INSTITUTE OF MEDICINE

REPORT OF A STUDY

Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feed

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HUMAN HEALTH RISKS WITH THE SUBTHERAPEUTIC USE OF PENICILLIN OR TETRACYCLINES IN ANIMAL FEED

Committee on Human Health
Risk Assessment of Using Subtherapeutic
Antibiotics in Animal Feeds

INSTITUTE OF MEDICINE
Division of Health Promotion
and Disease Prevention

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PREFACE

In 1980, at the request of the Food and Drug Administration (FDA), a committee of the Assembly of Life Sciences of the National Research Council (NRC) prepared a report evaluating the effects on human health of the use of penicillin and two tetracyclines (chlortetracycline and oxytetracycline) at subtherapeutic concentrations[1] in animal feed. That committee concluded that the postulated hazards to human health from such use of antimicrobials had been neither proved nor disproved. It drew the conclusion largely because a direct detailed epidemiologic investigation of the hazards had not been feasible and in part because it was impossible to ascertain prior antimicrobial exposures of individual animal sources of meat products for human consumption. The committee recommended various epidemiologic studies (especially of human illness due to salmonellae and pathogenic Escherichia coli) and monitoring and surveillance of the occurrence of antimicrobial resistance of enteric bacteria in humans, animals, and foodstuffs.

Several years later, in 1987, FDA asked the Institute of Medicine to conduct an independent review of the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. The Institute established a committee and gave it a tightly drawn charge: specifically, to perform "a quantitative risk assessment" of those consequences--to "assess the adequacy of existing human health data and use such data to arrive at an estimate of

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[1] The Center for Veterinary Medicine considers any extended use of antibiotics in feed at 200 g/ton or less beyond 2 weeks as "subtherapeutic use," whether it is for growth enhancement or disease prevention. "Use levels are generally 200 g or less of penicillin or tetracycline per ton of feed, but dosage units will vary by species. Levels approved for growth claims and disease prophylaxis are usually lower than those approved for disease treatment; however, there is some overlap in the claims for dose levels of 200 g per ton or less." There is more concern in the agency "with the length of time the antibiotic is used in feed than in the level of drug." (FDA personal correspondence, April 26, 1988)

risk, the basis of which will be justified." If complete quantification of human health risks was not possible because of inadequacies of the available data, the committee was to evaluate the scientific information that had become available since the 1980 report and make judgments about the magnitude of the risks. The committee has not addressed risk management, nor any aspects related to policymaking because this was not part of its charge.

In its risk assessment, the committee was to address the following questions:

- Does the subtherapeutic use of penicillin and the tetracyclines in animal feed result in an increased frequency of antimicrobial resistance in pathogens, particularly foodborne pathogens? If so, can the increase in frequency be reliably estimated and compared with the increases associated with other sources of resistance?

- Does antimicrobial resistance increase (or diminish) the ability of foodborne pathogens to cause disease, change the number of foodborne pathogens (dose) needed to produce disease, or alter the severity of disease caused by foodborne pathogens?

- Does the subtherapeutic use of penicillin and the tetracyclines in animal feed result in increased prevalence of pathogens in the animals so fed and in foods derived from them?

- Does antibiotic resistance attributable to subtherapeutic use in feed increase the incidence of foodborne infectious disease in humans or complicate its medical management?

The current committee is well aware of the longstanding uncertainty of the benefits of the subtherapeutic use of antimicrobials in animal feed and its possible restriction in this country and abroad, and it understands the need for a risk assessment as a foundation for risk management in FDA's decision-making (rule-making) regarding the use of feed additives.

It is inherently difficult to relate human morbidity and mortality associated with a specific antibiotic-resistant bacterial pathogen directly to that pathogen's origin in livestock (or poultry) on a farm or in a feedlot and to administration of subtherapeutic amounts (as opposed to treatment amounts) of penicillin and the tetracyclines to the animals. Unequivocal direct evidence linking mortality to the postulated initial events is not available--certainly not in sufficient quantity to establish a cause-and-effect relationship. For want of direct evidence, the committee has

approached its task indirectly by developing a risk model, using the most reliable data available for the individual elements involved, including annual numbers of reported cases of specified infections, fractions of cases due to bacterial strains that show antibiotic resistance, mortality rates, fractions of deaths associated with bacterial strains of farm origin, and fractions of antibiotic-resistant strains of farm origin caused by subtherapeutic use of antibiotics in animal feed. Although some bacterial pathogens (salmonellae, Campylobacter jejuni, enterohemorrhagic E. coli, and Yersinia enterocolitica) are commonly foodborne and of animal origin, salmonella infections are the only ones that have been reportable for many years and for which incidence figures and antimicrobial-susceptibility data have been collected. Salmonellosis has therefore been selected for the risk assessment model, although we acknowledge that several other human infections would also be relevant to our charge.

The committee is particularly conscious of the limitations and inherent weaknesses of the data base used in the risk assessment model. Where an assumption or estimate had to be made, we have stated its basis. We are aware that some estimates used in the model are weaker than others; for example, the fraction of antibiotic-resistant strains of farm origin attributable to subtherapeutic use of antibiotics (or penicillin and the tetracyclines specifically). Because some data for the model were only estimates, we considered a range of values (low, mid-range, and high) for each element and expressed the final risk estimates (deaths per year) as minimum, median, and maximum.

In addition to the risk assessment, the committee has reviewed further new information pertinent to human health that might be related to subtherapeutic use of antibiotics in animal feed. Some of the new information addresses study possibilities identified by the former NRC committee on subtherapeutic antibiotic use in animal feeds. Some of it deals with the biologic impact of antibiotic resistance in bacteria and the use of molecular biologic techniques in identifying clonal features of isolates obtained from farm animals, from foodstuffs derived from livestock and farm animals, and from infected humans. Some of it reflects followup experience in European countries that have, in the last 10-20 years, by regulatory action prohibited use in animal feed of subtherapeutic concentrations of antibiotics that are used in treatment of humans. Much of this information provides only circumstantial evidence bearing on the question under consideration. Some of the facts even appear to be mutually contradictory.

The committee has not addressed any cost-benefit aspects of the issues related to this problem, nor has it made any recommendations regarding regulatory strategies or policies. It hopes that its report on the subtherapeutic use of

penicillin and the tetracyclines in animal feed will be useful to FDA in its consideration of the risk involved and appropriate risk management. The committee stresses the continuing need for more extensive gathering of detailed epidemiologic information to define the human health risks more sharply.

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I

EXECUTIVE SUMMARY

In 1987, the Food and Drug Administration asked the Institute of Medicine to conduct an independent review of the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. The Institute established a committee and gave it a tightly drawn charge: perform "a quantitative risk assessment" of those consequences--to "assess the adequacy of existing human health data and use such data to arrive at an estimate of risk, the basis of which will be justified." If complete quantification of human health risks was not possible because of inadequacies of the available data, the committee was to evaluate the scientific information that had become available since the 1980 NRC report of a similar investigation and make judgments about the magnitude of the risks.

Since the introduction of the first antimicrobials into clinical medicine their use has exerted continuing selective pressure, resulting in an increase in the prevalence of antimicrobial-resistant strains of both primary pathogens and commensal "opportunistic" bacterial species. Succeeding generations of antimicrobials, often possessing broader spectra of activity, have compounded the problem. Over the years, medical practice has yielded abundant examples of the emergence to predominance of strains of common pathogens that bear resistance (often encoded on plasmids) to one or more antimicrobials. In view of this, many have speculated about the need to limit injudicious and unnecessary prophylactic and therapeutic use of antimicrobials in humans. The continued broad, subtherapeutic use of antimicrobials in animal feeds has added to the concern that such practices may contribute to the emergence of resistant strains of bacteria that pose a risk to human health.

