no. ISTN3X; 96% identical), comprising one of Tn3-like inverted repeats and putative coding regions for transposase, resolvase (also called repressor), and ampicillin resistance. The resistance gene encodes a TEM-1 type β-lactamase. (The sequence has been registered to DDBJ/GenBank/EMBL with accession no. AB103092.)

Conjugative transferability of p981123 between *S*. Enteritidis strains was examined by using the parental *S*. Enteritidis RDNC-a R-AS strain as a donor, and three independent *S*. Enteritidis strains (PT1; PT4; and PT21) resistant to nalidixic acid (R-N) as recipients. p981123 was transferable between *S*. Enteritidis strains at frequencies of 10^{-5} to 10^{-4}, and the resulting R-AN transconjugant showed the same lytic pattern of the typing phages as RDNC-a. Thus, transfer of p981123 could convert the phage types at least from PT1, PT4, and PT21 to RDNC-a. Pulsed-field gel electrophoresis (PFGE) was done by using *Xba*I or *Bln*I as well, and RDNC-a strains showed a variety of PFGE profiles. These results suggest emergence and prevalence of the 50-kb R-plasmid converting phage types to RDNC-a in *S*. Enteritidis in Japan.

Previous studies reported correlation between R-plasmids and phage types of *S*. Enteritidis, where, for example, a 34-MDa R-plasmid of incompatibility group N (IncN) (8) and a 36-MDa R-plasmid of IncX (pDEP57) (6) were described. Both kinds of plasmids encoded ampicillin resistance as well as that in this study, but both were identified in PT6a isolates. Preliminary sequence data of the region of p981123 essential for replication indicated a gene coding for a protein similar to protein p1 of R6K (IncX) plasmid (9), which suggests that p981123 may be related to pDEP57. However, the reactions to the typing phages in RDNC-a strains were different from those in PT6a. Therefore, the R-plasmid in this study seems to have different features from previous ones. In addition, *S*. Enteritidis PT6d resistant to ampicillin was recently reported (10). Relationship between RDNC-a in this study and PT6d is unknown, and further investigations will be needed.

Transfer of an R-plasmid is a common way for bacteria to acquire drug resistance, and it often affects other aspects such as sensitivity of bacteriophages, as described in this study. Molecular based surveillance for drug resistance in *S*. Enteritidis needs to continue.

Hidemasa Izumiya,* Naomi Nojiri,* Yoshiko Hashiwata,† Kazumichi Tamura,* Jun Terajima,* and Haruo Watanabe*

*National Institute of Infectious Diseases, Tokyo; and †Hiroshima City Institute of Public Health, Hiroshima, Japan

Acknowledgments

We thank all the municipal and prefectural public health institutes for providing us with *Salmonella enterica* serovar Enteritidis isolates. We also thank Public Health Laboratory Service, United Kingdom, for kindly providing the typing phages and the scheme.

