of genotyping patterns, multilocus sequence typing (ST) results, presence of Panton-Valentine leukocidin (PVL), staphylococcal cassette chromosome *mec* (SCC*mec*) type, and country of patient origin of 13 community-associated, methicillin-resistant *Staphylococcus aureus* isolates (CA-MRSA). The dendrogram illustrates the genetic relatedness of the 13 CA-MRSA in comparison to 1) 2 nosocomial MRSA isolates representing the prevailing endemic strain in the Geneva healthcare setting (strains B5-63, B5-64) and to 2) profiles obtained for strains MW2 and HT20030642 from the United States, 2 closely related CA-MRSA isolates used as controls.

more likely to have a permanent residency outside Switzerland. Compared to non-MRSA controls, no significant differences were noted in previous outpatient antimicrobial drug use and presence of coexisting conditions (Table). By contrast, CA-MRSA patients had fewer coexisting conditions and less prior exposure to antimicrobial drugs than patients with healthcare-associated MRSA. The presence of skin lesions on admission was not predictive of CA-MRSA carriage.

Conclusions

This study provides information about the epidemiology of CA-MRSA on admission to the largest hospital in Switzerland. It showed 1) a low prevalence of CA-MRSA, 2) a reservoir of asymptomatic persons colonized with PVL-containing CA-MRSA strains, 3) a high degree of CA-MRSA diversity, and 4) no readily modifiable risk factor for CA-MRSA carriage.

Several investigators have recently attempted to describe the epidemiology of CA-MRSA more precisely (5,14). These study findings are not consistent since they were conducted in different settings and used various case definitions (3). Typically, most studies used an epidemiologic case definition that excluded patients with recent healthcare system contact in whom MRSA was detected within 48 hours after hospital admission (15). This type of study, however, cannot prove that MRSA acquisition was unrelated to healthcare system contact. Therefore, how to define true community-acquired MRSA remains controversial. Although we added a molecular component to increase the validity of our case definition, we used the more conservative term "community-associated" MRSA, since we cannot exclude the fact that CA-MRSA case-patients may have previously been in contact with outpatient care or hospitalized family members.

Described risk factors associated with CA-MRSA infection include intravenous drug use, military training, jail exposure, team sport activities, homosexuality, low socioeconomic class, and being member of a "closed population" such as Native Americans and Australian aborigines (5,6). Two recent analyses found an increased risk in patients exposed to antimicrobial drugs (5,6). Our study did not confirm this hypothesis, making it unlikely that antimicrobial drug control measures will substantially decrease transmission of CA-MRSA.

Control of CA-MRSA remains a challenge, since transmission is linked to migration and travel (16). Our study showed that the ratio of 4:1 between colonization and infection with CA-MRSA possessing the *pvl* gene was larger than previously thought. Restricting surveillance to infected carriers will underestimate the prevalence of PVL-producing CA-MRSA. Thus, CA-MRSA surveillance should not rely on clinical specimens alone.

Several study limitations merit consideration. First, we may have underestimated CA-MRSA prevalence since our study was not truly population-based. Second, we may have misclassified CA-MRSA cases. Thorough review of medical charts minimized this potential bias. Finally, we were not able to elucidate the route of transmission for those CA-MRSA carriers living in the Geneva community. MLST results suggest that nonendemic hospital strains may already circulate in the Geneva community.

In summary, the prevalence of CA-MRSA remains relatively low in our community. Yet migration will likely increase CA-MRSA carriage in the near future. This development will influence clinical practice by changing the choice of empiric antimicrobial drug therapy for severe skin and soft tissue infections.

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Community-associated Methicillin-resistant *Staphylococcus aureus* in Pediatric Patients

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Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections increased from 2000 to 2003 in hospitalized pediatric patients in Houston. CA-MRSA was associated with greater illness than was infection with methicillin-susceptible strains. Children with CA-MRSA were younger and mostly African American. Of MRSA isolates, 4.5% had the inducible macrolide-lincosamide-streptogramin B phenotype.

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection in children is an increasing problem (1,2). However, we do not know whether CA-MRSA and the historically more common community-associated methicillin-susceptible *Staphylococcus aureus* (CA-MSSA) have similar pathogenesis and cause similar illness (3–5). In Houston, CA-MSSA infections were reported initially to be more severe than CA-MRSA infections (3), but further reports stated the opposite (4,5). Our clinical impression was that CA-MRSA infections were becoming more frequent and were more severe than CA-MSSA infections. To test the validity of our clinical impression, we performed a retrospective chart review of hospitalized pediatric patients with *S. aureus* infections during a 3-year interval. We determined prevalence, clinical characteristics, susceptibility patterns, and empiric antimicrobial regimens for CA-MRSA and CA-MSSA.

The Study

We performed a retrospective chart review of pediatric patients (≤18 years of age) who were admitted to Memorial Hermann Children's Hospital, Houston, Texas, in a 36-month period (July 2000 to June 2001 and January 2002 to December 2003; we excluded the second semester of 2001 from the analysis because the hospital and the microbiology laboratory were temporarily closed in July 2001). A laboratory report of isolation of *S. aureus* from an inpatient qualified the person as a candidate. From these candidates, patients with underlying illness predisposing to frequent hospitalization (immunodeficiency, cystic fibrosis, chronic renal failure, malignancy) and patients who had been previously hospitalized or underwent surgery within 3 months before *S. aureus* isolation were excluded. Patients from the neonatal intensive care unit and patients with mixed cultures were also excluded. For the remaining patients (N = 239), community-associated *S. aureus* was defined as the isolation of *S. aureus* from a culture obtained within 72 h of admission. Antimicrobial resistance testing was performed by broth microdilution MIC method by the Clinical Microbiology Laboratory (Pasco, Becton Dickinson, Sparks, MD, USA).

Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) standards and guidelines were used to interpret MICs for clindamycin, erythromycin, gentamicin, linezolid, minocycline, oxacillin, fluoroquinolones (ciprofloxacin, levofloxacin, gatifloxacin), rifampin, trimethoprim/sulfamethoxazole (TMP/SMX), and vancomycin. For MRSA isolates that were erythromycin resistant and clindamycin susceptible, inducible macrolide-lincosamide-streptogramin B (MLSB) resistance was determined by the disk diffusion method (6). Demographic and clinical characteristics between CA-MRSA and CA-MSSA were compared by Student *t* test or Wilcoxon signed rank for continuous variables and chi-square/Yates correction or Fischer exact test for categorical variables.

From 2000 to 2003, CA-MRSA accounted for 67% (159/239) of community-associated *S. aureus* infections in hospitalized pediatric patients (56% in 2000–2001, 57% in 2002, and 78% in 2003, $p<0.01$ for trend). Patients with CA-MRSA infections were significantly younger and more likely to be African American than patients with CA-MSSA infections, which is consistent with results from a previous study (3). Patients with CA-MRSA tended to have longer duration of bacteremia and significantly more surgical interventions (incision, aspiration, drainage, or débridement) (Table 1). Both groups had similar duration of hospitalization, intensive care unit treatment, proportion of positive blood culture, peripheral leukocyte counts, and erythrocyte sedimentation rates at admission (data not shown).

CA-MRSA infections were seen more frequently with abscesses and complicated pneumonias (Table 2). The locations of the abscesses were similar in both groups; the most common sites were the extremities, gluteal, and perirectal areas. Among deep abscesses, 2 mediastinal and

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Table 1. Demographic and clinical characteristics of hospitalized pediatric patients with CA-MRSA and CA-MSSA infections*

Demographic data	No. MRSA (n = 159), (%)	No. MSSA (n = 80), (%)	p value
Age, median (range)	1.6 y (1.5 mo–17.9 y)	2.6 y (2 mo–17.7 y)	<0.05
Female sex	86 (54.0)	38 (47.5)	NS
Ethnicity			
African American	75 (47.1)	27 (33.7)	<0.05
Hispanic	52 (32.7)	32 (40.0)	NS
White	21 (13.2)	14 (17.5)	NS
Other	11 (6.9)	7 (8.7)	NS
Clinical data			NS
Duration bacteremia (d)†, mean ± SD	2.4 ± 1.8	1.1 ± 0.3	0.06
Surgical intervention	140 (88.1)	57 (70.4)	<0.01
Hospital days, median (range)	3 (1–53)	4 (1–38)	NS
Intensive care‡	13 (8.2)	10 (12.5)	NS

*CA, community-associated; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; NS, not significant; SD, standard deviation.
†Positive blood cultures: 7 MRSA and 9 MSSA.
‡No. patients who required treatment in the pediatric intensive care unit.

1 retropharyngeal abscess were CA-MRSA, and 1 retropharyngeal abscess was CA-MSSA. Among patients with pneumonia, 12 of 17 CA-MRSA were complicated (9 empyemas and 3 pneumatocele/pneumothorax) versus 2 of 13 CA-MSSA (1 empyema and 1 pneumatocele). Only 2 patients had a documented viral pneumonia before the *S. aureus* pneumonia. Among patients with osteoarticular infections, both groups had similar involvement and complications. In the CA-MRSA group (10 patients) were 7 osteomyelitis, 5 septic arthritis, 1 myositis, 1 deep venous thrombosis, and 4 bacteremia cases. In the CA-MSSA group (8 patients) were 8 osteomyelitis, 2 septic arthritis, 3 myositis, 1 deep venous thrombosis, and 4 bacteremia cases.

CA-MRSA isolates were more likely to be resistant to erythromycin (92% vs. 45%, p<0.01) and fluoroquinolones (16% vs. 4%, p<0.01). Resistance to clindamycin was 5% in both groups. All CA-MRSA and CA-MSSA isolates were susceptible to gentamicin, linezolid, minocycline, rifampin, TMP/SMX, and vancomycin. A total of 3 (4.5%) of 66 CA-MRSA isolates had the inducible MLSB-resistant phenotype. Clindamycin was the most commonly used antimicrobial drug. It was the initial empiric treatment for 60% of both CA-MRSA and CA-MSSA infections when used alone or in combination with other antimicrobial drugs. The use of clindamycin increased over time (32% in 2001, 54% in 2002, and 66% in 2003, p<0.001). The empiric use of vancomycin was more frequent in the CA-MRSA group (25% vs. 12%, p<0.05) but did not increase over time. Nafcillin use was similar in both groups (8% vs. 11%). For 16% of the CA-MRSA cases, empiric therapy was with an agent to which the infecting isolate was later found not to be susceptible in vitro, regardless of the clinical outcome.

Conclusions

CA-MRSA is seen with increasing frequency in Houston; it is a more severe infection with more frequent serious complications, compared to CA-MSSA. The increasing frequency of severe *S. aureus* infection requires reassessing regimens of empiric therapy delivered on admission and added emphasis to timely and appropriate acquisition of specimens for culture.