Initially, the committee examined epidemiologic studies that employed recently developed molecular fingerprinting techniques capable of directly linking, by clonal characteristics of the etiologic agent, human illness due to a foodborne pathogen (Salmonella) to the same organism with these same clonal characteristics isolated in the food production chain and back on the farm. Although the committee did look at the data available on other infectious bacteria than salmonellae, it was not possible to find a substantial body of direct evidence establishing conclusively

the presence of a human health hazard that resulted from the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds. Nonetheless, the committee believes that important, although as yet sparse, data show the flow of distinct salmonella clones from farm animals medicated with antibiotics in subtherapeutic concentrations, through food products, to humans, who thus acquire clinical salmonellosis. For example, a multiple antibiotic-resistant strain of S. newport originated in farm animals exposed to chloramphenicol, a drug not approved for feed additive use, rather than to penicillin or the tetracyclines. We believe further that application of available methods for clonal analysis of bacterial isolates in future outbreaks of salmonellosis can provide the direct evidence required to relate use of specific antimicrobials on the farm to human infection with antimicrobial-resistant foodborne pathogens; or could show that no relationship exists.

This model estimates risks for salmonellosis, because adequate data were lacking to quantify the risk for other foodborne pathogens, such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli. Furthermore, this report deals only with the risk for mortality from salmonellosis, and does not estimate risk for morbidity due to lack of data.

In contrast to the paucity of direct evidence implicating subtherapeutic use of antimicrobials as a potential human health hazard, the committee found a considerable body of indirect or circumstantial evidence as follows:

o Data on the biologic properties of transposons and R plasmids. The use of antimicrobial agents for either therapeutic, prophylactic, or growth-promoting purposes generates a strong selective pressure for the emergence of drug-resistant bacteria in the environment, and this selection operates at several levels simultaneously by causing molecular expansion of the antimicrobial-resistance genetic elements as well. The basic genetic unit of resistance, the transposon, can occupy almost any location in the genome, but most typically is on a conjugative R plasmid. The ability of the drug-resistance gene to transpose, as a transposon, and to move to other strains and species provides the potential to confer resistance to the selecting antimicrobial on a large population of bacteria.

Studies of bacterial strains differing only in the presence of an R plasmid have generally revealed little difference in virulence. However, the results of R plasmid acquisition can be striking when a conjugative R plasmid contains virulence genes such as ones encoding enterotoxin, aerobactin, hemolysin, or colonization factors in addition to

antimicrobial-resistance determinants. Selection by antimicrobials can promote spread of virulent strains in instances in which R plasmids have incorporated virulence genes or in which virulence plasmids have acquired drug-resistant transposons. Thus, the mere presence of the drug-resistant phenotype may be enough to enhance the disease-producing potential of the pathogen. Most species of Enterobacteriaceae pathogenic for humans and animals carry at least one essential determinant of pathogenicity on a plasmid.

o There is evidence of extensive use on farms and in feedlots of subtherapeutic concentrations of penicillin, the tetracyclines, and other antimicrobials (see Chapter IV). Over 31 million pounds of antibiotics are produced annually in the United States. Although accurate data on antibiotic use in animal feeds are not available, estimates indicate that almost half the total annual production of antibiotics is directed to use in farm animals. The tetracyclines used in livestock and poultry feeds represent almost 50% of the total of antibacterial use in feeds. Almost 90% of all antibiotics used in farm animals and poultry is administered in subtherapeutic concentrations. About 70% of the total of all antibiotics used in subtherapeutic concentrations in animal feeds is given for the purpose of disease prevention (prophylaxis), and the remainder of this amount is administered for growth promotion.

o Ample evidence exists of a high prevalence of antimicrobial resistance among isolates of salmonellae from farm animals. The frequency of resistance to any of the commonly tested antimicrobials among farm-animal isolates of salmonellae ranges from 69 to 80%; of resistance to ampicillin, from 15 to 72%; and of resistance to tetracycline, from 37 to 81%. These frequencies of resistance among animal isolates are 3-5 times greater than those among strains isolated from human beings. Surveys of resistance to various antimicrobials among salmonella and E. coli isolates from farm animals in the United States generally have shown that feeding antibiotics in subtherapeutic concentrations increases resistance to antibiotics. The prevalence of resistance can vary considerably, probably with temporal and regional differences, and differences among farm animal species. The variations may also reflect differences in antibiotic practices, such as the use of specific antibiotics and subtherapeutic vs. therapeutic dosages, the relative proportions of resistance in bacterial isolates from each of the farm animal species, and environmental factors, such as production methods, and stress on the animals.

o Animal and poultry carcasses in meat-processing plants are often contaminated with intestinal pathogens and E. coli (see recommendations in Chapter XI). Contamination with salmonellae has been found in up to 45-50% of samples of ready-to-cook poultry obtained from food markets at different times and in different parts of the United States. Although few data are available on the prevalence of antimicrobial resistance among salmonellae isolated from animal and poultry carcasses at meat-processing plants, it is likely that the prevalence would be similar among isolates from animals on the farm. The limited data available on salmonella isolates from retail-marketed poultry would show about 30-40% are resistant to the tetracyclines and about 65% to one or more antimicrobials.

o Human infection with salmonellae or other enteric bacteria may follow handling and ingestion of improperly cooked, packaged, frozen, or refrigerated meat or poultry contaminated with these organisms.

o The results of several studies have shown the selection of drug-resistance in coliform bacteria due to the use of antibiotics in feed and subsequent spread from farm animals to humans. The potential exposure of members of a farm family to various enteric bacteria indigenous to farm animals has been investigated to determine the temporal frequency with which antibiotic-resistant E. coli strains from such contact spreads to them. Following the use of tetracycline-supplemented feed in flocks of chickens, the intestinal coliform flora in these chickens became largely tetracycline-resistant; within 2 weeks 90% of the coliform isolates, predominantly E. coli, from the chickens were tetracycline-resistant. The prevalence of tetracycline-resistant coliform organisms also increased in the intestinal tracts of the 11 members of the farm family caring for the chickens, but not in members of neighboring families. After 5-6 months of use of subtherapeutic concentrations of the tetracyclines in chicken feed, 31% of fecal samples taken at weekly intervals from members of the family contained over 80% tetracycline-resistant coliform bacteria, in contrast to 7% of the samples from neighbors. Multiple antimicrobial resistance, most likely plasmid-mediated, to unselecting antimicrobials (streptomycin, ampicillin, sulfonamides), and to the tetracyclines, developed in over 50% of the E. coli strains isolated from chickens fed tetracycline-containing feed for over 10 weeks. Another study, described in Chapter VI, indicating that antibiotic-resistant E. coli of farm origin can spread to humans involved the use in pigs of a nonabsorbable antibiotic of the streptothricin group, nourseothricin, which had not been used in humans and therefore could not have spread initially from humans to farm

animals. After 2 years of use of this antibiotic in pig feed, E. coli that contained plasmids bearing nourseothricin resistance were present in the feces of 33% of pigs with diarrheal illness, 18% of workers on the pig farms, 17% of family members of the farm workers, and 16% of outpatients from the same geographic region where nourseothricin had not been used in feed. Even though no nourseothricin had been used in treatment of humans in the region, 1% of the E. coli urinary tract infections of outpatients were due to nourseothricin-resistant strains. In contrast, intestinal E. coli isolated from outpatients in neighboring regions, where nourseothricin had not been used in pig feed, were not nourseothricin-resistant. Although much of the important information needed to evaluate these observations is lacking, the available information does appear to suggest nourseothricin-resistant E. coli from pigs were transmitted to humans and probably from humans to other humans. Whether nourseothricin-resistant isolates are more or less virulent than susceptible ones is not known.