This work was partly supported by grants from Ministry of Health, Labor and Welfare, and Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. National Institute of Infectious Diseases. Salmonellosis in Japan as of June 2000. Infectious Agents Surveillance Report, vol. 24;2003:162. Available from: URL: http://idsc.nih.go.jp/iasr/24/282/tpc282.html
2. Threlfall EJ. Epidemic *Salmonella* Typhimurium DT 104-a truly international multiresistant clone. J Antimicrob Chemother 2000;46:7–10.
3. Matsune W, Ishikawa K, Hayashi KI, Tsuji M, Izumiya H, Watanabe H. Molecular analysis of *Salmonella* Enteritidis isolates resistance to ampicillin and streptomycin from three outbreaks of food poisoning in Shiga prefecture. Jpn J Infect Dis 2001;54:111–3.
4. Ward LR, de Sa JD, Rowe B. A phage-typing scheme for *Salmonella enteritidis*. Epidemiol Infect 1987;99:291–4.
5. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests; approved standard-7th ed. NCCLS document M2-A7, Wayne (PA): The Committee; 2000.
6. Ridley AM, Punia P, Ward LR, Rowe B, Threlfall EJ. Plasmid characterization and pulsed-field electrophoretic analysis demonstrate that ampicillin-resistant strains of *Salmonella enteritidis* phage type 6a are derived from *Salm. enteritidis* phage type 4. J Appl Bacteriol 1996;81:613–8.
7. Helmuth R, Stephan R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect Immun 1985;48:175–82.
8. Vatopoulos AC, Mainas E, Balis E, Threlfall EJ, Kanelopoulou M, Kalapothalki V, et al. Molecular epidemiology of ampicillin-resistant clinical isolates of *Salmonella enteritidis*. J Clin Microbiol 1994;32:1322–5.
9. Kelley WL, Bastia D. Conformational changes induced by integration host factor at origin gamma of R6K and copy number control. J Biol Chem 1991;266:15924–37.
10. Eurosurveillance. Upsurge in *Salmonella* Enteritidis outbreaks in England and Wales, September to November 2002. Eurosurveillance Weekly, vol. 6; 2002. Available from: URL: http://www.eurosurveillance.org/ew/2002/021205.asp

Address for correspondence: Haruo Watanabe, Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan; fax: +81-3-5285-1171; email: haruwata@nih.go.jp

Factors Influencing Fluoroquinolone Resistance

To the Editor: Recently, Scheld summarized factors that he considered to have an influence on the efficacy of fluoroquinolones (1). In the review, ciprofloxacin was presented as the most active fluoroquinolone against *Pseudomonas aeruginosa*

with MICs typically two- to eightfold lower than those for levofloxacin, moxifloxacin, or gatifloxacin. However, because the National Committee for Clinical Laboratory Standards (NCCLS) MIC interpretative breakpoints are fluoroquinolone-specific, percent susceptibility is considered to be a better measure by which to compare fluoroquinolone activities. Our company has conducted annual investigations called TRUST (Tracking Resistance in the United States Today) since 1996. These surveillance studies have consistently shown similar susceptibility rates for levofloxacin (67.7% in 2002) and ciprofloxacin (67.4% in 2002) against *P. aeruginosa* (2,3). Both agents show higher in vitro activity against *P. aeruginosa* than gatifloxacin and moxifloxacin (2–4). A critique of antipseudomonal fluoroquinolone activity should also consider peak achievable fluoroquinolone levels at a site of infection, the area under the serum concentration curve in 24 hours (AUC_{24h}), and the AUC_{24h}/MIC ratio (5). At equivalent dosages for nosocomial pneumonia, levofloxacin (750 mg intravenously, once daily) has a threefold higher peak serum level (C_{max}) and threefold higher AUC_{24h} than ciprofloxacin (400 mg intravenously, every 8 hours) (package inserts for Levaquin and Cipro). While certain *P. aeruginosa* isolates have lower ciprofloxacin than levofloxacin MICs, the two fluoroquinolones have equivalent activity against *P. aeruginosa* because of their equivalent AUC_{24h} /MIC ratios (6). We agree strongly with Scheld's suggestion that the fluoroquinolone used clinically should be the fluoroquinolone tested by the laboratory and reported; surrogate testing of fluoroquinolones may lead to major errors in reporting, particularly for *Enterobacteriaceae* (2,3,7).

The review also stated that levofloxacin-resistant strains of *P. aeruginosa* emerge at a significantly higher rate than with ciprofloxacin. However, a recent study of *P. aeruginosa* isolated from cystic fibrosis patients reported that fewer resistant mutants were isolated after exposure to levofloxacin (11 mutants) than to ciprofloxacin (28 mutants) (8).