Since 2000, rates of pediatric CA-MRSA in our hospital have increased from 56% to 78%; these infections are associated with greater illness, especially empyema and necrotizing pneumonia, compared to CA-MSSA infections. A similar increase in MRSA frequency and severity has been reported from another pediatric hospital in Houston (4,5). Of all CA-MRSA isolates reported from

Table 2. Site of infection with CA-MRSA or CA-MSSA*

Site	No. MRSA (n = 159), (%)	No. MSSA (n = 80), (%)	p value
Abscess	80 (50.3)	23 (28.7)	<0.01
Lymphadenitis	35 (22.0)	13 (16.2)	NS
Pneumonia	17 (10.7)	13 (16.2)	NS
Complicated pneumonia†	12/17 (70.6)	2/13 (15.4)	<0.01
Cellulitis	12 (7.5)	8 (10.0)	NS
Osteoarticular‡	10 (6.3)	8 (10.0)	NS
Other§	5 (3.1)	15 (18.7)	ND

*CA, community-associated; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; NS, not significant; ND, not done (various diagnosis grouped).
†Empyema, necrotizing pneumonia, pneumatocele, or pneumothorax.
‡Osteomyelitis or septic arthritis.
§Sinusitis, preseptal and septal cellulitis, retropharyngeal and mediastinal abscess, urinary tract infection, toxic shock syndrome, isolated bacteremia.

that hospital, 94% are of the same clone (7). The similar characteristics and rates across institutions support the hypothesis that CA-MRSA in Houston are related, but molecular genetic analysis of our strains would be necessary to confirm this hypothesis.

Emerging CA-MRSA is a global problem (1,2). For some regions, direct evidence shows an association between clonality of CA-MRSA and severity (5,8–10). This association seems to be related to specific virulence factors, such as the Panton-Valentine leukocidin, among others (10,11) . In a recent study from Houston, strains carrying the *pvl* gene were associated with severe staphylococcal sepsis in adolescents (5) and with CA-MRSA musculoskeletal infection in children (8). The presence of the *pvl* gene may be related to an increased likelihood of complications in children with *S. aureus* infections. The present study lacks molecular genetic analysis of the strains to support this hypothesis.

Treatment of MRSA infections is challenging. Empiric treatment usually includes the use of clindamycin or vancomycin (2,4). MRSA strains that are clindamycin-susceptible but erythromycin-resistant may have the in vitro inducible MLSB-resistance phenotype with potential for treatment failure (12–14). Rates of inducible MLSB resistance among pediatric MRSA isolates vary widely. Our results are similar to those from previous studies from Houston (2%–8% inducible MLSB resistance) (3,4) and different from reports from cities such as Baltimore (43%) (13) and Chicago (94%) (12). Awareness of local resistance patterns is required to select adequate empiric therapy. Trends in clindamycin use could indicate physician awareness of MRSA resistance patterns. The increasing penetration of CA-MRSA in the community requires disseminating information to primary care providers about the potential severity of this infection, methods for rapid and accurate diagnosis, and need to rapidly implement appropriate empiric and definitive treatment regimens.

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Erythromycin-nonsusceptible *Streptococcus pneumoniae* in Children, 1999–2001

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After increasing from 1995 to 1999, invasive erythromycin-nonsusceptible *Streptococcus pneumoniae* rates per 100,000 decreased 53.6% in children from Baltimore, Maryland (USA) from 1999 to 2001, which was partially attributed to strains related to the *mef*E-carrying England^{14}-9 clone. The decline in infection rates was likely due to the pneumococcal 7-valent conjugate vaccine.

From 1995 to 1999, the prevalence of macrolide resistance among invasive pneumococci doubled to 20% in the United States (1). The rise in the 1990s was primarily due to strains with an M phenotype, a surrogate marker for the *mef*E gene (1,2). In 1999, children <5 years of age and white persons had a higher proportion of M phenotype strains causing invasive disease than did older persons and black persons (1). Most macrolide-resistant strains in the United States were also penicillin-nonsusceptible (1); modeling suggested that strains resistant to both drug classes would increase without a vaccine or other intervention (3). Since the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in 2000, the overall incidence of macrolide-resistant infections, including serotype 14 strains, has decreased in Atlanta, Georgia (4). It is unclear whether these changes are caused by shifts in a small number of clones. Most drug-resistant infections in the United States are related to a small number of international clones (5).

The Study

The Baltimore metropolitan area is one of the sites in the Active Bacterial Core system that conducts active, laboratory surveillance for invasive pneumococcal disease. Approximately 15 million people residing in Maryland, Georgia, California, Minnesota, Oregon, Tennessee, and Connecticut were included in the multicenter study (1). In this study, we calculated the rates of invasive erythromycin-nonsusceptible *S. pneumoniae* disease, *mef*E-associated disease, and disease due to *mef*E-carrying clones in the Baltimore metropolitan area in 1995, 1999, and 2001. These years were chosen to validate the earlier multicenter study in the Baltimore metropolitan area (1995 and 1999) and include 1 year after licensure of PCV7 (2001). PCV7 includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. We also assessed whether the *mef*E-carrying strains were equally distributed in all populations, in all locations, and during both respiratory and nonrespiratory seasons from 1995 to 2001. Cases of invasive pneumococcal disease with an erythromycin MIC ≥0.5 μg/mL isolated from January 1,1995, to December 31, 2001, were included. Strains with an erythromycin MIC ≥0.5 μg/mL and penicillin MIC ≥0.12 μg/mL were defined as erythromycin-nonsusceptible and penicillin-nonsusceptible *S. pneumoniae*, respectively (6). Pneumococcal serotypes were determined at the Centers for Disease Control and Prevention by the latex agglutination test and confirmed with Quellung reaction. The presence of *mef* and/or *ermB* was determined by using a duplex polymerase chain reaction (PCR) (7), and *mefA* was differentiated from *mefE* by PCR-restriction fragment length polymorphisms (8). Pulsed-field gel electrophoresis (PFGE) was performed on all strains (9). Dendrograms were created in Bionumerics (Applied Maths, Sint-Martens-Latem, Belgium) with a position tolerance of 1.5%. Strains were compared to the first 25 international clones (http://www.sph.emory.edu/PMEN/) (7). Multi-locus sequence typing was performed on 87 (24.5%) of the 349 strains (10,11), and included the spectrum of PFGE patterns. Based on PFGE patterns with ≥80% relatedness by dendrogram, and/or 5 identical alleles, strains were classified into sequence type (ST)-complexes. The following 4 ST-complexes were classified as the clones in this analysis: ST9-complex (related to England^{14}-9 clone), ST81-complex (related to Spain^{23F}-1 clone), ST156-complex (related to Spain^{9V}-3 clone), and ST236-complex (related to the Taiwan^{19F}-14 clone).

Rates were calculated by using population estimates from US Census Bureau data for the Baltimore metropolitan area for 1995, 1999, and 2001. Chi-square and Fisher exact tests were used to compare the proportion of the population with disease in 1995, 1999, and 2001 (SAS 8.2, SAS Institute, Cary, NC, USA). Cochran-Armitage trend test was used to compare the proportion of erythromycin-nonsusceptible *S. pneumoniae* strains carrying the *mef*E gene from 1995 to 2001. Age groups were defined as children <5 years of age and persons ≥5 years of age; races were defined as persons of white and black race (1);

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respiratory and nonrespiratory seasons were defined as November–April and May–October, respectively (12).

Most cases of invasive pneumococcal disease occurred in 3 geographic regions: 61.0% (2,976/4,885) in Baltimore City, 18.3% (895/4,885) in Baltimore County, and 9.8% (480/4,885) in Anne Arundel County. From January 1, 1995, to December 31, 2001, a total of 4,885 pneumococcal cases were detected in the Baltimore metropolitan area, of which 85.8% (4,192/4,885) were available for MIC testing. Ninety-seven percent (349/360) of the erythromycin-nonsusceptible *S. pneumoniae* isolates were available for further analysis. Of these isolates, 255 (73.1%) carried only the *mef*E gene, 61 (17.5%) carried only the *erm*B gene, 8 (2.3%) carried both the *mef*E and *erm*B genes, 6 (1.7%) carried the *mef*A gene, and 19 (5.4%) had unknown resistance mechanisms. All isolates carrying both the *erm*B and *mef*E genes were serogroup 19 strains that were related to the Taiwan^{19F}-14 clone. The *mef*A-carrying strains were either serotype 6B or serotype 14. The serotype 6B strains, belonging to ST146, were detected in Baltimore City during a 3-month period in 1998; the serotype 14 strains were detected in Howard County in 3 different years.

The incidence of invasive pneumococcal disease significantly increased from 1995 to 1999 before dramatically decreasing from 1999 to 2001. From 1995 to 1999, the overall rates of erythromycin-nonsusceptible *S. pneumoniae* and *mef*E-associated disease increased, and then stabilized from 1999 to 2001 (Table). While the overall rates of erythromycin-nonsusceptible *S. pneumoniae* were stable, the proportion of pneumococcal strains with reduced susceptibility to erythromycin increased from 5.1% (26/510) in 1995 to 13.6% (77/567) in 2001 ($p<0.01$). Moreover, the proportion of erythromycin-nonsusceptible *S. pneumoniae* strains carrying the *mef*E gene with an erythromycin MIC ≥16 μg/mL increased from 0% (0/12) in 1995 to 12.3% (8/65) in 2001 (χ^2 for linear trend, $p = 0.02$). The proportion of erythromycin-nonsusceptible *S. pneumoniae* strains carrying the *mef*E gene increased from 48.0% (12/25) in 1995 to 85.5% (65/76) in 2001 ($p<0.01$). Of the erythromycin-nonsusceptible *S. pneumoniae* strains, the proportion of *mef*E-carrying strains that were penicillin-nonsusceptible *S. pneumoniae* rose from 20.0% (5/25) in 1995 to 72.4% (55/76) in 2001 ($p<0.01$). In 3 counties, in both age groups, in both races, and during both seasons, the proportion of *mef*E-carrying strains increased from 1995 to 2001 (Figure; p values for all analyses were ≤0.02). Sixty-nine percent (182/263) of the *mef*E-carrying strains were related to 4 international clones (percent serotype): 30.0% England^{14}-9 clone (100% 14); 16.0% Spain^{23F}-1 clone (83.3% 23F); 14.1% Spain^{9V}-3 clone (97.3% 9V); and 9.1% Taiwan^{19F}-14 clone (95.8% 19F).

Among children <5 years old, the incidence of erythromycin-nonsusceptible *S. pneumoniae* increased from 1995 to 1999 before declining by 53.6% ($p = 0.03$) between 1999 and 2001. Likewise, the incidence of *mef*E-associated disease rose in the 1990s before decreasing by 51.5% from 1999 to 2001 ($p = 0.07$). The rates of disease due to strains related to the England^{14}-9 clone mirrored the age-specific trends from 1995 to 2001 ($p\leq0.05$; Table). In contrast, in persons ≥5 years of age, the rate of erythromycin-nonsusceptible *S. pneumoniae* disease remained stable after increasing in the 1990s. From 1999 to 2001, the rate of *mef*E-associated resistance increased

Table. Annual rates of erythromycin-nonsusceptible pneumococcal disease*

		1995		1999		2001		1995 vs. 1999		1999 vs. 2001	
Age group	Disease	No. of cases	Rate	No. of cases	Rate	No. of cases	Rate	Change in rate (%)	p value	Change in rate (%)	p value
All	All invasive	683	28.1	762	31.1	599	23.6	10.7	0.05	–24.1	<0.01
	Erythromycin-nonsusceptible *S. pneumoniae*	26	1.4	71	3.2	77	3.2	125.3	<0.01	–0.7	1.0
	*mef*E	12	0.7	51	2.3	65	2.7	250.7	<0.01	16.7	0.4
	England^{14}-9, *mef*E	5	0.3	19	0.9	23	1.0	213.6	0.01	10.8	0.8
<5 y	All invasive	135	76.7	146	89.3	63	38.1	16.4	0.2	–57.4	<0.01
	Erythromycin-nonsusceptible *S. pneumoniae*	8	6.1	23	15.6	11	7.2	153.5	0.01	–53.6	0.03
	*mef*E	3	2.3	18	12.2	9	5.9	429	<0.01	–51.5	0.07
	England^{14}-9, *mef*E	1	0.8	11	7.4	4	2.6	870	<0.01	–64.7	0.05
≥5 y	All invasive	548	24.3	616	26.9	536	22.6	10.8	0.08	–16.1	<0.01
	Erythromycin-nonsusceptible *S. pneumoniae*	18	1.1	48	2.3	66	2.9	119	<0.01	25.2	0.2
	*mef*E	9	0.5	33	1.6	56	2.5	201.2	<0.01	54.6	0.04
	England^{14}-9, *mef*E	4	0.2	8	0.4	19	0.8	64.3	0.4	116.3	0.06

*Rate calculations: For cases where an isolate was not available, erythromycin susceptibility, presence of *mef*E gene, and serotype were assumed to be similar to those for which an isolate was available. For each disease category, rates >2 for ≥1 year in any age group are included in the table.