Epidemiologic approaches have been used to address further the question of whether exposure of human beings to antibiotic-resistant bacteria of farm-animal origin enhances the risk of subsequent infection with such strains. In one such study (see Chapter VI), the possible acquisition of E. coli urinary tract infections due to antibiotic-resistant strains of farm origin was examined in a population of about 700 female employees in poultry processing plants. E. coli were isolated in 95% of the cultures from poultry; 96% of these strains were resistant to one or more antimicrobials and 87% were resistant to two or more drugs. Eleven percent of the female workers who had not recently taken antimicrobial therapy had bacteriuria, with E. coli accounting for two-thirds of the isolates. Of the latter, 17% were resistant to one or more antibiotics. The antibiograms of the E. coli from the bacteriuria workers and from the poultry to which they were heavily exposed seldom were similar or identical. The E. coli strains from both sources were examined for commonly occurring R plasmids with restriction-endonuclease-digestion patterns to trace spread of drug resistance from poultry to workers. The presence of a unique plasmid pattern endemic in poultry also found in the human isolates might support the concept of such spread. However, because the same plasmid could be demonstrated in two isolates in only a few instances, the results had too little power to exclude the possibility of spread. Unfortunately, intestinal E. coli from the workers were not studied, and, therefore, possible spread to their gastrointestinal tracts could not be ascertained. The validity of using urinary tract infection as an epidemiologic approach to the question of whether exposure of humans to

antimicrobial-resistant bacteria of farm-animal origin enhances the risk of subsequent infection by such strains can be seriously questioned. E. coli strains causing urinary tract infections appear to represent a selected group of clones not included among those equally likely to colonize animals and humans.

Monitoring of infections among farm workers and their families caused by antimicrobial-resistant salmonellae or other gastrointestinal pathogens of farm animal origin might provide evidence of spread of these organisms to humans with ensuing illness. Extensive data bearing on this issue are not available. In one study, salmonellosis or diarrheal illness did not occur at a higher frequency in farm children regularly exposed to poultry than in a control group lacking such exposure. Although scattered clinical case reports have documented transmission of salmonellae from farm animals to humans, this does not appear to occur commonly, or at least is not often recognized.

In the United Kingdom, since about 1970 all antimicrobials used in humans have been banned for use in animals, thus only selected antimicrobials can be used for animal growth promotion. The Swann Committee report of 1969 made this recommendation and prohibited penicillin, tetracycline, and certain antimicrobials from being used as feed additives. However, these drugs could be used for therapy or prophylaxis of disease only when prescribed by a veterinarian and then only for a limited time period. Alternatively, antimicrobials such as Zinc Bacitracin, Virginiamycin, and Avoparcin have been used as feed additives but not for therapeutic indications. Use of these drugs does not select for bacterial strains resistant to penicillin, tetracycline, or other antimicrobials used in human medicine. Some investigators in the United Kingdom insist that the selection of resistant salmonellae (and other bacterial strains) is due to the therapeutic use of antimicrobial drugs in humans as well as animals. The concentration of 200 grams/ton is a therapeutic or prophylactic dose in the United Kingdom and must be prescribed by a veterinarian. In contrast, this concentration in the USA is considered a subtherapeutic dose when it is administered for 2 weeks or longer and does not require a prescription from a veterinarian. Consequently, comparison of the effects of this concentration of antimicrobials on the selection of resistant enteric pathogens, especially Salmonella, is difficult because of the difference in applications in these two countries. Although penicillin and the tetracyclines have been banned as feed additives in the U.K., both can be used when prescribed for therapy or prophylaxis of disease. The amounts of antimicrobials used in veterinary practice has increased since the Swann Report. The surveys conducted subsequently indicate a fairly constant and low incidence of

resistance among salmonella isolates except for S. typhimurium phage type 204C, an exceptional strain that has become the most commonly isolated strain from cattle, especially calves. Not only has it been reported in increasing numbers since its isolation in 1979, but each year new antimicrobials have been added to the list to which it is resistant. In a few human cases this strain has developed serious treatment problems because of the limited choice of effective antimicrobials. Although in 1985 it was responsible for 4% of all reported cases of salmonellosis in humans, most of them were self-limiting.

It is difficult to assess the effect of banning penicillin and the tetracyclines as feed additives in the U.K. after 1969 when the Swann Report recommendations were implemented. There were no systematic collections of strains prior to the report to serve as a baseline. Also, there have been significant changes in the methods of raising and marketing animals and these have been important factors in the spread of resistant Salmonella, e.g., S. typhimurium phage type 204C. The multiple antimicrobial resistance profile of this particular strain presumably has been caused by the many antimicrobials used therapeutically for scours in calves and not by subtherapeutic doses used in the U.K. Other strains of Salmonella have not shown this tendency to acquire resistance plasmids under similar circumstances. Various other salmonella species, of animal origin, that cause human disease have fluctuated widely in incidence during this same period but there has not been any dramatic increase in resistance to antimicrobials in bacterial isolates. Some experts in the U.K. are convinced the Swann Committee recommendations have had no impact in reducing one hazard this Committee was charged to address, the selection of resistant bacteria by antimicrobials in animal feed.

Because the committee was unable to find data directly implicating the subtherapeutic use of feed antimicrobials in human illness and that much of the available evidence was primarily circumstantial, often ambiguous, and sometimes conflicting, the committee proceeded to develop a risk model and perform a quantitative risk assessment. Even though salmonellosis may contribute only a small portion of the total incidence of possible human disease due to subtherapeutic use of antibiotics in animal feed, our model was restricted to salmonellosis, because adequate data were lacking to quantify the risk for other foodborne pathogens, such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli.

The model consists of a sequence of five mathematically derived quantitative estimates that are linked in a cascade fashion:

(1) Annual number of cases of salmonellosis reported in the U.S. (the committee has used a mid-range estimate of 50,000 cases in developing the model in Chapter VII)

(2) Fraction of salmonella strains from human cases showing resistance to--

(a) any antimicrobial (mid-range estimate, 24%)

(b) penicillin/ampicillin and/or tetracycline (mid-range estimate, 15%)

(3) Death rate associated with infection by salmonella strains with various resistance patterns--

(a) susceptible to all antimicrobials (mid-range estimate, 0.5%)

(b) resistant to any antimicrobial (mid-range estimate, 1.0%)

(c) resistant to penicillin/ampicillin and/or tetracycline (mid-range estimate, 1.0%)

(4) Fraction of deaths due to salmonellosis that are associated with salmonella strains of farm origin (mid-range estimate, 70%)

(5) Proportion of the above fraction (4) resulting from the subtherapeutic use of antimicrobials in animal feed--

(a) any antimicrobial (mid-range estimate, 88%)

(b) penicillin/ampicillin and/or tetracycline (mid-range estimate, 90%

In view of the nature of the link between individual estimates described above, the five estimates can be multiplied to indicate the number of annual deaths. With appropriate modification of the relevant estimates, the number of deaths due to use of antimicrobials in feed only for the purpose of growth promotion, rather than for all subtherapeutic uses (both growth promotion and disease prevention), can be calculated.

It is extremely important to recognize that the numbers of annual deaths estimated by multiplying the five individual estimates listed above are not necessarily excess deaths. It is possible that, even if all subtherapeutic uses of antibiotics were stopped, a like number of deaths might replace those produced by resistant strains as a result of infections by drug-susceptible salmonellae.

It is possible to estimate the number of excess deaths due to subtherapeutic uses of antibiotics by introducing into the risk model the so-called "etiologic fraction." This fraction represents the proportion of cases that would almost certainly not occur in the absence of drug-resistant strains. These excess cases are estimated by taking into account the proportion of the population that is taking antibiotics at any given time and the documented excess risk of infection following such antibiotic administration. Estimates based on inclusion of the "etiologic fraction" are more certain than those estimated without its inclusion in the sense that these represent true excess cases.

Because the data available for use in the risk model are scanty, were likely to have been collected for other purposes and retrospectively, and sometimes require extensive extrapolation, the inherent limitations of the model must be appreciated. Furthermore, it should be recognized that the figures used in the model result in a low estimate of the actual number of deaths, because possible unreported deaths from salmonellosis are not estimated and deaths due to other foodborne intestinal pathogens, such as Campylobacter jejuni, have not been taken into account. Moreover, there is no doubt an additional burden of disease (morbidity) should be considered part of the characterization of risk. Insufficient data were available to allow quantification of this burden.

The committee believes that it has employed the best available data to make a series of low, mid-range, and high estimates for each sequential step in the model. A similar approach is taken to derive three estimates of the etiologic fraction. Because values for five variables (described above) have been multiplied to provide specific risk quantification, and because there are three different estimates of each variable (low, mid, and high), there are 243 different possible estimates of risk for each linked sequence of steps. The committee tends to place greatest reliance on estimates near the mid-range (median) value as being the "likeliest" estimate reported below.