With regards to *S. pneumoniae*, the review stated that in vitro studies have demonstrated that ciprofloxacin (1–4 mg/L) and levofloxacin (1–2 mg/L) are not as active as moxifloxacin (0.06–0.25 mg/L) and gatifloxacin (0.5–1 mg/L) against pneumococci. As with *P. aeruginosa*, fluoroquinolone comparisons against *S. pneumoniae* should not be limited to MICs alone because pharmacokinetic and pharmacodynamic characteristics differ for each fluoroquinolone. Pneumococcal time-kill studies with levofloxacin, gatifloxacin, and moxifloxacin in a pharmacodynamic model have demonstrated that these three agents possess equal bactericidal activity and are equally effective in preventing resistance development because the lower in vitro MICs for gatifloxacin and moxifloxacin were offset by the higher serum and tissue levels of levofloxacin (9). In the same study, ciprofloxacin did not exhibit rapid killing and selected for resistance faster than the other three agents (9). TRUST and other U.S. surveillance studies, using the NCCLS-recommended broth-dilution method, have shown that *S. pneumoniae* remain highly susceptible to levofloxacin with resistance rates in the United States of <1%; the MIC_{90} for levofloxacin in these studies has remained at 1 mg/L from 1997 through 2002 (10–15). Further, levofloxacin, gatifloxacin, and moxifloxacin are equally effective in rates of clinical cure and microbiologic eradication of pneumococcal respiratory infections (16, and FDA website; available from: URL: http://www.fda.gov/cder/foi/nda/99/21061_Tequin.htm and http://www.fda.gov/cder/foi/nda/2001/21277_Avelox.htm)

The review implied that, in general, higher AUC_{24h}/MIC ratios were associated with better patient outcomes. For *S. pneumoniae*, several pharmacodynamic studies have demonstrated that a target AUC_{24h}/MIC ratio of 30 to 35 for fluoroquinolones is the best correlate for successful bacteriologic eradication, clinical cure, and prevention of emergence of resistance during therapy (5,9,17–19). Levofloxacin, gatifloxacin, and moxifloxacin all achieve this AUC_{24h}/MIC ratio (9). Zhanel et al. demonstrated that AUC_{24h}/MIC ratios above the target value of 30 to 35 did not improve bacteriologic eradication or reduce the emergence of resistance (9). Moreover, no clinical data support the claim that higher AUC_{24h}/MIC ratios correlate with better patient outcomes.

The review discusses the question of whether C-8-methoxyquinolones (moxifloxacin and gatifloxacin) have a lower propensity to select resistant mutants of *S. pneumoniae* compared with levofloxacin. Mutation prevention concentration is a theoretical laboratory concept based on agar dilution methodology, and no published data have shown any clinical correlation between this theory and clinical outcomes. NCCLS does not recommend agar dilution for susceptibility analysis of *S. pneumoniae*. Moreover, the extremely low levels of resistance in *S. pneumoniae* (<1%) after many years of fluoroquinolone use do not support the theory of mutation prevention concentration. The review did not reference an analysis of 16 penicillin-resistant *S. pneumoniae* strains by Kolhepp et al. (20). In that broth-dilution study, in vitro resistance developed in a greater proportion of strains exposed to gatifloxacin (11/16) and moxifloxacin (8/16) than to levofloxacin (2/16). Similarly, in a study by Klepser et al. that used an in vitro pharmacodynamic model, levofloxacin was less likely than moxifloxacin to select for resistant isolates

of *S. pneumoniae;* moreover, after 24 hours of exposure, levofloxacin MICs remained unchanged while moxifloxacin MICs increased two- to eightfold (21).

Levofloxacin, gatifloxacin, and moxifloxacin all have susceptibility rates >99% for *S. pneumoniae* (22,23). Although resistance is rare, considerable cross-resistance among fluoroquinolones is observed once two or more key mutations (e.g., Ser^{79} in ParC, Ser^{81} in GyrA) are detected (24,25). Using topoisomerase IV-selecting fluoroquinolones (ciprofloxacin and levofloxacin) in the same patient population as DNA gyrase-selecting fluoroquinolones (gatifloxacin and moxifloxacin) could potentially accelerate the development of double mutants (ParC and GyrA) and clinically important class resistance because selective pressure would be applied to both enzyme targets (26).