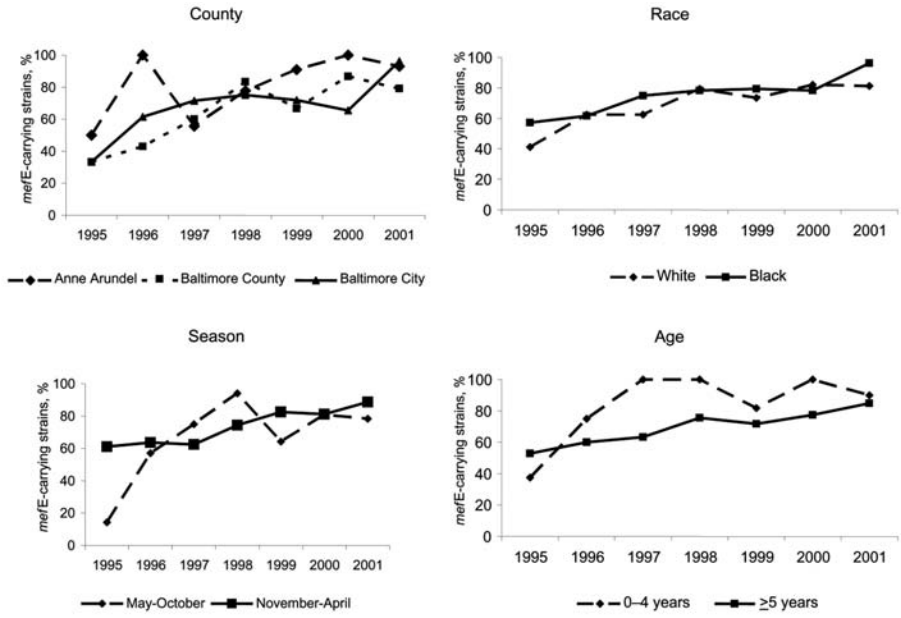

Figure. From 1995 to 2001, the proportion of erythromycin-nonsusceptible pneumococcal strains carrying the *mef*E gene increased over time by county, season, race, and age group. p<0.01 for all trends except for Anne Arundel County and May–October season (p = 0.02 for those comparisons).

by 54.6% (p = 0.04; Table). Strains related to the England[14]-9 clone partially accounted for this increase.

Conclusions

The incidence of invasive disease in the Baltimore metropolitan area increased in the 1990s before declining from 1999 to 2001 (13). Likewise, the rate of invasive erythromycin-nonsusceptible *S. pneumoniae* disease increased in the 1990s (1). From 1999 to 2001, the overall incidence of erythromycin-nonsusceptible *S. pneumoniae* disease and *mef*E-associated disease stabilized. The proportion of erythromycin-nonsusceptible *S. pneumoniae* strains carrying the *mef*E gene dramatically increased from 1995 to 2001. The increase in the proportion of erythromycin-nonsusceptible *S. pneumoniae* strains carrying the *mef*E gene over time was detected in patients residing in all 3 geographic locations, from both races, from both age groups, and during both the respiratory and nonrespiratory seasons.

Both the incidence of invasive erythromycin-nonsusceptible *S. pneumoniae* disease and *mef*E-associated disease declined by >50% from 1999 to 2001 in children <5 years of age. This decrease was partially due to serotype 14 strains related to the England[14]-9 clone. Strains related to this clone may also account for the substantial decrease in macrolide-resistant serotype 14 infections noted in Atlanta (4). In contrast to the Atlanta study, the rates of *mef*E-associated disease increased among persons ≥5 years. The differences in rates detected in Baltimore compared to Atlanta may reflect regional variation and the inclusion of 2002 in the Atlanta analysis (4). In this study, the decrease in the incidence of erythromycin-nonsusceptible *S. pneumoniae* in children may have been due to variation in antimicrobial drug use or to introduction of PCV7.

In summary, after increasing in the 1990s, the rates of invasive erythromycin-nonsusceptible *S. pneumoniae* disease stabilized overall and decreased in children from 1999 to 2001. This remarkable decline was most likely due to PCV7, although differential antimicrobial drugs may have been a contributor. Unfortunately, the lack of decline in the rate of invasive erythromycin-nonsusceptible *S. pneumoniae* infections among persons ≥5 years of age, coupled with the marked increase in dual resistance and the increase in the proportion with erythromycin MICs ≥16 μg/mL from 1995 to 2001 (14), is cause for concern. Public health initiatives that focus on judicious use of antimicrobial drugs and the PCV7 vaccine (13) may be beneficial in slowing these trends (15).

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Community-acquired Methicillin-resistant *Staphylococcus aureus*, Uruguay

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A novel, multidrug-resistant *Staphylococcus aureus* clone (Uruguay clone) with a non–multidrug-resistant phenotype caused a large outbreak, including 7 deaths, in Montevideo, Uruguay. The clone was distinct from the highly virulent community clone represented by strain MW2, although both clones carried Panton-Valentine leukocidin gene and *cna* gene.

Since the 1990s, multidrug-resistant *Staphylococcus aureus* (MRSA) infections have been increasingly recognized in the community, and MRSA strains isolated from patients with community-associated cases have been called community-associated MRSA (CA-MRSA) (1). CA-MRSA strains have been reported to differ from isolates from hospitals (healthcare-associated MRSA; HA-MRSA) in many characteristics such as susceptibility to antimicrobial drugs, types of staphylococcal cassette chromosome (SCC) *mec* element, and repertoires of exotoxin gene. In Uruguay, MRSA strains are among the most prevalent nosocomial pathogens. In late 2001, we observed a case in a young man with recurrent boils who visited an outpatient clinic. An MRSA strain that was susceptible to other drugs was isolated from the patient. After that, pediatric infections associated with similar strains were observed (2). The initial sporadic cases were followed by an epidemic increase of infections in the community, hospitals, and jails. We began to record the microbiologic data and analyze cases together with the National Antimicrobial Resistance Surveillance Network belonging to the Public Health Ministry in Uruguay, and we concluded that a large outbreak of CA-MRSA strains occurred in Uruguay. Here we report the emergence of a novel CA-MRSA clone, which has been shown by multilocus sequence typing (MLST) and SCC*mec* type to be distinct from the midwestern CA-MRSA strain.

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The Study

We studied patients with non–multidrug-resistant MRSA infections identified at 2 hospital centers in the metropolitan area of Montevideo, Uruguay, Hospital Maciel and Centro de Asistencia del Sindicato Médico del Uruguay, from January 2002 to October 30, 2003. A total of 125 *S. aureus* strains that were resistant to oxacillin alone or to erythromycin in addition were isolated from outpatients and inpatients. Since 1 of our members noticed some cases of pyogenic infections in a prison, we conducted a sentinel study of skin and soft tissue infection (SSTI) in the 2 main prisons from May to June in 2003. We isolated 40 non–multidrug-resistant MRSA strains from 58 inmates with SSTIs. Of these 40 strains, 17 were randomly selected to be analyzed. Susceptibilities to 8 antimicrobial drugs (oxacillin, vancomycin, gentamicin, rifampin, ciprofloxacin, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole) were tested by the Kirby Bauer disk diffusion test (Becton Dickinson, Cockeysville, MD, USA). Production of PBP2′ and protein A were verified by MRSA Screen latex PBP2′ (Denka Seiken-Oxoid Ltd, London, UK) and latex slide agglutination kits (Oxoid, Hampshire, UK), respectively. Most (133/142, 94%) showed heterogeneity in the degree of resistance to oxacillin, since double halos or haze zones were observed around the disk containing 1 μg of oxacillin.

The course of the outbreak during the 22 months is shown in Figure 1. The number of MRSA infections increased greatly in 2003. The Table summarizes the cases in which non–multidrug-resistant MRSA strains were isolated. Of the 125 case-patients, 112 were adults. The mean age was 39.7 years, which was lower than that of case-patients infected with HA-MRSA strains (mean age 59 years) reported previously (3). We classified the cases as community-associated if MRSA was isolated from cultures performed within 48 hours after admission to hospitals and excluded patients who had previously noted criteria for risk factors of HA-MRSA acquisition: recent hospitalization (within the last 6 months); use of medical devices (such as a permanent indwelling catheter or percutaneous medical device); exposure to healthcare services, including invasive or surgical procedures; residence in a long-term care facility; and any known antimicrobial drug use within the past year (4,5). Community-associated cases were dominant (78%). The predominant infection type in adults was skin and soft tissue infection (n = 86)

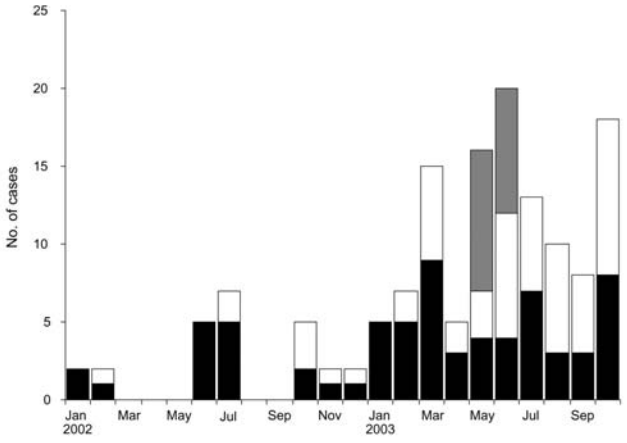

Figure 1. The monthly accumulation of cases of infections due to non–multidrug-resistant *Staphylococcus aureus* strains from January 2002 to October 2003. Black blocks indicate numbers of strains that were isolated from patients in the public hospital (Hospital Maciel), white indicates strains from a private hospital (Centro de Asistencia del Sindicato Médico del Uruguay), and gray indicates strains from 2 prisons (Libertad and Comcar).

such as abscesses, boils, and cellulitis, followed by respiratory tract infections, among which 12 of 14 were pneumonia. Four of 14 adult patients with respiratory tract infections exhibited symptoms of acute severe pneumonia, with histopathologic findings of "necrotizing pneumonia," and all died after bacteremia developed. Besides developing in these 4 patients with fatal cases, bacteremia developed in 9 other patients, and 3 of them died. The sites of infection preceding the bacteremia for these 3 patients were skin and soft tissue, bone and joint (septic arthritis), and unknown (classified as septic syndrome in Table), respectively. Bacteremia also developed in 3 pediatric patients. In total, bacteremia developed in 17 patients, and 7 died during the study period. We studied the molecular microbiologic characteristics of 68 isolates: 16 from the patients in whom bacteremia developed, 35 randomly selected from all case-patients, and 17 from the inmates.