With this risk model, a series of estimates of annual numbers of deaths from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed were made:

- The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion is in the range of 40 per year.

- The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for growth promotion is in the range of 15 per year.

It must be emphasized that neither of the above estimates is certain to represent the true excess number of deaths due to subtherapeutic uses of antibiotics in animal feed. It is possible that stopping such uses will not reduce these numbers. Inclusion of the etiologic fraction allows estimation of the excess numbers of annual deaths:

o The likeliest estimate of excess deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion is in the range of 6 per year.

o The likeliest estimate of the excess deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines only for growth promotion only is in the range of 2 per year.

It is also possible to estimate the numbers of deaths due to "increased difficulty of treatment." These estimates, which are more uncertain than the others, represent cases due to possibly increased virulence of resistant strains, presence of resistance to antimicrobials ordinarily employed in treatment of such infections when they are severe or occur in particularly vulnerable persons, some other factor, or a combination of these. These estimates probably include those in the etiologic fraction estimated above.

o The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion and arising because of "increased difficulty of disease treatment" is 20 per year.

o The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for growth promotion only and arising because of increased difficulty of disease treatment is 8 per year.

The currently available data are an incomplete "patchwork" from a variety of sources; they are not collected systematically for the nation, they are complex, they are frequently of poor quality and require extrapolation for use in risk assessment, and they are not focused on the specific points of direct interest.

Complete evaluation of these risk estimates of mortality from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed requires consideration of the possible benefits to food production that might accrue from such antimicrobial use. Consideration must also be given to the question of whether overall human deaths from salmonellosis (attributable to both antimicrobial-

susceptible and -resistant strains) would be changed by the discontinuance of subtherapeutic use of penicillin and/or tetracycline. The committee believes that, although some deaths attributable to antibiotic-resistant strains may be substituted for by deaths from susceptible strains, the total number of deaths would decrease. However, this cannot be proved at present.

Using all the resources noted above, the committee was unable to find a substantial body of direct evidence that established the existence of a definite human health hazard in the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds.

The committee does not offer recommendations for risk management or policy making, because this was not part of its mandate. However, a series of recommendations for strengthening the data bases for future risk analyses have been made by the committee. Many of these would warrant implementation in order to monitor antibiotic use and the changes that might ensue in drug resistance in isolates from animals and humans with salmonellosis and other foodborne diseases. These recommendations would seem particularly appropriate in view of the fact that debate on the benefits of use of subtherapeutic doses of penicillin and the tetracyclines in animal feed has gone on for over two decades.

II

INTRODUCTION

Subtherapeutic concentrations of antimicrobials in feed have been used for decades in the raising of animals for food production. The concentrations are lower than those usually chosen to treat established infections in animals, but high enough to affect growth of bacterial components of the gastrointestinal flora. Subtherapeutic concentrations are used to improve growth and to prevent infection during periods of increased susceptibility in rearing. In 1980, a committee in the Division of Medical Sciences of the National Research Council prepared a report entitled The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds[2]. The committee concluded that hazards to human health associated with subtherapeutic use of antimicrobials in animal feeds had been neither proved nor disproved; that was not to say that the postulated hazards did not exist. That committee made a number of observations that are still relevant:

o Subtherapeutic uses of antimicrobials in animals increase the prevalence of antimicrobial resistance in some bacteria, such as salmonellae and Escherichia coli. Persons in close contact with animals treated with antimicrobials are more likely to harbor antimicrobial-resistant E. coli than are persons not so exposed. However, in studies of antimicrobial-resistant E. coli in humans with animal contact, the dosage and duration of antimicrobial use in the animals have not been clearly defined. Thus, subtherapeutic use usually cannot be distinguished from therapeutic use.

o Slaughterhouse workers carry some of the same phage types of Enterobacteriaceae as are found in slaughtered animals and in the slaughterhouse environment. However, the relevant studies were not conclusive, because too few persons were examined. In addition, the animals to which the workers had been exposed had probably received both therapeutic and subtherapeutic doses of antimicrobials.

o A link could not be established between illness due to antimicrobial-resistant pathogenic bacteria and contact with animals given only subtherapeutic antimicrobials or ingestion of meat from such animals.

o Therapeutic and prophylactic doses of antimicrobials in humans increase the prevalence of antimicrobial-resistant microorganisms in their bacterial flora and in the flora of their close contacts.

o Data that would allow measurement of the frequency of transfer of R factors (resistance factors) from the bacterial flora of animals to the flora of humans were not available; nor were quantitative data on the frequency of R-factor transfer among components of the human microbial flora.

o Available data were insufficient to determine any relationship in the general human population between ingestion of meat from animals fed subtherapeutic amounts of antimicrobials and the prevalence of drug-resistant E. coli. The limited available data suggested that antimicrobial-resistant E. coli were as prevalent in vegetarians as in meat-eaters.

The purpose of the present study, initiated 7 years later by the Institute of Medicine at the request of the Food and Drug Administration (FDA), was to develop a formal quantitative assessment of human health risk associated with the subtherapeutic use of penicillin and the tetracyclines in animal feed.

HISTORICAL BACKGROUND

It is helpful to review briefly the history of federal policies regarding the use of penicillin and the tetracyclines in animal feeds.[6,7] FDA approved the use of penicillin and chlortetracycline as feed additives in 1951 and the use of oxytetracycline in 1953. In 1972, FDA issued a policy statement regarding the use of antimicrobial drugs in feeds.[6,7] In 1977, the agency issued a Notice of Opportunity for Hearing (NOOH) on penicillin- and tetracycline-containing premixes to help to determine whether the previously approved New Drug Applications for the drugs should be withdrawn because of possible adverse effects on human health.[4,5] In 1978, before any action on these NOOHs, Congress stipulated that the FDA should seek rigorous evaluation of the available scientific evidence of human health hazards associated with the use of the drugs in subtherapeutic concentrations in animal feed. That stipulation led to FDA's request that resulted in the 1980 National Research Council report. Congress, in its fiscal 1981 appropriations hearings, requested that FDA hold any proposed withdrawal proceedings on the New Drug Applications in abeyance until the research recommended in the report could be done and evaluated.

Between the congressional request and the study reported here, the National Resources Defense Council (NRDC) in December 1984 submitted a petition asking the Secretary of Health and Human Services to suspend approval of the drug applications for subtherapeutic use of penicillin and the tetracyclines in animal feeds.[8] NRDC alleged that the use of the drugs presented an "imminent hazard" to the public health. A decision to invoke the imminent-hazard provision would have resulted in immediate withdrawal of the drugs from the market. FDA reviewed the petition and the scientific evidence submitted in support of the claim of imminent hazard. In summary, the FDA stated that it believed that NRDC has not established that antibiotic-resistant Salmonella, whose resistance results from subtherapeutic uses of penicillin and the tetracyclines in animal feed, have a significant impact on the outcome of human salmonellosis. Moreover, the figures used by NRDC to calculate mortality and morbidity rates were derived, in part, from a study that was not designed for that purpose and, therefore, could be biased. The FDA recommended that the Secretary deny NRDC's petition on the grounds that an "imminent hazard" has not been demonstrated.