The review stated that, since 1999, at least 20 case reports of pulmonary infection that did not respond to levofloxacin therapy have been published. This number is remarkably small considering that >250 million patients have been treated with levofloxacin worldwide. A number of the treatment failures cited had documentation of prior ciprofloxacin use and ciprofloxacin failure, and many isolates were not tested for levofloxacin susceptibility before treatment (27). We agree with the recommendation in the cited Davidson et al. reference: a patient's failure to respond to one fluoroquinolone is sufficient reason not to use other fluoroquinolones (27). Isolated clinical failures will occur with the use of any antimicrobial agent when treating pneumococcal pneumonia.

The notion that fluoroquinolone therapy can be "targeted" for an indication requires challenge as fluoroquinolone therapy will always result in systemic drug levels. Evidence does not indicate that the use of two fluoroquinolones, such as ciprofloxacin and moxifloxacin, minimizes fluoroquinolone resistance. Targeted fluoroquinolone therapy may in fact have adverse implications for the patient and for overall institutional resistance patterns. For example, the use of ciprofloxacin for urinary tract infections exposes resident streptococci in the respiratory tract to an agent that has demonstrated weaker activity against pneumococci, thus potentially selecting for pneumococcal resistance (9). Moreover, 20%-35% of ciprofloxacin is excreted through the intestinal tract (Cipro package insert), compared to 4% of levofloxacin (Levaquin package insert). Studies have shown that ciprofloxacin displays weaker in vitro activity (lower percentage of isolates susceptible) than levofloxacin for several gram-negative enteric bacteria (2,3). Stepwise adaptive changes towards fluoroquinolone resistance in enteric bacteria may be selected by fluoroquinolones with weaker in vitro activity and higher levels of exposure in the intestinal tract. Therefore, ciprofloxacin would have a greater potential than levofloxacin for the selection of resistant strains of intestinal gram-negative pathogens. A recent report stated that ciprofloxacin-resistant *Escherichia coli* were isolated from the feces of 48% of patients treated with ciprofloxacin for prostatitis; before ciprofloxacin therapy, only ciprofloxacin-susceptible *E. coli* were isolated from the feces of these patients (28). Further, given that 25% of moxifloxacin is excreted through the intestinal tract (Avelox package insert), the use of moxifloxacin for respiratory infections exposes bacteria in the intestinal tract to a fluoroquinolone with greater activity against *Bacteroides fragilis* and other intestinal anaerobes than levofloxacin (29,30). Moxifloxacin has a greater potential than other fluoroquinolones to alter the normal intestinal flora and select for vancomycin-resistant enterococci (31) and intestinal gram-negative strains with increased fluoroquinolone resistance.

In conclusion, we believe that the data we have briefly presented here supplements the previous discussion by Scheld (1) and will help facilitate an improved understanding of the factors influencing the maintenance of fluoroquinolone efficacy.

Focus Technologies is the central testing laboratory for the TRUST antimicrobial susceptibility testing surveillance program, sponsored by Ortho-McNeil Pharmaceutical.

Daniel F. Sahm,*
Clyde Thornsberry,*
Mark E. Jones,*
and James A. Karlowsky*

*Focus Technologies, Herndon, Virginia, USA

References

1. Scheld WM. Maintaining fluoroquinolone class efficacy: review of influencing factors. Emerg Infect Dis 2003;9:1–9.
2. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Evangelista AT, Critchley IA, et al. Susceptibility to fluoroquinolones among commonly isolated Gram-negative bacilli in 2000: TRUST and TSN data for the United States. Int J Antimicrob Agents 2002;19:21–31.
3. Blosser-Middleton RS, Sahm D, Evangelista AT, Thornsberry C, Jones ME, Critchley IA, Karlowsky JA. Antimicrobial susceptibilities of common pathogens causing nosocomial pneumonia: 2001–2002 TRUST surveillance. Annual Meeting Infectious Disease Society of America, 2002, abstract 71.
4. Milatovic D, Schmitz F-J, Brisse S, Verhoef, Fluit AC. In vitro activities of sitafloxacin (DU-6859a) and six other fluoroquinolones against 8,796 clinical bacterial isolates. Antimicrob Agents Chemother 2000;44:1102–7.
5. Craig WA. Does dose matter? Clin Infect Dis 2001;33(Suppl 3):S233–7.
6. MacGowan AP, Wootton M, Holt HA. The antibacterial efficacy of levofloxacin and ciprofloxacin against *Pseudomonas aeruginosa* assessed by combining antibiotic exposure and bacterial susceptibility. J Antimicrob Chemother 1999;43:345–9.