Pulsotypes, coagulase isotypes, SCC*mec* types, and exotoxin gene repertoires were examined by the methods indicated in the footnotes of the online Appendix Table (available at http://www.cdc.gov/ncidod/eid/vol11no06/04-1059_app.htm). Among 6 pulsotypes identified in the Uruguay strains, 38 (74.5%) of 51 isolates from patients and all 17 isolates from the inmates, had related pulsotypes within 4 band differences designated A1–A4 (Appendix Table). All of them produced type-4 coagulase and carried type IVc SCC*mec* element, 53 of 55 carried *lukS,F-PV* genes, and 51 of 55 carried the *cna* gene. Isolates from 11 of 16 bacteremic case-patients, including 6 who died, belonged to pulsotype A. Evidence shows that a clone

Table. Clinical presentation of 125 MRSA-infected case-patients, Montevideo, Uruguay*

	Adult patients infected in†			Pediatric patients infected in‡	
Clinical feature	Community	Hospital	Unknown	Community	Hospital
Skin and soft tissue					
Abscess	26 (4) ¶			3 (3)	
Boils	20 (5)			1(1)	
Cellulitis	15 (3)	2 (2) ¶		2 (1)¶	
Hidradenitis	3 (2)				
Myositis	1 (1)				
Wound infection	8 (3)	11 (3)			
Infected atopic dermatitis					1 (1)¶
Respiratory tract					
Upper respiratory tract infection				4 (1)	
Necrotizing pneumonia	4 (3)¶#				
Pneumonia	1 (1)	3 (1) ¶			
Ventilator-associated pneumonia§		4(4)			
Colonization in respiratory tract		2(2)			
Catheter-associated infection					1 (1) ¶
Cerebrospinal fluid shunt					1(1)
Bone and joint infection	2 (1)¶	1 (1)¶			
"Sepsis" syndrome	5 (4) ¶		4 (2)		
Total	85 (27)	23 (13)	4 (2)	10 (6)	3 (3)

*MRSA, methicillin-resistant *Staphylococcus aureus*; parenthesis indicate the numbers of case-patients whose MRSA isolates were analyzed in this study.
†The range and mean age were 16–82 years and 39.7 years, respectively. The number of male and female case-patients were 65 (58%) and 47 (42%), respectively. Twenty-nine case-patients required hospitalization.
‡The range and mean age were 16–82 years and 6 years, respectively. The numbers of male and female case-patients were 8 and 5, respectively. One patient required hospitalization.
§Ventilator-associated pneumonia of the patients in an intensive care unit.
¶Besides 9 cases of sepsis syndrome, some of the other case categories were also bacteremic. They were 1 abscess, 4 necrotizing pneumonia, 2 bone and joint, 2 cellulitis, 1 infected atopic dermatitis, and 1 catheter-associated infection.
#A strain isolated from 1 of the patients was lost for analysis in this study.

(pulsotype A-SCC*mec* IVc), which possessed both *cna* and *lukS,F-PV* genes, caused the outbreak in Uruguay.

Other pulsotype strains carried primarily other SCC*mec* elements, such as type-II, type-IVa, or type-V, and produced type-2, -5, or -7 coagulase and did not carry *lukS,F-PV* genes. Notably, 49 (96%) of 51 strains isolated from both community-associated and healthcare-associated cases carried either type-IV or type-V SCC*mec* element, which have been found in CA-MRSA strains (6–8).

We compared characteristics of outbreak strains with those of previously investigated CA-MRSA strains isolated in the United States (MW2) and Australia (A803355, A823549, and E802537), and a strain isolated from an outpatient in Japan in 1981 (81/108) (Figure 2). All of them possessed both *cna* and *lukS,F-PV* genes as well. The dominant outbreak strains belonged to ST-30, which was the same as Australian and Japanese strains and distinct from MW2 (ST-1). In addition, we found that the pulsotype of strain A803355 (reported previously as H1) was identical to pulsotype A1, the most representative pulsotype among tested strains. Pulsotypes of other 2 Australian strains, A823549 and E8025347, were classified into the same cluster as A, while that of 81/108 showed a similar type on pulsed-field gel electrophoresis (PFGE) to pulsotype A. In contrast, only 2 Uruguay isolates, UR20 and UR41, had a PFGE pattern similar to that of MW2.

Conclusions

The outbreak of CA-MRSA in Uruguay involved >1,000 patients and ≥12 deaths, when the data after this study period are added. According to a follow-up survey conducted at jails from May to October 2003, 890 of 1,142 inmates were infected with similar pyogenic infections after an outbreak of scabies (10). Five patients required hospitalization. Boils and abscesses in the buttocks and neck were the most prevalent infections (85%), followed by hidradenitis and cellulitis. The prevalence of a new clone represented by UR6 (Uruguay clones) is considered to have been the cause of this large outbreak. We are conducting a further study of isolates from patients after this study period and isolates from inmates in a follow-up survey, and a final conclusion awaits those results.

We have suggested 2 genes as important candidates for high virulence in the midwestern CA-MRSA strains represented by strain MW2 (11), which is distributed in the United States and Europe (12,13). Since the Uruguay clone shared *lukS,F-PV* genes and *cna* gene with the midwestern CA-MRSA clone, this study strengthened the likelihood that these 2 genes are contributors for high virulence. Their genotypes, however, were completely different, and we now appear to have 2 distinct clones of highly virulent CA-MRSA. That certain CA-MRSA strains, identified in Australia as carrying the *luk-PV* and *cna* genes, had an identical PFGE pattern with UR6 does not imply that the same CA-MRSA clone has been disseminated between Uruguay and Australia because these 2 clones had distinct SCC*mec* elements, IVc and IVa, respectively. Since the MRSA clone originated when the SCC*mec* was integrated into the chromosome of a *S. aureus* strain, the 2 MRSA clones are understood to have originated independently by acquiring different SCC*mec* genes in their respective countries (14,15). In this regard, the difference in the SCC*mec* type was not reflected in the PFGE pattern. This example provides an excellent illustration of the fact that clonality cannot be judged on PFGE pattern alone.

Nonetheless, CA-MRSA strains with identical PFGE and MLST patterns possessing *lukS,F-PV* genes and *cna* genes exist in both Uruguay and Australia. This finding may indicate the existence of a genetically stable, virulent, multidrug-susceptible *S. aureus* (MSSA) clone in a community that extends beyond country borders and across the ocean. The MSSA clone, which has *lukS,F-PV* and *cna* genes, is established in the community and occasionally acquires SCC*mec* when the use of β-lactam antimicrobial drugs is increased to a stressful level for the survival of the MSSA clone in the community. Since we have also found MRSA strains isolated in the 1980s in Japan with a similar

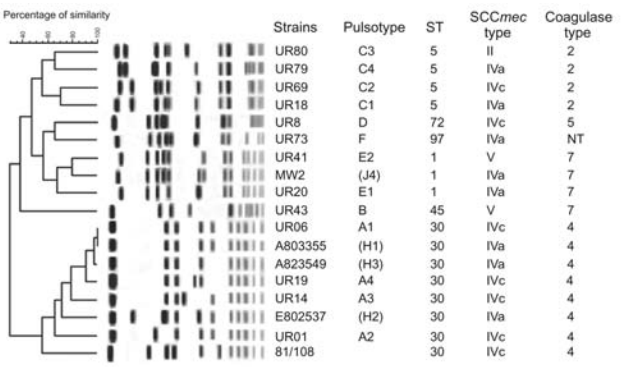

Figure 2. Dendrogram of pulsed-field gel electrophoresis (PFGE) banding pattern of representative Uruguay clone. Pulsotypes of representative Uruguay strains, a CA-MRSA strain isolated in the United States (MW2), 3 CA-MRSA strains isolated in Australia (A803355, A823549, and E802537), and a Japanese strain isolated from an outpatient (81/108) were compared by using a BioNumerics software program (Applied Maths, Sint-Martens-Latem, Belgium). Similarity coefficient was calculated by using Pearson correlation with position tolerance of 5%, and cluster analysis was performed by the unweighted pair-group method. Pulsotypes in parentheses indicate the types previously reported (7). PFGE was performed for 22 h with a CHEF MAPPER (Bio-Rad, Hercules, CA, USA) with a pulse time of 5 s to 40 s. *Sma*I-restriction patterns of the tested strains and reference strains were compared by using BioNumerics software. Genotypes of representative strains were determined by multilocus sequence typing as described by Enright et al. (9). Sequence type (ST) and clonal complex were assigned using programs in the *S. aureus* multilocus sequence typing database (http://www.mlst.net).

genotype to that of UR6 and possessing *PVL* and *cna* genes, the outbreak observed in Uruguay could occur in any part of the world if social or medical predisposing conditions are met.

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Correction: Vol. 11, No. 5

In "Adenovirus Type 7 Peptide Diversity during Outbreak, Korea, 1995–2000," by Eun Hwa Choi et al., errors occurred. In Table 2, column 3, amino acid sequences should be identified at position 197.

Also, Figures 1 and 2 in this article do not show all information. The online figures correctly represent the findings.

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol11no05/04-1211.htm

Correction: Vol. 11, No. 5

In "Osler and the Infected Letter," by Charles T. Ambrose, an error occurred. Yellow fever swept through Philadelphia in 1793.

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol11no05/04-0616.htm

Correction: Vol. 11, No. 5

In "Probable Tiger-to-Tiger Transmission of Avian Influenza H5N1," by Roongroje Thanawongnuwech et al., an error occurred. The GenBank accession nos. for HA and NA gene initiated from influenza A virus (A/Tiger/ Thailand/CU-T3/04) are AY842935 and AY842936.

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol11no05/05-0007.htm

We regret any confusion these errors may have caused.

Rifampin-resistant Meningococcal Disease

Jean Rainbow,* Elizabeth Cebelinski,* Joanne Bartkus,* Anita Glennen,* Dave Boxrud,* and Ruth Lynfield*

Rifampin-resistant meningococcal disease occurred in a child who had completed rifampin chemoprophylaxis for exposure to a sibling with meningococcemia. Susceptibility testing of 331 case isolates found only 1 other case of rifampin-resistant disease in Minnesota, USA, during 11 years of statewide surveillance. Point mutations in the RNA polymerase β subunit (*rpoB*) gene were found in isolates from each rifampin-resistant case-patient.

Chemoprophylaxis is recommended for close contacts of persons with invasive meningococcal disease to prevent secondary cases. In the 1960s, rifampin replaced sulfonamides as the recommended agent for chemoprophylaxis of household members and other close contacts of persons with invasive meningococcal disease when sulfonamide-resistant meningococci became common (1). In recent years, ciprofloxacin and ceftriaxone have been established as acceptable alternatives to rifampin for prophylaxis of meningococcal disease. However, rifampin remains a popular choice due to its low cost, ease of administration, and well-established record among infants and children.

Pharyngeal colonization with rifampin-resistant meningococci following chemoprophylaxis with rifampin of persons exposed to meningococcal disease was documented soon after treatment was initiated (2) and has continued to be observed over time (3). However, although rifampin has been used routinely worldwide for more than 30 years, few cases of rifampin-resistant meningococcal isolates in cases of invasive disease have been reported (4–7), and reports of only 3 instances in the United States could be found (8–10).