COMMITTEE APPROACH

The present Committee on Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animal Feeds has sought data that could be used in making a quantitative assessment of hazards and risk to human health. It has sought the results of peer-reviewed scientific studies on several pathogenic organisms that infect both humans and animals, that cause disease and death in humans, and for which antimicrobial susceptibility testing is commonly performed. The need for data on mortality (reports of human infectious disease reported to state and federal agencies) further limited the bacterial infections that might be included in this study. Few data on morbidity were available. The committee needed a definitive end point (survival or death) that was not afforded in any published evaluations of the effects of salmonellae in causing morbidity in humans. Several other diseases were considered, and these are discussed in Chapter VI, but morbidity and mortality data on the listed diseases other than salmonellosis were unavailable or insufficient in quantity and quality for risk assessment. (Morbidity was not assessed in the present model, because of the lack of data on the cases studied, and the current model has no provisions for morbidity.) Infection with Salmonella species (other than S. typhi) was selected for several reasons: salmonellae are often isolated from farm animals and foodstuffs derived from them; salmonellae are pathogens for

both farm animals and humans, some data are available on antimicrobial resistance among farm-animal and human isolates of salmonellae; the Centers for Disease Control (CDC) conducts a national surveillance for salmonellae and receives numerous human salmonella isolates from all parts of the country for identification and serotyping, and CDC has conducted studies on the incidence of human salmonellosis in selected U.S. urban and rural counties.[1,3] Requisite data related to salmonella infections as either the "underlying cause" or a "contributing cause" of human deaths were sought, and the relevant bacterial isolates were categorized as to antimicrobial susceptibilities. The drug-resistance profiles of the clinical isolates have been examined with a view to determining the sources of drug resistance and whether drug-resistant isolates could be traced to farm origin or, even further, to subtherapeutic use of penicillin or the tetracyclines in animal feed. The task has been formidable, because of the sparseness of data that link drug-resistant clinical isolates to primary sources of infection, whether human or animal, and of data that identify the specific drugs used on the farm, their form of administration (for growth promotion or prevention of infection), and their dosages. The committee has recognized several weaknesses in the data needed for risk assessment and its findings have highlighted these weaknesses.

Bacterial antimicrobial resistance can be natural or acquired, and the growth of bacteria with either type of resistance will be selectively favored when antimicrobials are used in humans, in animals, or in other environments. Natural resistance to an individual antimicrobial pertains to resistance of an entire species to that antimicrobial, e.g., resistance of Pseudomonas aeruginosa to chloramphenicol. Such resistance usually arises from a lack of permeability to the drug or the lack of a susceptible target site for the drug. Acquired resistance occurs as a result of mutation or as a result of transfer of resistance (R) plasmids. Exposure of large bacterial populations to various antimicrobial agents results in selection of antimicrobial-resistant microorganisms, provided that the concentrations of the agents are above the minimal inhibitory concentrations (MICs) for the exposed bacteria. That selection occurs in bacterial cultures in vitro, in humans receiving antibiotics either prophylactically or therapeutically, and in animals whose feed contains antibacterial agents for growth enhancement, for treatment of established infection, or for disease prevention. One would like to know particularly the extent to which the human "pool" of drug-resistant enteric microorganisms is increased by the aggregate of disease-producing pathogenic strains, bowel flora, and gastrointestinal "transients" of animal origin that are

generated by subtherapeutic uses of penicillin and the tetracyclines on the farm and in feedlots.

General questions that might reasonably be raised concerning the importance of the subtherapeutic use of penicillin and the tetracyclines in current problems of human infection with antimicrobial-resistant bacteria include the following:

o What are the relative (quantitative) contributions to drug resistance, in bacterial species pathogenic for humans, of antimicrobial use in humans and in animals?

o What are the relative contributions to bacterial drug resistance of subtherapeutic and therapeutic uses of antimicrobial agents?

o Does bacterial resistance to penicillin and the tetracyclines foster resistance to other antimicrobial agents used in the treatment of animal or human infections? More specifically, what is the relationship of the subtherapeutic use of these drugs in animals to resistance to other drugs?

o If subtherapeutic (but not therapeutic) use of penicillin and the tetracyclines were eliminated, would the frequency of antimicrobial resistance among enteric pathogens be likely to increase, to decrease, or not to change?

The committee hopes that newly developed molecular fingerprinting techniques that pinpoint the sources of infections with antibiotic-resistant pathogens and that trace routes of transmission (animal to human or human to animal) will be used regularly in epidemiologic investigations and that a database will be established for use in future assessment of risk. The pathways of infective bacteria from animals to humans or vice versa and the relationship of the development of antimicrobial resistance in these bacteria to antibiotic use thus might be more clearly identified.

THE REPORT

The committee considered the available data on several bacterial organisms and selected nontyphoidal salmonella infections as the most appropriate infections to consider in making the human health risk assessment, because relevant human case reports, antibiotic resistance profiles in bacterial isolates, and other data needed were available. This report is organized according to individual steps or issues of importance that the committee felt might directly

or indirectly provide insights into current health risks (or might be identified as targets for future investigation).

The report deals with the biologic impact of resistance to antimicrobial agents first, because the mechanisms of antimicrobial resistance, population genetics and the overgrowth of resistant microorganisms in the presence of antimicrobial agents, and bacterial resistance transfer are well-studied topics relevant to antimicrobial drug effects. Furthermore, definition of specific antimicrobial-resistance genes and other genes of plasmids provides a potential means of establishing the common clonal identity of isolates obtained at geographically distant yet epidemiologically related sites.

The next chapter describes antimicrobial production in the Unites States as a means of estimating total antimicrobial use, whether for humans or animals and whether for treatment, disease prevention, or growth enhancement (in farm animals). Particular attention was paid to estimating the use of penicillin and the tetracyclines in livestock and poultry production. To the extent possible, the committee has estimated that portion of the total use of antimicrobials on the farm and in feedlots accounted for by penicillin and the tetracyclines. Such estimates have been analyzed to apportion the total amounts to therapeutic and subtherapeutic use and to growth promotion and disease prevention.

Central to any consideration of possible adverse effects on human health are the patterns and prevalence of antimicrobial resistance in salmonellae and E. coli isolates of animal and human origin. Chapter V discusses possible differences in prevalence and patterns of resistance between isolates from healthy animals and from meat and poultry products and isolates from veterinary institutions that treat ill animals. Studies of the effects of long-term administration of subtherapeutic antibiotics to farm animals (and later discontinuation) on the prevalence of antimicrobial resistance in their E. coli strains are reviewed, and temporal trends in resistance among isolates of human origin are surveyed.

The possible transmission of antimicrobial-resistant pathogens or intestinal commensals of farm origin to humans is examined in the next chapter. Data bearing on the issue have come from experimental field studies, from epidemiologic studies of outbreaks of salmonellosis in which molecular biologic techniques have been used to establish clonality of isolates along the food chain from production to human consumption, and from investigations of the occurrence in farm and slaughterhouse workers of infections due to antimicrobial-resistant microorganisms of farm origin.

The principal focus of this report is the development of a risk model and its use for establishing an estimate of

risk. The model described in Chapter VII is based on human salmonella infection and incorporates data on the following:

- o The numbers of cases reported annually.
- o The fraction of cases due to organisms resistant to more than one antimicrobial or resistant to ampicillin (the penicillin congener that is used in treatment of susceptible human salmonella infections) or the tetracyclines.
- o The fraction of cases that result in death.
- o The fraction of cases of farm origin.
- o The fraction of cases that might be attributable to the subtherapeutic use of antimicrobials in animal feeds.
- o The role of recent ingestion of antimicrobial agents for unrelated reasons in predisposing to infection by smaller inocula of antimicrobial-resistant pathogens than would ordinarily be required to produce disease (the so-called etiologic fraction), i.e., the excess of cases attributable to the effects of previously administered antimicrobial agents.

Several European countries have placed restrictions on the use of antimicrobials in subtherapeutic quantities in animal feeds over the last 2 decades. Their experience regarding use of antimicrobials, patterns of antimicrobial resistance in E. coli and salmonellae, and incidence of disease due to enteric pathogens in livestock and humans has been reviewed for insights into the consequences of such restrictions.

The final chapters of the report present the conclusions reached by the committee and its recommendations for future research.

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III

BIOLOGIC IMPACT OF RESISTANCE TO ANTIMICROBIAL AGENTS

BRIEF HISTORY OF CLINICAL DEVELOPMENT OF DRUG RESISTANCE

The discovery of sulfonamides and antibiotics in the first half of the twentieth century led to at least two biologic "revolutions." The first was the ability to treat infectious diseases. The second was the use of antibiotics to gain insights into the genetics of bacteria. In 1943, Luria and Delbruck[70] first demonstrated that the emergence of bacterial resistance to a single antibiotic after exposure to it was a result of chromosomal mutation (independent of antibiotic exposure), rather than an adaptive change. In the years that followed, it became conventional to consider that the emergence of any drug resistance in any bacteria was due to a selected mutation.