7. Sahm DF, Thornsberry C, Jones ME, Blosser R, Critchley IA, Evangelista AT, Karlowsky JA. Antimicrobial susceptibility of *Enterobacteriaceae* and *Pseudomonas aeruginosa* from inpatient infections in the U.S.: 1999–2002 TRUST surveillance. Critical Care Congress, 2003, Abstract 22015.
8. Gillespie T, Masterton RG. Investigation into the selection frequency of resistant mutants and the bacterial kill rate by levofloxacin and ciprofloxacin in non-mucoid *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Int J Antimicrob Agents 2002;19:377–82.
9. Zhanel GG, Walters M, Laing N, Hoban DJ. In vitro pharmacodynamic modeling simulating free serum concentrations of fluoroquinolones against multidrug-resistant *Streptococcus pneumoniae*. J Antimicrob Chemother 2001;47:435–40.
10. Thornsberry C, Ogilvie PT, Holley HP Jr, Sahm DF. Survey of susceptibilities of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolates to 26 antimicrobial agents: a prospective U.S. study. Antimicrob Agents Chemother 1999;43:2612–23.
11. Biedenbach DJ, Barrett MS, Croco MA, Jones RN. Bay 12-8039, a novel fluoroquinolone, activity against important respiratory tract pathogens. Diagn Microbiol Infect Dis 1998;31:45–50.
12. Jones RN, Pfaller MA. In vitro activity of newer fluoroquinolones for respiratory tract infections and emerging patterns of antimicrobial resistance data from the Sentry antimicrobial surveillance program. Clin Infect Dis 2000;31(Suppl 2):S16–23.
13. Doern GV, Heilmann KP, Huynh HK, Rhomberg PR, Coffman SL, Brueggemann AB. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in the United States during 1999-2000, including a comparison of resistance rates since 1994–1995. Antimicrob Agents Chemother 2001;45:1721–9.
14. Thornsberry C, Sahm DF, Kelly LJ, Critchley IA, Jones ME, Evangelista AT, et al. Regional trends in antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the United States: results from the TRUST surveillance program, 1999-2000. Clin Infect Dis 2002;34(Suppl 1):S4–16.
15. Sahm DF, Thornsberry C, Jones ME, Blosser RS, Critchley IA, Evangelista AT, et al. Correlation of antimicrobial resistance among *Streptococcus pneumoniae* in the U.S.: 2001–2002 TRUST surveillance. Interscience Conference on Antimicrobial Agents and Chemotherapy, 2002, Abstract C2-1640.
16. Zhanel GG, Ennis K, Vercaigne L, Walkty A, Gin AS, Embil J, et al. A critical review of the fluoroquinolones: focus on respiratory infections. Drugs 2002;62:13–59.
17. Lacey MK, Lu W, Xu X, Tessier PR, Nicolau DP, Quintiliani R, Nightingale CH. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin, and ampicillin against *Streptococcus pneumoniae* in an in vitro model of infection. Antimicrob Agents Chemother 1999;43:672–7.
18. Nightingale CH, Grant EM, Quintiliani R. Pharmacodynamics and pharmacokinetics of levofloxacin. Chemotherapy 2000;46 (Suppl 1):6–14.
19. Ambrose PG, Grasela DM, Grasela TH, Passarell J, Mayer HB, Pierce PF. Pharmacodynamics of fluoroquinolones against *Streptococcus pneumoniae* in patients with community-acquired respiratory tract infections. Antimicrob Agents Chemother 2001;45:2793–7.