Rifampin targets the β subunit of DNA-directed RNA polymerase by inhibiting extension of the RNA strand. The β subunit is encoded by the *rpoB* gene. Previous studies have demonstrated that one of the mechanisms of rifampin resistance in *Neisseria meningitidis* is associated with single point mutations of the *rpoB* gene that result in amino acid substitutions (11–13). The data presented in this study confirm the rapid development of rifampin resistance upon exposure of meningococci to rifampin as a result of point mutations in the *rpoB* gene.

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The Study

Cases of invasive meningococcal disease in Minnesota residents are required to be reported to the Minnesota Department of Health (MDH). Laboratories throughout the state routinely submit isolates from patients with this disease to the MDH Public Health Laboratory, where they are serogrouped by slide agglutination (Difco, Detroit, MI, USA). In 1995, the MDH began routinely testing antimicrobial susceptibilities on meningococcal isolates and retrospectively conducted susceptibility testing on all available meningococcal isolates that had been submitted since 1993.

Antimicrobial susceptibilities were determined by using broth microdilution. Panels contained cation-adjusted Mueller-Hinton broth with 2%–5% lysed horse blood (PML Microbiologicals, Wilsonville, OR, USA) and were incubated at 35°C in CO_2 for 20–24 h. An Etest (AB Biodisk, Solna, Sweden) was also used for isolates that demonstrated resistance to further quantify degree of resistance. MIC breakpoints have recently been established by the Clinical and Laboratory Standards Institute for *N. meningitidis* (14). An MIC ≥2 μg/mL is considered resistant to rifampin.

Molecular subtyping of the sibling isolates was done by pulsed-field gel electrophoresis (PFGE) as described previously (15). The *rpoB* genes from rifampin-resistant and rifampin-sensitive isolates (Table 1) were amplified by polymerase chain reaction and sequenced by using primers described previously (13). DNA and peptide sequences were analyzed with BioNumerics (Applied Maths, Austin, TX, USA) and Vector NTI Suite (InforMax, North Bethesda, MD, USA).

The first known case of rifampin-resistant invasive meningococcal disease in Minnesota occurred in 1996. A 5-month-old infant had a clinical syndrome consistent with meningococcemia. He was hospitalized for 10 days, received antimicrobial drug therapy, and survived. By Etest, his serogroup B *N. meningitidis* isolate had a rifampin MIC ≥32 μg/mL. This was a sporadic case with no apparent links to any other previous or subsequent cases.

In 2002, fever, vomiting, and irritability developed in a 2-month-old infant, followed 12 hours later by labored breathing and a generalized rash. She was taken to a clinic where she experienced cardiac arrest and underwent cardiopulmonary resuscitation. She was transferred to a nearby emergency room where she died ≈1 hour later. Meningococcemia was suspected and household members were given prescriptions for rifampin. Waterhouse-Friderichsen syndrome was noted on autopsy, and

Table 1. Rifampin phenotype and genotype of *Neisseria meningitidis* isolates

Strain	Description	Rifampin MIC (μg/mL)	Amino acid change*
MDH02-2342	Sporadic rifampin-susceptible serogroup C case isolate	0.004†	None (WT)
MDH02-2271	Sporadic rifampin-susceptible serogroup B case isolate	<0.002†	None (WT)
MDH97-498	Isolate from sporadic rifampin-resistant serogroup B case in 1996	>4,† >32‡	$His_{552}Tyr$§
MDH02-2398	First sibling's isolate: rifampin susceptible, serogroup C	0.008†	None (WT)
MDH02-2408	Second sibling's isolate: rifampin resistant, serogroup C	>1,† >32‡	$Ser_{548}Phe$§

*WT, wildtype.
†Determined by broth microdilution.
‡Determined by Etest.
§Numbering based on the entire *N. meningitidis rpoB* gene (GenBank accession no. Z54353). Accession numbers of isolate sequences submitted to GenBank: MDH02-2342 (AY746965), MDH02-2271 (AY746964), MDH97-498 (AY746963), MDH02-2398 (AY746966), MDH02-2408 (AY746967).

N. meningitidis was isolated from a swab of brain tissue. Three days after the death of the case-patient and 1 day after completing a 2-day course of rifampin, a fever and lethargy developed in the case-patient's 6-year-old sister. Blood cultures were obtained and she was hospitalized, given antimicrobial drug treatment (ceftriaxone), and observed. No cerebrospinal fluid was collected. Blood cultures were subsequently positive for *N. meningitidis.* She responded to ceftriaxone and continued treatment as an outpatient after a short hospitalization. Household contacts, along with other close contacts of the 6-year-old girl, again received chemoprophylaxis. It was recommended that adults be treated with ciprofloxacin and children be treated with ceftriaxone because of concerns that 1 or both siblings could have had rifampin-resistant meningococcal infections. No additional related cases were identified over the following weeks.

Isolates from both siblings were identified as serogroup C. The PFGE patterns were indistinguishable and had, in fact, the most common PFGE pattern seen for that serogroup in Minnesota. Antimicrobial susceptibility testing showed that the isolate from the case-patient was susceptible to ceftriaxone, penicillin, chloramphenicol, ciprofloxacin, and rifampin. The MIC for rifampin was 0.008 μg/mL. The isolate from the 6-year-old patient was susceptible to the same drugs, except for rifampin, which had an MIC >1 μg/mL by broth microdilution and an MIC >32 μg/mL by Etest.

A comparison of the nucleotide sequence of the *rpoB* gene of both sibling isolates showed they were identical except for a single nucleotide change. This change resulted in a substitution of serine for phenylalanine at amino acid position 548. This substitution has previously been associated with rifampin resistance in *N. meningitidis* (12).

The PFGE subtype of the isolate from the rifampin-resistant case in 1996 differed from that of the siblings' isolates. Sequencing of the *rpoB* gene from this isolate showed an amino acid substitution of histidine for tyrosine at position 552. This substitution has also been previously associated with rifampin resistance in *N. meningitidis* (Table 1; MDH97-498) (11,13).

Susceptibility results on meningococcal isolates from 1993 to 2003 for other antimicrobial agents are shown in Table 2. Using the newly established breakpoints, we observed that 92% (303/331) of the isolates were susceptible to penicillin, 100% (205/205) were susceptible to ceftriaxone, 100% (331/331) were susceptible to meropenem, 100% (205/205) were susceptible to ciprofloxacin, 100% (331/331) were susceptible to chloramphenicol, and 48% (158/ 331) were susceptible to trimethoprim-sulfamethoxazole.

Conclusions

Primary cases of rifampin-resistant meningococcal disease are rare. While more common, secondary cases with rifampin resistance can develop following chemoprophy-

Table 2. Antimicrobial drug susceptibilities for meningococcal invasive disease *Neisseria meningitidis* isolates, Minnesota, USA, 1993–2003*

Antimicrobial drug susceptibility†	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Rifampin	100	100	100	97	100	100	100	100	100	97	100
Ceftriaxone	NA	NA	NA	NA	NA	100	100	100	100	100	100
Ciprofloxacin	NA	NA	NA	NA	NA	100	100	100	100	100	100
Chloramphenicol	100	100	100	100	100	100	100	100	100	100	100
Meropenem	100	100	100	100	100	100	100	100	100	100	100
Penicillin	92	100	81	89	92	83	96	95	96	86	93
Trimethoprim-sulfamethoxazole	30	55	74	62	32	42	29	36	52	58	66
No. of cases	27	23	30	40	40	36	56	22	27	36	29
No. of isolates tested (%)	13 (48)	11 (48)	27 (90)	37 (93)	38 (95)	36 (100)	55 (98)	22 (100)	27 (100)	36 (100)	29 (100)

*NA, not available.
†Values for antimicrobial drugs are % of isolates susceptible by broth microdilution.

laxis with rifampin. All *N. meningitidis* isolates tested at MDH were susceptible to ceftriaxone and ciprofloxacin. Ceftriaxone must be given parenterally but is the recommended prophylactic agent for infected pregnant women. According to the 2003 American Academy of Pediatrics Report of the Committee on Infectious Diseases, ciprofloxacin may be used by persons >15 years of age. While few instances of ciprofloxacin resistance have been reported, its widespread use may result in greater resistance in *N. meningitidis* (as has occurred in related pathogens such as *Neisseria gonorrhoeae*) (16,17). Persons receiving chemoprophylaxis should be advised about the potential of meningococcal disease developing, even though they have taken antimicrobial agents as prescribed. If a close contact who has been treated with rifampin becomes ill with meningococcal disease, alternative antimicrobial agents should be used for prophylaxis until rifampin sensitivity of the secondary infection can be established. Although rifampin-resistant meningococcal disease is still rare after 30 years of using rifampin for chemoprophylaxis and ciprofloxacin resistance has rarely been observed, susceptibilities to chemoprophylactic agents should be monitored to ensure that recommendations are sufficiently effective to minimize the occurrence of secondary cases.

Acknowledgments

We thank the hospital infection control practitioners and local public health agencies of Minnesota for their cooperation in reporting cases; the microbiology laboratory personnel for submitting isolates to the Minnesota Department of Health; Richard Danila and Harry Hull for thoughtfully reviewing this manuscript; Nancy Rosenstein for guidance; and James Jorgenson for input on recommended breakpoints for *N. meningitidis* susceptibility.

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Diversification in *Salmonella* Typhimurium DT104

To the Editor: Multidrug-resistant (MDR) *Salmonella* Typhimurium definitive phage type (DT) 104 with chromosomally encoded resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides, and tetracyclines (ACSSpSuT) was first identified and characterized in the United Kingdom in the early 1990s (1). MDR DT104 has subsequently caused numerous outbreaks throughout the world (1). The organism is characterized by a distinctive *Xba* I-generated pulsed-field profile (PFP), designated Xtm 1 (2), carriage of the 90-kb *S.* Typhimurium serovar-specific plasmid (SSP), and presence of the 43-kb *Salmonella* genomic island 1 (SGI 1), which is composed of integrons containing, respectively, the ASu (bla_{CARB-2} and *sul1*) and SSp (*aadA2*) genes, with intervening plasmid-derived genes coding for chloramphenicol/florfenicol (*cmlA*) and tetracycline resistance (*tetG*) (1,3,4). The same genetic characteristics have been observed in MDR strains of the closely related DTs 12 and 104b (5) and in some strains of phage type U302 (4). All isolates of MDR DT104 ACSSpSuT contain the same gene cassettes irrespective of source or country of origin. Although MDR DT104 has declined during the last 5 years, the organism remains the most common MDR *Salmonella* in the United Kingdom and many other European countries (6).

Since 1998, MDR DT104 has undergone changes in both resistance spectrum and genetic structure. In the United Kingdom, outbreaks of MDR DT104 have been caused by new subclones with additional resistance to trimethoprim (Tm) (R-type ACSSpSuTTm) (7), by clones with decreased susceptibility to ciprofloxacin (Cp_L) (R-type ACSSpSuTCp_L) (1), and by strains of R-type SSpSu. In 2002, an outbreak of MDR DT104 ACSSpSuTTm with >200 cases was recognized (7). The outbreak strain was characterized by 3 plasmids of 6.8, 3.0, and 1.5 kb. The 6.8-kb plasmid coded for resistance to sulfonamides and trimethoprim, with trimethoprim resistance being mediated by *dhfr1b*. The outbreak strain lacked the *S.* Typhimurium SSP and was negative by polymerase chain reaction (PCR) for a 437-bp internal fragment of the *Salmonella* plasmid virulence (*spv*)*C* gene. The absence of the SSP was reflected in the PFP, which was identical to Xtm 1 but lacked a fragment of ≈90 kb that corresponds to the presence of the SSP (4). A strain of R-type ACSSpSuT that also lacked the SSP and with a PFP indistinguishable from that of the ACSSpSuTTm strain caused a simultaneous outbreak with >40 cases (7). This strain was also characterized by 2 plasmids of 3.0 and 1.5 kb but did not possess the 6.8-kb sulfonamide-trimethoprim resistance plasmid.