Conventional beliefs concerning the importance of mutation in drug resistance were overturned by events in Japan in the years just after World War II. There, physicians treating patients in epidemics of shigella dysentery used sulfonamides extensively. A substantial proportion of those bacteria soon became resistant to sulfonamides. The widespread sulfonamide resistance caused physicians to switch from sulfonamides to new drugs--streptomycin, tetracycline, and chloramphenicol--to treat shigella dysentery. By the late 1950s, many of the isolates of shigellae were drug-resistant, not only to sulfonamides, but to all drugs. That finding led investigators to question, on theoretical grounds, mutation as the explanation for drug resistance. Mutation had previously been shown to take place in about 1 in 10^7 cells, and it strained credulity to assume that cells of a single strain could spontaneously develop drug resistance to four antibiotics over a short period. Moreover, several serotypes of Shigella spp. were shown to have developed multiple-drug resistance virtually simultaneously, and this multiple-drug-resistance pattern was actually more common than resistance to a single antimicrobial.

Japanese investigators showed in the late 1950s that multiple-drug resistance could be transferred from one bacterial species to another. In light of discovery of bacterial conjugation in the preceding decade, it was possible to demonstrate that multiple-drug resistance was

being transferred by the process of conjugation. Shortly thereafter, other workers demonstrated that these conjugative packages of genetic information, labeled R factors (now called R plasmids), were prevalent not only in Shigella spp., but also in Salmonella spp. and Escherichia coli. Since the time of their initial discovery, R plasmids have been shown--in complementary studies by epidemiologists, molecular geneticists, and bacterial physiologists--to be widely transmissible and specifically selectable when antimicrobial drugs are present in the environment. Increasing drug resistance among human isolates of clinically relevant bacteria during this period has been shown repeatedly to be due primarily to the proliferation of R plasmids.

MECHANISMS OF ACQUIRING ANTIMICROBIAL RESISTANCE IN BACTERIA

CHROMOSOMES

Most drug resistance in clinically relevant bacteria is due to conjugative transfer of R plasmids and their clonal expansion during exposure to antimicrobial drugs. But chromosomal inheritance and the transfer of chromosomal mutant genes by transformation also play a role.

Bacteria can acquire new genetic information in three known ways: conjugation, transduction, and transformation. Conjugation requires that the donor bacterium possess both the means to duplicate part of its genetic information and the means to attach itself to a recipient bacterium for DNA transfer. The donor must mate with a recipient bacterium that is physiologically capable of permitting the new DNA to enter and replicate autonomously as a plasmid or permitting it to become incorporated by recombination into the recipient's chromosome. Transduction, a process much less important for the transfer of drug resistance, depends on bacterial viruses known as bacteriophages to "package" pieces of the chromosome or plasmid of the donor organism and inject the package into the appropriate bacterium for uptake and incorporation of the foreign DNA. Transformation is the process by which DNA in solution is taken up directly by a bacterial cell. Plasmid (circular) DNA is usually taken up more efficiently than chromosomal (linear) DNA, in that many bacteria have enzymes, known as exonucleases, that attack only linear fragments of DNA. Nevertheless, in some organisms, transformation of chromosomal contents has been shown to be capable of transferring drug resistance.

Chromosomal drug resistance, in most cases, is due to mutation of pre-existing DNA. Although there are others, three antimicrobial drugs stand out for their ability to select chromosomal mutants that have acquired drug

resistance: streptomycin, nalidixic acid, and rifampin. Their common characteristic is that they work by binding to protein targets within the bacterial cell. Each of the protein targets provides an important vegetative function for the cell--the streptomycin target is the S12 ribosomal protein, the nalidixic acid target is DNA gyrase, and the rifampin target is RNA polymerase. Those targets perform essential functions for the cell: protein synthesis, the required winding of DNA, and the transcription of DNA into messenger RNA, respectively.

The specific sites of antibiotic binding in the targets can occasionally be altered by mutation in such a way that the antibiotic no longer binds to the target and the target retains most of its function. Thus, the appropriate mutations in the genes for the S12 ribosomal protein, DNA gyrase, and RNA polymerase will lead to resistance to streptomycin, nalidixic acid, and rifampin, respectively. The bacterium pays a price for the mutations, in that it acquires a less than optimal "housekeeping" protein in the process of evading the effect of the antibiotic; thereafter, it is typically not as hardy as its nonmutant parents. Although chromosomal resistance usually involves resistance that is specific for the selecting antimicrobial drug, it is occasionally responsible for simultaneous resistance to several antibiotics of different structures and sites of action, e.g., the mar locus in E. coli affects uptake of tetracycline, cefoxitin, and chloramphenicol. Extensive epidemiologic investigations of drug resistance in enteric bacteria have yielded little evidence of the importance of chromosomal mutation in the acquisition of drug resistance or of transformation as a means by which drug resistance can be exchanged. Most enteric bacteria have been shown to have low efficiency in taking up DNA (a property known as competence), unless they are treated so as to damage their permeability barriers temporarily.

R PLASMIDS AND TRANSPOSONS

Since their discovery about 30 years ago, R plasmids have been extensively studied epidemiologically and molecularly and have been shown to play a predominant role in drug resistance among bacteria.[7,22-24,27,29,34,55,85,91,92,98] Like other self-replicating nonchromosomal units of DNA, R plasmids carry "machinery" for efficient replication and genes for particular drug-resistance phenotype. By definition, R plasmids carry genes that encode products that confer drug resistance in a bacterium. Often a single R plasmid contains multiple genes, each encoding a different kind of resistance. Some individual resistance genes encode resistance to multiple related antibacterial drugs. Almost

every drug-resistance determinant is carried on a genetic unit (usually small, occasionally large), called a transposon, that can move from its location on the R plasmid to other locations--typically, but not exclusively, other plasmids. That form of DNA rearrangement, or transposition, requires special genes and stretches of DNA that are parts of the transposon.[16,103] It is useful to consider transposons as freely movable genetic modules that can be assorted, reassorted, and added to and subtracted from evolving R plasmids as environmental pressures dictate.

The ability of most drug-resistance genes to transpose provides R plasmids with an extraordinary degree of genetic plasticity. Although it is not clear that the presence of antibiotics in the environment has any influence on the extent of transposition of resistance determinants, antibiotics exert a profound influence on the selection and persistence of R plasmids with multiple drug-resistance determinants. Studies of indigenous soil bacteria in the preantibiotic era showed not only that R plasmids were less prevalent, but also that recovered R plasmids typically contained only one or two resistance determinants each.[24,29] More recently, in sharp contrast, bacteria recovered in environments exposed to antibiotics have had a high prevalence of R plasmids with multiple drug-resistance determinants.[29,34,91,92] It is important to note that these R plasmids are similar in many genetic respects to the preantibiotic-era plasmids that did not have multiple drug-resistance determinants. Transposons, whose drug-resistance genes evolved as "protection" against the natural antibacterial substances in the soil, thus used plasmids already available.[7,29] Moreover, the same transposon can be found in an array of different plasmids; in nature, drug-resistance elements are indeed promiscuous and can locate in a broad spectrum of genomes.