20. Kolhepp SJ, Grunkemeier G, Leggett JE, Dworkin RJ, Slaughter SE, Gilbert DN. Phenotypic resistance of penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* after single and multiple in vitro exposures to ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and trovofloxacin. Annual Meeting Infectious Diseases Society of America, 2000, Abstract 97**.**
21. Klepser M, Ernst E, Petzold CR, Rhomberg P, Doern GV. Comparative bactericidal activities of ciprofloxacin, clinafloxacin, grepafloxacin, levofloxacin, moxifloxacin, and trovafloxacin against *Streptococcus pneumoniae* in a dynamic in vitro model. Antimicrob Agents Chemother 2001;45: 673–8.
22. Low D, de Azavedo J, Weiss K, Mazzulli T, Kuhn M, Church D, et al. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in Canada during 2000. Antimicrob Agents Chemother 2002;46:1295–301.
23. Brueggemann AB, Coffman SL, Rhomberg P, Huynh H, Almer L, Nilius A, et al. Fluoroquinolone resistance in *Streptococcus pneumoniae* in United States since 1994–1995. Antimicrob Agents Chemother 2002;46:680–8.
24. Evangelista AT, Loeloff M, Pfelger S, Davies T, Bush K, Mauriz Y, et al. Cross-resistance among fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. J Antimicrob Chemother 2001;47 (Suppl 1):29, Abstract P50.
25. Davies TA, Pfleger S, Goldschmidt R, Bush K, Sahm DF, Evangelista AT. Characterization of U.S. clinical *Streptococcus pneumoniae* strains from 2000–2001 that are cross-resistant to ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin. Annual Meeting Infectious Disease Society of America 2002, Abstract 78.
26. Davies TA, Evangelista A, Pfleger S, Bush K, Sahm DF, Goldschmidt R. Prevalence of single mutations in topoisomerase type II genes among levofloxacin-susceptible clinical isolates of *Streptococcus pneumoniae* isolated in the United States in 1992–1996 and 1999–2000. Antimicrob Agents Chemother 2002;46:119–24.
27. Davidson R, Covalcanti R, Brunton JL, Bast DI, de Azavedo JC, Kibsey P, et al. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N Engl J Med 2002;346:747–50.
28. Horcajada JP, Vila J, Moreno-Martínez A, Ruiz J, Martínez J, Sánchez M, Soriano E, et al. Molecular epidemiology and evolution of resistance to quinolones in *Escherichia coli* after prolonged administration of ciprofloxacin in patients with prostatitis. J Antimicrob Chemother 2002;49:55–9.
29. Hoellman DB, Kelly LM, Jacobs MR, Appelbaum PC. Comparative antianaerobic activity of BMS 284756. Antimicrob Agents Chemother 2001;45:589–92.
30. Ednie LM, Jacobs, Appelbaum PC. Activities of gatifloxacin compared to those of seven other agents against anaerobic organisms. Antimicrob Agents Chemother 1998;42: 2459–62.
31. Zhanel GG, Laing NM, DeCorby M, Nichol KA, Hoban DJ. Pharmacodynamic activity of fluoroquinolones in a mixed infection simulationg an artificial bowel: effect of eradicating *Bacteroides fragilis*. American Society for Microbiology, 2002, Abstract A-145.

Address for correspondence: James A. Karlowsky, Focus Technologies, 13665 Dulles Technology Drive, Suite 200, Herndon, VA 20171-4603, USA; fax: (703) 480-2654; email: jkarlowsky@focusanswers.com

International Travel and Sexually Transmitted Disease

To the Editor: Recent articles in the professional literature (1–3) have offered advice regarding the importance of taking a careful travel history, particularly in this time of unprecedented levels of international travel