Decreased susceptibility to ciprofloxacin coupled with resistance to nalidixic acid (Nx) was first reported in MDR DT104 in 1996 in the United Kingdom (1). Four mutations in *gyrA*, each giving rise to resistance to Nx/Cp_L, were subsequently identified in MDR DT104. The most common mutation was aspartate (Asp)-87 to asparagine (AAC) and involved a change from aspartate (GAC) to AAC. An identical mutation was identified in a MDR DT104 strain responsible for an outbreak in Denmark in 1998 (8). In 1999, an Asp-87 to glycine mutation was identified in a Cp_L MDR DT104 strain responsible for a major outbreak in northwestern England (1). Subsequently, >500 sporadic MDR DT104 infections in the United Kingdom have involved strains with Cp_L.

In 2004, 2 simultaneous outbreaks of DT104 were recorded in the United Kingdom in which the strains were of R-type SSpSu, 1 involving >100 cases and 1 involving >50 cases. Isolates were characterized by pulsed-field gel electrophoresis coupled with plasmid profile analysis. Resistance genes were identified by using PCR with primers specific for bla_{TEM} (A), bla_{CARB-2}, *cmlA*, *catI* (C), *catIII* (C), *aadA2*, *sul1*, and *tetG* (4). Testing for SGI1 was conducted by using primers U7-L12 and LJ-R1 for the left junction and primers 104-RJ and 104-D for the right junction (9). All isolates exhibited the Xtm 1 PFP, but strains from the 2 outbreaks could be differentiated by the presence or absence of an additional plasmid of ≈3 kb. By using PCR, all isolates possessed *aadA2* and *sul1* but were negative for bla_{TEM}, bla_{CARB-2}, *cmlA*, *catI*, *catIII*, and *tet*G. Similarly, all strains were positive for the left junction of SGI1. These results indicate that the SGI1 was in the same chromosomal location as for DT104 ACSSpSuT but was lacking the right junction.

These findings demonstrate that evolutionary changes have occurred involving both loss and acquisition of drug resistance genes, the development of chromosomal mutations conferring resistance to Nx/Cp_L, and in certain subclones, the loss of the SSP. None of these changes has altered the virulence of the organism, with bloodstream invasion in only 1.6% of 408 cases in the United Kingdom. These findings contrast with those in the United States, where MDR nontyphoidal *Salmonella* have been associated with excessive numbers of bloodstream infections (10).

All facets of the organism should be explored for epidemiologic investigations. These should include phage type, antibiogram, plasmid profile, and PFP, coupled with the identification of resistance genes and integrons, and, when appropriate, the characterization of mutations conferring resistance to quinolone antimicrobial agents.

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Extended-spectrum β-Lactamase-producing Flora in Healthy Persons

To the Editor: Extended-spectrum β-lactamase (ESBL)–producing gram-negative bacilli are endemic in hospitals. In intensive care units, 2% prevalence of ESBL-producing organisms has been reported (1). Exceedingly high rates of ESBL-producing bacteria in Indian hospitals prompted us to look at the fecal carriage of ESBL in the community (2).

One hundred healthy executives received a comprehensive health check at our tertiary care center in central Mumbai from August to September 2004. The predominant isolates from stool samples obtained for routine examination were cultured, and initial screening for ESBL production was conducted by using the disk diffusion method according to NCCLS guidelines (3). For these isolates, the ESBL phenotypic confirmation was performed with ceftazidime-clavulanate for an increase in zone diameter by 5 mm (disk potentiation). In addition, the ATB BLSE strip (bioMérieux, Lyon, France) was used to confirm the presence of inhibitor (sulbactam)-susceptible enzymes and to differentiate the strains from those that were either inhibitor resistant or harboring other β-lactamases, such as those of AmpC derivation. The ATB BLSE strip consists of a varying concentration of ceftazidime, 0.5–32 mg/L, and aztreonam, 0.5–8 mg/L, with varying combinations of these agents with a β-lactamase inhibitor, i.e., + sulbactam, 0.06–1 mg/L. Cefotetan (4 and 32 mg/L) and imipenem (4 and 8 mg/L) were also included in the strip. The test was considered positive when a variation of ≥4 dilutions was observed between the antimicrobial agent tested alone and the agent combined with the inhibitor. Eleven of the 100 samples screened were positive for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*. Seven of the 11 were confirmed by using the ATB BLSE strip. The MIC of ceftazidime and aztreonam in all 7 isolates was 8 μg/mL. We might be underreporting ESBL producers in these cases by not including the cefotaxime-clavulanate combination in addition to the ceftazidime-clavulanate concentration. The percentage resistance to ciprofloxacin was 45%. All isolates were susceptible to amikacin and the carbapenems. None of the executives gave a history of hospitalization in the last year or history of antimicrobial drug consumption in the last 6 months.

This trend in patients with no apparent risk factors for ESBL carriage calls for urgent attention. Unknown environmental factors are likely playing a key role in maintaining this selective pressure. Larger studies are required to substantiate these findings.

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Measuring Impact of Antimicrobial Resistance

To the Editor: *Staphylococcus aureus* and *Enterococcus faecium* commonly cause healthcare-associated bloodstream infections (BSI) in the intensive care unit (ICU). Antimicrobial resistance is increasing in both organisms. The impact of antimicrobial resistance on dying of BSI has been studied extensively (1,2). Many studies have concluded that BSI caused by an antimicrobial-resistant organism results in higher death rates (1,3–8). However, as discussed in a recent report by Kaye et al., "outcome studies of antimicrobial drug resistance are notoriously hard to perform because of confounding variables related to coexisting conditions" (9). Indeed, almost all studies have shown that infections with antimicrobial-resistant organisms occur later in hospitalization than infections with antimicrobial-susceptible organisms, which suggests that differences in death rates may be, at least in part, caused by a difference in the patients' underlying illnesses and protracted hospital course. We report 2 additional methodologic issues that can affect estimates of the impact of antimicrobial resistance: combining different organisms and combining populations from different types of ICUs.

The original objective of our multicenter observational study was to quantify the clinical impact of antimicrobial resistance in *S. aureus* and *E. faecium* infections when these bacteria cause a specific type of infection: a monomicrobial, ICU-attributable, central vascular catheter–associated bloodstream infection (CVC-BSI). We studied 187 adult ICU patients with BSI caused by *S. aureus* and *E. faecium* at 3 tertiary care institutions from 1994 to 1999. The institutional review boards of each institution and the Centers for Disease Control and Prevention approved this study. Severity of illness was measured with an APACHE II score at ICU admission and on day 7 in the ICU (if applicable). The score would indicate the patient's risk of dying in the hospital before a BSI developed by using a measure validated for predicting in-hospital deaths in ICU patients (10).

The study population stratified by organism is shown in the Table. Fifty-eight percent of patients had CVC-BSI with *S. aureus*, and 42% had CVC-BSI with *E. faecium.* Overall, 58% of the organisms causing CVC-BSI were resistant to oxacillin if *S. aureus* or to vancomycin if *E. faecium*. However, patients with *E. faecium* CVC-BSI were more likely to be infected with antimicrobial-resistant bacteria (69% versus 50%, p<0.01), and had a higher mortality rate (54% versus 34%, p<0.01) than patients with *S. aureus* CVC-BSI. This finding indicates that the type of organism (*E. faecium* versus *S. aureus*) confounds the association between resistance and death. In addition, the distribution of ICU type by organism varies, which suggests that patient populations infected with these 2 different organisms were different in other

Table. Description of 187 adult patients with central vascular catheter-associated bloodstream infections with *Staphylococcus aureus* or *Enterococcus faecium* attributable to the intensive care unit*

Characteristics	*S. aureus* (n = 109)	*E. faecium* (n = 78)	p value
Patient demographics			
Male (%)	74	56	0.02
Mean age, y (SD)	58 (17)	56 (16)	0.32
Type of ICU			<0.01
Cardiac (%)	20	10	
Cardiothoracic surgery (%)	6	6	
Medical (%)	20	40	
Neurologic/neurosurgical (%)	6	0	
Surgical (%)	20	37	
Trauma (%)	28	6	
Severity of illness			
Mean APACHE II score at ICU admission (SD)	19 (8)	21 (9)	0.12
Mean APACHE II score within 7 days of BSI (SD)	17 (8)	20 (8)	0.05
Resistant infections (%)	50	69	0.01
In-hospital death rate (%)	34	54	<0.01

*SD, standard deviation; ICU, intensive care unit.

ways. Thus, confounding factors for the association between resistance and death may differ for *E. faecium* and *S. aureus*, and analysis of the 2 organisms should be conducted separately. This is consistent with the results of Kaye et al. who showed that the effect of resistance was higher for *S. aureus* (odds ratio [OR] 3.4) than for *E. faecium* (OR 2.5) by using separate analyses to show death rates (9). Furthermore, these researchers found different confounding factors in the adjusted analysis of *S. aureus* than in the adjusted analysis of *E. faecium*. Because of the need to conduct separate analyses, which reduced our statistical power, our study was ultimately unable to show a difference in death rates if it existed.

In summary, future studies measuring the impact of antimicrobial resistance on death rates should be restricted to a specific type of infection cause by a single organism in a uniform setting using a validated system to predict mortality in that setting. As such, future studies should involve multiple study sites.

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Antimicrobial Resistance in *Campylobacter*

To the Editor: Iovine and Blaser (1) write, "This therapeutic use [of enrofloxacin] was withdrawn (2) but is now under appeal" and "Despite the restrictions on enrofloxacin use, emergence of fluoroquinolone-resistant *Campylobacter* species, with poultry as an important source, has been documented in the United States… Therefore, our conclusion remains: use of enrofloxacin in poultry materially contributed to increase in human infection by fluoroquinolone-resistant *Campylobacter* species."

These claims propagate the following important errors. First, the therapeutic use of enrofloxacin was not withdrawn. Judge Davidson's order to withdraw the approval was an initial decision, to which exceptions were filed in 2004. A final decision rests with the US Food and Drug Administration Commissioner.

Second, poultry has not been identified as an important source of fluoroquinolone resistance in human *Campylobacter* isolates. The raw data of the cited Smith et al. article (3) indicate a nonsignificant negative association between chicken consumption and fluoroquinolone resistance in human isolates. Substantial resistance levels in Northern Hemisphere countries with and without enrofloxacin use, which occurred well before fluoroquinolones were ever used in animals (3–5), also suggest that attribution of such resistance to enrofloxacin is simplistic.

Finally, rational decision-making is based on probable future consequences of a decision, not past history or causes of the current situation. Iovine and Blaser's claim, "Thus the decision to withdraw therapeutic use of enrofloxacin (3) was warranted," is not implied, even if enrofloxacin use

caused the emergence of fluoroquinolone resistance. If withdrawing enrofloxacin increases campylobacteriosis from airsacculitis-positive chickens, withdrawal may greatly harm human health. A rational withdrawal decision cannot be justified. In summary, Iovine and Blaser's view that enrofloxacin should be banned is not supported by the data that they have cited or by principles of sound risk management and decision-making.