Most bacteria, given the appropriate supplemental genes, are potentially capable of transferring DNA to other bacteria by conjugation. Usually the supplemental genes are carried on a plasmid. When present on an R plasmid, the supplemental genes together make up what is called the resistance transfer factor, or RTF. Most R plasmids contain an RTF, which enables them to be conjugatively transferred between bacteria.[29] Just as the transposon, at the level of the single drug-resistance determinant, is capable of movement to a different R plasmid, the RTF-containing R plasmid is capable of movement to other strains and other species of bacteria. Although initial studies on conjugative R plasmids were limited to facultative gram-negative bacteria,[29] conjugation clearly plays a major role in the transfer of drug resistance among facultative gram-positive bacteria[55,81,105] and among anaerobic bacteria.[15,96,114] Recently, it has been found that some transposons in some

gram-positive bacteria can bypass the need for RTF-containing R plasmids in conjugation.[15] These "conjugative transposons" contain the equivalent of the RTF, as well as the R factor. Other studies involving anaerobic bacteria have shown that sublethal concentrations of tetracycline in vitro actually promote R-plasmid transfer, in addition to selecting bacteria that carry the drug-resistance determinant.[119]

Of equal or greater concern from the standpoint of drug-resistance proliferation is the finding that the tetracycline-resistance determinant (Tc^r) can be conjugatively transferred back and forth between bacteroides (anaerobes) and E. coli (facultative gram-negative bacilli).[119] Inasmuch as anaerobic bacteria, especially species of Bacteroides, are the predominant flora in the mammalian gastrointestinal tract, the presence of back-and-forth transfers suggests that the reservoir for maintenance, persistence, and spread of at least one drug-resistance determinant, Tc^r, is enormous.

The latter example of interspecies conjugative transfer, of which there are many examples (see Odelson et al.[96] for a review), brings up the issue of plasmid host range. In addition to the genetic attributes already discussed, R plasmids contain a vegetative origin of replication, or oriV, which enables them to replicate autonomously in host bacteria. Plasmids can be cataloged into "incompatibility" groups based on their oriV; those with the same oriV cannot coexist within a bacterium, because of competition for identical replication factors.[29] The specific oriV carried by a plasmid constitutes another element of its phenotype: its host range. Narrow-host-range plasmids can replicate in only a few species of bacteria, usually because only those bacteria provide additional factors required for plasmid replication. In contrast, broad-host-range plasmids can transfer to and replicate in a large variety of bacteria. Results of transfer studies in E. coli and bacteroides indicate that antibiotic exposure--in this case to tetracycline--can increase the transfer and selection of at least one form of broad-host-range plasmid resistance to tetracycline.

In early studies of conjugative transfer of R plasmids among bacteria common in the gastrointestinal tracts of humans and farm animals, E. coli was found to act as a good donor and recipient of R plasmids; Salmonella spp. were not as good donors and recipients.[29] In a more recent study involving S. typhimurium and E. coli recovered from calves, strains of S. typhimurium were extremely proficient R plasmid donors--even better than strains of E. coli.[124] Certain plasmids, belonging to a particular group of incompatible plasmids, Inc H2, found in most strains of salmonellae and preferentially in S. typhimurium, showed a peak efficiency of transfer at 30°C and were conjugatively transferred in

calves' feces after excretion.[124] Inc H2 plasmids have other critical features: they carry resistance to both penicillin and the tetracyclines, they transfer to E. coli, and they carry other non-drug-resistance determinants that increase the ability of the bacteria to colonize the gastrointestinal tract.[124] In every general aspect of drug resistance studied (i.e., expression of resistance genes, maintenance of R plasmids, and transfer of plasmids), Salmonella spp. and E. coli have been found to be quite similar.[29] Although Salmonella spp. might behave quite differently from E. coli in the gastrointestinal tract, because of the former organism's ability to invade entrocytes and thereby avoid antibiotics that cannot penetrate cells well (i.e., penicillin and aminoglycosides, but not the tetracyclines or chloramphenicol), evidence suggests that R plasmid transfer occurs with ease in Salmonella spp. in vivo. For example, in an outbreak of gastroenteritis caused by S. typhimurium that affected 1,900 persons who ate contaminated turkey meat, the source strain of bacteria isolated from the meat was antibiotic-susceptible, as were bacterial organisms isolated from persons who had not taken any antibiotics. In sharp contrast, a high proportion of the persons who were given chloramphenicol, ampicillin, or one of several other antibiotics had R plasmids bearing S. typhimurium in their stools; that proves the ability of salmonellae to acquire R plasmids from the human gut.[2a] Thus, strains of salmonellae, as well as E. coli, can act as reservoirs for conjugative R plasmids and thus gain an enormous selective advantage over other bacteria in the face of antibiotics in the environment.

Epidemiologic surveys of R plasmids in bacteria that are clinically important have shown that multiple-drug resistance has increased progressively since the beginning of the antibiotic era.[34,81,91,92] Among individual R plasmids, the number of drug-resistance genes per R plasmid and the likelihood that a given R plasmid is conjugative have also been increasing.[63,89,114] Although early studies had indicated that the problem of drug resistance was most pronounced in the Enterobacteriaceae, the more recent studies show that the problem has spread. Drug resistance has now been found in bacteria of virtually all genera that are important.[15,22,27,34,53,55,81,85,91,92,96,98,102,105,114,119,122]

In summary, R plasmids affect the microbial populations of humans in several ways:

- o R plasmids confer drug resistance on a great number of bacteria, including pathogens and commensals.

- o R plasmids typically confer drug resistance to several antimicrobial drugs simultaneously.

o R plasmids are capable of being transferred among strains of the same species and among different species through the process of conjugation. Transfer can occur from commensal to pathogenic bacteria in the presence of antibiotics.

o R plasmids can carry additional genes, including virulence factors (discussed later).

o Exposure to antimicrobial drugs causes an increase in the number and the spread of R plasmids by preferentially selecting bacterial offspring that are drug-resistant and, in some cases, possibly by increasing the efficiency of conjugative transfer.

ROLE OF ANTIMICROBIAL DOSAGE IN SELECTION OF DRUG-RESISTANT BACTERIAL POPULATIONS

Antimicrobials are used in three ways in agriculture. In the first, high (therapeutic) doses are used for brief periods (usually no longer than about a week to 10 days) for the treatment of infectious disease. In the second, low (growth-enhancing) doses are used for long periods in livestock to promote growth. In the third, low (prophylactic) doses are used for a period up to 2 weeks to prevent disease. Although the duration of antimicrobial use differs between growth-promoting and prophylactic purposes, the dosages for both "subtherapeutic" uses are typically the same, i.e., less than 200 grams/ton. The selection of antimicrobial-resistant bacteria is a consequence of therapeutic use and of both types (growth-enhancing and prophylactic) of subtherapeutic use of antimicrobial agents.

Dosages of drugs that would be classified as subtherapeutic might well produce concentrations in the gastrointestinal tract that are sufficient to inhibit susceptible species of bacteria. In Danish studies examining the incidence of drug resistance in feces of pigs given only intermittent therapeutic courses of antimicrobial drugs (none of the antibiotics was fed), a high proportion (53%) of *E. coli* strains were found to be resistant to at least one commonly used antimicrobial drug.[115] Moreover, 53% of the pigs carried tetracycline resistant *E. coli* despite the fact that none of the animals had been exposed to tetracycline within the past year.[115] Using various methods of analysis, Corpet recently found that continuous subtherapeutic doses of antimicrobial drugs caused a profound alteration in the fecal flora of mice, with a significantly increased proportion of resistant *E. coli*.[17]

Although the data are overwhelming in showing that use of antimicrobial agents promotes the emergence of drug

resistance, the differential effects of the three kinds of use in selecting drug resistance either in animals or in vitro have not been well studied. Nevertheless, in a recently reported study, when three groups of pigs were examined for the presence of drug resistance in their fecal coliform bacteria, a non-antibiotic-treated herd had a lower proportion of drug-resistant bacteria than a herd given antibiotics only intermittently and at a high (therapeutic) dose; isolates from the latter herd had a lower proportion of drug-resistant coliform bacteria than those from a herd exposed continuously to antibiotics at a subtherapeutic dose.[58] Specifically, the proportion of the tetracyclines resistance among the fecal isolates was found to be 26% for the first herd, 76% for the second herd, and 100% for the third herd.[58]

An earlier study by the same group of investigators yielded nearly identical result.[59] The feeding of one of the tetracyclines at subtherapeutic doses resulted in a linear increase in tetracycline-resistant coliform bacteria, and the numbers of drug-resistant bacteria eventually equaled or exceeded those of drug-resistant bacteria from animals treated therapeutically. These studies support the hypothesis that any form of antibiotic exposure increases the prevalence of drug resistance. Moreover, they provide limited evidence that drug resistance is at least as prevalent after continuous subtherapeutic use of antibiotics as after intermittent therapeutic use.