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In Reply: Cox and colleagues raised 3 major points. For the first point, we stated (1) "This therapeutic use was withdrawn but is now under appeal." The actual language of US Federal Drug Administration Judge Davidson's ruling is "Enrofloxacin found not shown to be safe under the conditions of use upon the basis of which the application was approved as required under § 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (the Act) [21 U.S.C. § 360 b(e)(1)(B)]. Approval of NADA[1] for enrofloxacin ordered withdrawn" (1). The drug manufacturer now is appealing the ruling.

For the second point, the authors state that poultry has not been identified as an important source of fluoroquinolone resistance in human *Campylobacter* isolates. In both Denmark and Spain, introduction of fluoroquinolones into poultry led to a rapid rise in resistance to *Campylobacter* in both poultry and human isolates (2–5), and banning their use in Denmark led to a rapid fall in resistance (6). Cox and colleagues may maintain that there is no "proof of a causal relationship," but the relationship is sufficiently strong, temporally restricted, biologically plausible, and coherent to convince disinterested observers, including Judge Davidson and ourselves, otherwise.

For the third point, that decisions must consider probable consequences, we agree. However, Cox et al. appear to use "possible" as their standard. In fact, nearly everything is possible, including the reasoning that they offer. However, in our opinion, based on experience as scientists and microbiologists, we deem the possible consequences described by Cox et al. as insubstantial compared to the clear and present danger to human health of continuing fluoroquinolone use in poultry. Obfuscation and delay have been effective tactics used to maintain profitability even when the facts indicate a different course of action. We hope that the FDA Commissioner will carefully weigh the actual evidence of the risk to human health imposed by the use of fluoroquinolones in poultry.

[1]New Animal Drug Application

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Quinolone Safety and Efficacy

To the Editor: Richard Frothingham should be commended for providing added perspective on the matter of quinolone selection. His letter to the editor emphasizing the paramount importance of a well-established safety profile and documented clinical efficacy in severe infections before a "wholesale change" to the newer quinolones is an appropriate response to Michael Scheld's essay on maintaining quinolone class efficacy in which a "correct spectrum" strategy of using the most potent quinolone to treat the presumed or confirmed pathogen was described and advocated (1). In his article, Frothingham reminds us that serious adverse drug effects in patients led to the withdrawal or restriction of 4 quinolones in the last decade and that safety may differ substantially among the quinolones discussed in Scheld's review (ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin) (2).

With the exception of labeling changes regarding glucose homeostasis abnormalities associated with gatifloxacin therapy, the subject of quinolone safety is centered on torsades de pointes. Data published in 2001 are cited; these consist of a review of crude rates of US cases of torsades de pointes from January 1996 through May 2, 2001 (3). However, these data only capture adverse drug reports for the first full year gatifloxacin and moxifloxacin were widely available in the United States. The last several years have seen dramatic uptake of all 3 respiratory quinolones. Use of these agents is pervasive in both community and hospital settings. Indeed, the Infectious Diseases Society of America, American Thoracic Society, and Sinus and Allergy Health Partnership have since published revised consensus statements calling for the use of these agents earlier in therapy for community-acquired pneumonia and bacterial sinusitis (4–6).

December 2004 marked 5 years since the Food and Drug Administration approved gatifloxacin and moxifloxacin and 8 years since the approval of levofloxacin. As a result of tens of millions of patient exposures, we now have more robust data to work with and are better able to make informed and meaningful safety comparisons, particularly with respect to torsades de pointes, a rare, life-threatening cardiac arrhythmia infrequently associated with quinolone therapy.

With respect to efficacy, Frothingham writes that ciprofloxacin and levofloxacin have been studied in patient populations with more severe illness, and trials of the newer quinolones have enrolled patients with predominantly mild or moderate community-acquired infections and low overall death rates in comparison. However, a cursory review of the literature suggests otherwise. As with gatifloxacin and moxifloxacin, few peer-reviewed, published data support the use of levofloxacin in the treatment of severe, life-threatening infections at the currently approved doses of 500 mg or 750 mg.

Indeed, the 2 references cited raise serious concern about the suitability of levofloxacin at currently recommended doses for severe and life-threatening infections. In File et al. (7) levofloxacin was studied in only 16 patients classified as having severe community-acquired pneumonia; in Norrby et al. (8) a dose of levofloxacin 500 mg every 12 hours was studied in severe community-acquired pneumonia. At this time, other published studies support the use of levofloxacin at a dose of 500 mg every 12 hours in severe and life-threatening infections: an approved regimen in Europe but not yet approved in the United States (9,10).

In summary, differences in quinolone safety are evidenced by labeling changes to gatifloxacin, the only quinolone to carry a specific warning regarding glucose homeostasis abnormalities. However, the incidence of torsades de pointes associated with each of these agents is ripe for further investigation as we pass the 5-year mark of approval for the new respiratory quinolones. An update of those data on the rate of torsades cited by Frothingham and published in 2001 would provide meaningful guidance to clinicians. Currently, with the exception of ciprofloxacin, each of these quinolones contains labeling guidance in the form of a warning (gatifloxacin, moxifloxacin) or a precaution (levofloxacin), and concurrent use with class IA (e.g., quinidine, procainamide) or class III (e.g., amiodarone, sotalol) antiarrhythmics should be avoided to reduce the risk of torsades de pointes per current product labeling.

Ciprofloxacin remains the only quinolone to date based on multiple, head-to-head, well-controlled, published trials to have established efficacy and safety in a severely ill patient population at approved doses. A paucity of published clinical data exist on the use of gatifloxacin, levofloxacin and moxifloxacin in hospitalized patients with severe, life-threatening infections. Therefore, the respective manufacturers must establish safety and efficacy in well-controlled studies with the resultant data made available in peer-reviewed journals before these agents are fully embraced for these infections.

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In response: I agree. Expanding clinical experience makes a reappraisal of quinolone safety and efficacy timely (1). Through December 2004, >160,000,000 ciprofloxacin, 69,000,000 levofloxacin, 12,000,000 gatifloxacin, and 11,000,000 moxifloxacin prescriptions were filled at US retail pharmacies.

Dr. Bellomo requests an update of my previous report on torsades de pointes adverse drug events (ADEs) (2). I reviewed 16,868 U.S. Food and Drug Administration ADE reports associated with these 4 quinolones from November 1997 to September 2003 (3). My review identified the following numbers of unique US torsades de pointes ADEs: 3 ciprofloxacin, 51 levofloxacin, 37 gatifloxacin, and 20 moxifloxacin. Ciprofloxacin or nonquinolone antimicrobial drugs should generally be selected for patients with risk factors for QT interval prolongation (4-7).

Numerous published trials and extensive clinical experience support the safety and efficacy of ciprofloxacin. Generic oral ciprofloxacin is inexpensive. These factors make ciprofloxacin the quinolone of choice for nonrespiratory infections.

Gatifloxacin is associated with hypoglycemia and hyperglycemia ADEs, including death, at rates greatly exceeding those of other quinolones (3). A causal relationship between gatifloxacin and these ADEs is supported by animal data (8), manufacturer safety cohort studies (5), in vitro assays (9), a large randomized trial (10), and >20 published case reports. Gatifloxacin has no meaningful cost or efficacy advantage to balance this excess risk.

Clinical experience continues to support the safety of levofloxacin and moxifloxacin. Apart from torsades de pointes, my review did not identify specific safety concerns. Both drugs are effective for community-acquired respiratory infections, although clinical experience and published data are more extensive of levofloxacin than moxifloxacin. Levofloxacin has received FDA approval for nosocomial pneumonia (6). Dr. Bellomo notes that levofloxacin trials have used a variety of dosages; the optimal dosage for serious infections is unknown. Moxifloxacin has greater activity against *Streptococcus pneumoniae*, which could possibly prevent the emergence of resistance or lead to faster clinical responses in pneumococcal infections. Both moxifloxacin and levofloxacin are appropriate choices for community-acquired respiratory infections.

Acknowledgments

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R. Frothingham has received honoraria or consulted for Bayer, Bristol-Myers Squibb, Ortho-McNeil, Otsuka, and Pfizer.

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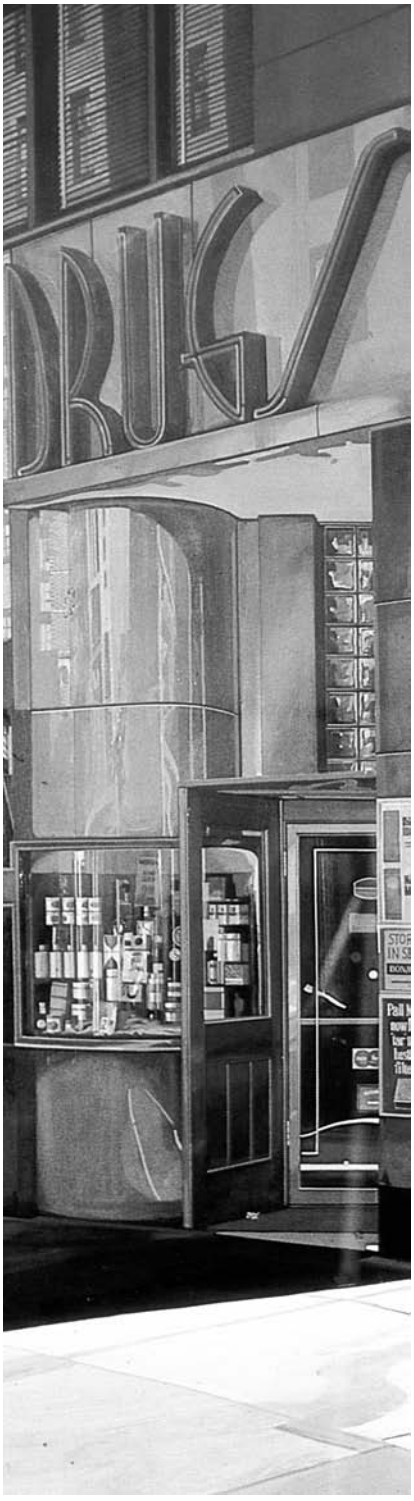

Richard Estes (b. 1932).
DRUGS (1970)
Oil on canvas
(152.4 cm × 112.71 cm)

Drugs, Microbes, and Antimicrobial Resistance

Polyxeni Potter*

"If America is to produce great painters," wrote great American painter Thomas Eakins (1844–1916), young artists should "remain in America to peer deeper into the heart of American life" (1). Eakins traveled abroad, where he became familiar with the work of 19th-century greats Gustave Courbet, Édouard Manet, and Edgar Degas, but returned home to become a master of realism whose exactness and precision influenced many 20th-century American painters, one of them Richard Estes.

Estes, a native of Kewanee, Illinois, grew up in Chicago and attended the city's famed Art Institute. Upon graduation in 1956, he moved to New York to pursue a career in graphic design as freelance illustrator for magazines and advertising agencies. In the next 10 years, he crossed over to fine art and had his first exhibit around 1968 (2).

An admirer of Eakins, Estes peered deeply into the American cityscape for a new style of art, true not only to his native culture but also to his times. This style evoked the traditions of *trompe l'oeil* (fooling the eye through photographic illusion) and of 17th-century Dutch painting to create a contemporary version of reality (3). "When you look at a scene or an object you tend to scan it. Your eye travels around and over things. As your eye moves the vanishing point moves," Estes said in an interview. "…to have one vanishing point or perfect camera perspective is not realistic" (4).

Drawing from his surroundings rather than the imagination, Estes used photography to collect images or frozen moments of light on surfaces to complement his own recollection of places and objects. He did not reproduce photographic scenes. From multiple images, he selected certain elements, abstracting and arranging them to best advantage, exaggerating angles and omitting extraneous detail. This innovative perception and composition of visual reality came to be called superrealism or photorealism, a new art movement co-founded by Estes in the late 1960s (5).

Photorealists, many of them influenced by Estes, painted varied images, portraits as well as landscapes, in exquisite detail. Their subjects were diners and storefronts, gum-ball machines, neon lights, pickup trucks, and other trappings of 1970s American life. Estes was captivated by the contemporary urban landscape, particularly of New York, where he has lived much of his life, although he has also worked in Chicago, Venice, and Paris. One in a long line of artists to know and paint New York, he has worked in terms that seem architectural in their emphasis on structure and design and created of this landscape a veritable visual spectacle for posterity. Buildings, bridges, traffic patterns, city curbs were manipulated and transformed from commonplace scenes into grand theater, much more intense and "real" on canvas than ever in their own existence.

Yet, even as he has created an archivist's treasure of Downtown Manhattan and Manhattan's Upper West Side, Estes is not interested in nostalgia or future archaeologic records. And as much as he has been compared with 18th-century Italian artists (e.g., Canaletto), who painted palaces, piazzas, and canals, he

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is not interested in urban scenery for its beauty or underlying social commentary. He paints for the sake of painting, usually with acrylic color overlaid with oils, lovingly reinventing the scenes he explores (6).

"Daily life has a reputation for being banal, uninteresting, boring somehow. It strikes me that daily life is baffling, mysterious, and unfathomable" (7). These are the words of George Segal, Estes' contemporary and colleague, who saw magic in the mundane. Estes walks around the streets of New York until something catches his eye. He returns to the scene on weekends or evenings when the streets are deserted to take photos. Later in his studio, he reconstructs what he saw and collected, in a scene become more fiction than reality. Unlike Segal's work, which witnesses a moment of human existence, Estes' witnesses the moment itself and celebrates its visual presence with clarity and exactness.

In his meticulous reconstructions, Estes eliminates clutter, shadows, people—as if by scrubbing the scene, he can extract its essence and verify its existence. Singling out the structural, he elaborates on it from multiple angles, under a uniformly glaring light, and produces a sharp image much more compelling and deliberate than any captured by the naked eye. In the process, a perfectly dull building, an anonymous row of telephone booths, a street corner become arresting and memorable.

In DRUGS, on this month's cover, Estes' penetrating eye examines an icon of contemporary city life, the corner drugstore. Expertly cropped, central, and direct, the structure invites inspection on several levels: storefront and curb, window displays, and nearby buildings mirrored on shiny surfaces. A prominent column, neatly plastered with ads, blocks visual access to the interior, even with the entrance doors propped wide open against the sidewalk. The windows upstairs are shut. Elaborate glass facets and distortions of light restrict the viewer to the exterior.

Intentionally or not, Estes' drugstore, with its pristine appearance, reflects more than the block across the street. A cornerstone in the life of the city and the development of modern medicine, the institution it represents has held tricks of the medical trade, from camphor to penicillin to telithromycin. This shining apothecary symbolizes human efforts to improve health and control disease, efforts often stymied by the complexity of the task.

Not unlike artists, scientists in disease control seek order in a complicated universe. With their powerful microscopes, they too focus on the details as they construct clear, artificially uncluttered versions of a crowded microbial world. Singling out microbes that cause disease, scientists scrutinize, isolate them, and neutralize their effects on human health through powerful drugs. For their part, the microbes expel, modify, or exclude the drugs, prompting a new cycle of drug development, also destined for obsolescence. Antimicrobial drug resistance, begun with the first antimicrobial drug (8), threatens the single-microbe approach to disease control and the venerable institution Estes immortalized in DRUGS.

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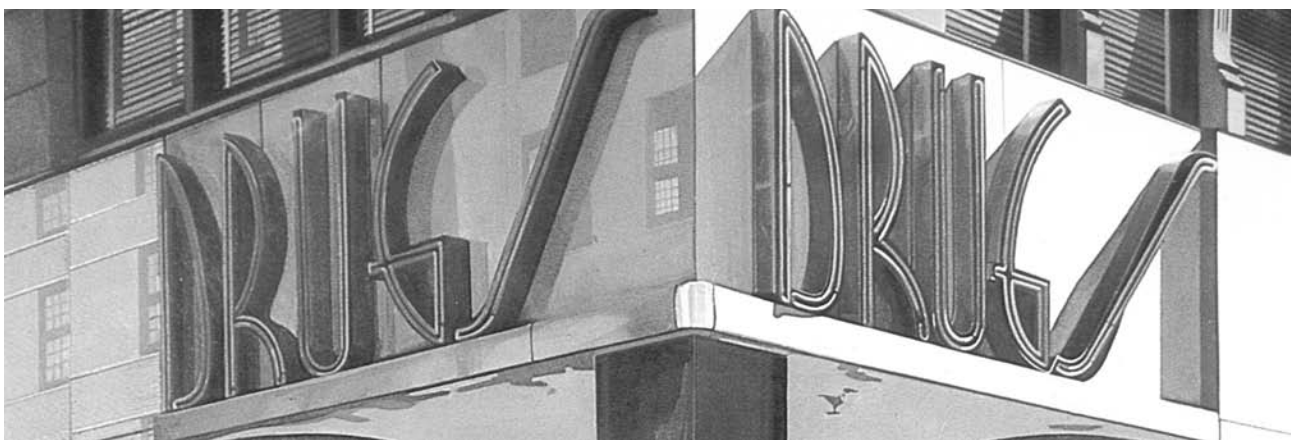

EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends
Vol. 11, No. 7, July 2005

Upcoming Issue

Look in the July issue for the following topics:

Linking Illness to Food

Occupational Mortality in Healthcare Workers

Wildlife Trade and Disease Emergence

Drug-resistant *Neisseria gonorrhoeae*, Michigan

SARS Vaccine Development

West Nile Virus–associated Flaccid Paralysis,

Primate-to-Human Retroviral Transmission, Asia

Nipah Virus in Lyle's Flying Foxes, Cambodia

Leptospirosis in Germany, 1962–2003

Tickborne Infections in Denmark

Influenza A H5N1 Replication Sites in Humans

Risk Factors for Pediatric Invasive Group A Streptococcal Disease

Complete list of articles in the July issue at http://www.cdc.gov/ncidod/eid/upcoming.htm

Upcoming Infectious Disease Activities

June 5–9, 2005
105th General Meeting of the American Society for Microbiology
Georgia World Congress Center
Atlanta, GA, USA
Contact: 800-974-3621
http://www.asm.org/Meetings/index.asp?bid=697

June 18–21, 2005
4th International Conference on Rickettsiae and Rickettsial Diseases
Logroño, Spain
http://www.rickettsia.net

July 23–28, 2005
Microbes in a Changing World
Moscone Convention Center
San Francisco, CA, USA
http://www.iums2005.org

August 28–September 2, 2005
5th International Conference on Ticks and Tick-borne Pathogens
University of Neuchâtel
CH-2000 Neuchâtel, Switzerland
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6th International Conference on Anthrax
La Fonda Hotel
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Abstract deadline: August 15, 2005
Contact: tandongmei112@yahoo.com.cn or yyyjin@126.com

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JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
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The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
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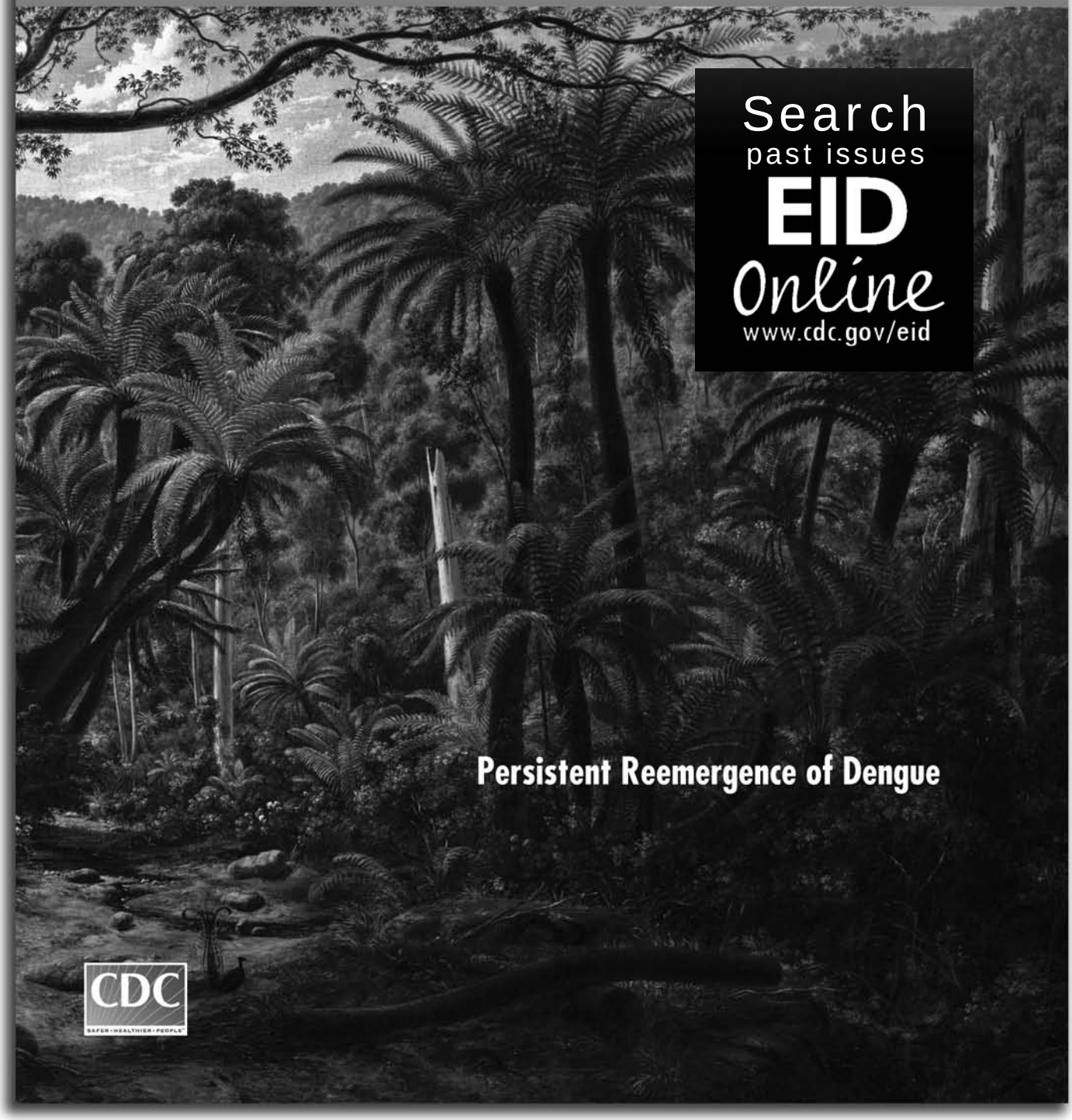
Vol.11, No.5, May 2005



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Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

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Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch of first author—both authors if only two. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be 1,000–1,500 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only (effective January 2005). Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.

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