EXPERIENCE WITH ANTIBIOTIC RESISTANCE AFTER ANTIBIOTIC USE IN HUMANS

The experience gained in the use of antimicrobial drugs since the 1940s provides strong evidence of the effects of such agents in the selection of antimicrobial-resistant commensals and pathogens in humans. That experience emphasizes the importance, constantly reiterated in clinical teaching, of avoiding unnecessary, prolonged, inadequate (subtherapeutic dosing), or inappropriate (for the etiologic bacteria) treatment or prophylaxis with antimicrobial drugs.

The current prevalence of antimicrobial-resistant strains of bacteria is a consequence of extensive antimicrobial use in the last 40-50 years. The prevalence was much lower in the pre-antibiotic era.

Baseline Levels of Antibiotic Resistance--the Status in the Pre-antibiotic Era

Insights into the prevalence of antibiotic resistance in the pre-antibiotic era come from examination of bacterial

isolates from primitive societies unexposed to antimicrobial therapy and from study of stored isolates predating the introduction of penicillin.

In an examination of 21 human stool specimens and 19 soil specimens from an "antibiotic-virgin" population in the Solomon Islands in 1968, R plasmids (mediating streptomycin and tetracycline resistance) were found in only two specimens (5%).[35] In a study of human and animal communities in Rhodesia, about 10% of 47 fecal specimens from Kalahari bushmen and 540 from animals contained gram-negative bacilli resistant to one or more antibiotics.[74] Isolates were frequently resistant to only a single drug (often ampicillin), and none contained R factors. However, the initial fecal cultures were streaked on media containing low concentrations of antibiotics, which may have been inhibitory to occasional R-plasmid-containing strains and thus might have caused their prevalence to have been somewhat underestimated.

In a study of another antibiotic-unexposed population, in North Borneo, 50 multiple-antibiotic-resistant strains were found among 1,017 fecal isolates (more than half identified as E. coli) from 128 persons. Of the latter, six strains (all E. coli), contained R plasmids;[24] those strains represented 0.6% of the original isolates. Skerman and Falkow in 1969 studied the incidence of drug-resistant fecal E. coli in members of a "pre-antibiotic" society in Australia and found that 16% of 247 E. coli isolates were antimicrobial-resistant and that less than 1% contained R factors.[30,110]

In a stored collection of enterobacteriaceae from widely scattered parts of the world (Europe, the Middle East, and North America), originally isolated between 1917 and 1954, very little antibiotic resistance was found on susceptibility testing decades later.[45] Of 433 strains, 11 (2.5%) were resistant to antibiotics (9 to tetracyclines, 2 to ampicillin). No transferable antibiotic-resistance plasmids were detected. None of the 210 Salmonella spp. and none of the 32 E. coli strains in the study showed resistance to any of seven antibiotics, to sulfonamides, or to trimethoprim. In contrast, 24% of strains were able to mobilize a nonconjugative plasmid in a recipient strain, indicating the presence, in the host, of a conjugative plasmid that was lacking antimicrobial-resistance determinants. That prevalence is roughly comparable with that (17%) noted in 300 fecal E. coli strains isolated from nonhospitalized persons[65] and that (33%) noted in 60 E. coli strains obtained from human, porcine, and bovine sources.[113]

Thus, it appears that conjugative plasmids were as common among enterobacteriaceae before the introduction of antibiotics as they are in the current antibiotic era. In contrast, under the selective pressure of antibiotic use in

humans and animals, plasmid transfer and gene exchange have markedly expanded the population (strains and species) of resistant bacteria.

Increases in Resistance After Widespread Clinical Use of Antibiotics

Experience over the last 4 decades of antimicrobial use in treatment and prophylaxis of human infections indicates the profound effect of these practices on the prevalence of antimicrobial resistance in a variety of bacterial species. Experience time and again has emphasized the importance of limiting the use of antimicrobials to necessary and reasonable therapeutic indications and, indeed, even to holding some highly effective drugs in reserve. Before 1946, when penicillin became generally available, 85% of clinical isolates of Staphylococcus aureus at the Boston City Hospital were highly susceptible to penicillin.[33] Within 3 years, most of the strains there had become highly resistant to penicillin. In most developed countries today, only about 10% of S. aureus isolates, whether of community or hospital origin, are susceptible to penicillin.

Isolates of another gram-positive human bacterial pathogen, Streptococcus pneumoniae, collected before 1950 were tested years later and found to be uniformly susceptible to penicillin. The situation is entirely different in Barcelona, Spain, where recently about half of a small number of pneumococcal isolates were either moderately or highly resistant to penicillin, and 62% of the same isolates were resistant to chloramphenicol.[60] This high degree of antibiotic resistance might well be related in part to widespread use of those antibiotics, prevalent for many years in Spain.[66]

Problems of antimicrobial resistance in bacterial species associated with widespread antimicrobial use in humans have been evident among gram-negative bacillary species notorious for causing infections in hospitals. For example, whereas serratia strains isolated in the 1950s were uniformly susceptible to kanamycin, nalidixic acid, and gentamicin and a minority of strains were resistant to streptomycin, a large proportion of strains were resistant to all but gentamicin by 1967.[33]

Prophylactic use of antimicrobial drugs, as well as use in therapeutic concentrations, might similarly exert a selective effect in humans, increasing the prevalence of antimicrobial-resistant strains. The experience on the Burn Service at Grady Hospital where prophylactic topical use of gentamicin was extensive from 1964 to 1969, offers a dramatic example.[108] In 1968, more than 1,400 lb of that drug was used topically on burns at that center. From 1965 through

1967, 80-90% of all Pseudomonas aeruginosa isolates from patients in that burn unit were susceptible to gentamicin. By 1969, only nine percent were susceptible. Almost all the resistant isolates were almost all of a single pyocin type--a type that had in the past been seen only infrequently in the burn unit and had previously been susceptible to gentamicin. In mid-1969 routine topical use of gentamicin was discontinued, and it was replaced with other topical drugs that would not have been used in systemic treatment of bacterial infections. In mid-1970, 95% of P. aeruginosa isolates were susceptible to gentamicin--back to baseline values that existed before the routine use of topical gentamicin.[108]

Effect of Reduced Use of Antibiotics in Humans on Antimicrobial Resistance

Quantitative aspects of overall antimicrobial use in humans contribute to selection of resistant strains. Thus, rational choices of drugs for appropriate therapeutic and prophylactic indications are of continuing importance in treating humans. The same rational approach seems warranted in antimicrobial use for animals. If one accepts the concept that populations of bacteria represent a common gene pool with considerable genetic fluidity and that some of the genetic information segregated for much of the time in individual components of the population are capable of being transferred among members of the population, then the effects of antibiotic use on one component (e.g., indigenous flora of farm animals) might be relevant to another component (e.g., commensals and pathogens for humans).

It is not known whether the current widespread prevalence of antimicrobial resistance genes among bacterial isolates from human and farm animal sources is too great to be reduced by any reasonable reduction of overall antimicrobial use; the issue warrants careful consideration. The extensive reduction in gentamicin resistance in P. aeruginosa over a 1- to 2-year period in the Grady Hospital Burn Unit[108] cannot be generalized, because of the relatively short period of use of the drug and the confined location of its extensive use. Since the early 1950s, scattered studies have reported temporal relationships between decreased use of specific antimicrobial agents in a given hospital or hospital ward and decreased prevalence of nosocomial bacterial pathogens resistant to those drugs.[78] However, caution must be exercised in interpreting such data, because many of the reported studies dealt with nosocomial outbreaks. Other epidemic-control measures that were instituted might have contributed as much as the changes in antibiotic use to the decreased prevalence of the resistant strain in some of the

outbreaks. Nonetheless, the data suggest the potential for reduction of antimicrobial resistance by limiting the use of some drugs.

When antimicrobial use in humans has been decreased, usually in a hospital setting, resistant strains did not necessarily disappear quickly. Resistant bacteria can sometimes persist despite the absence of antimicrobial selective pressures. In a study of 56 infants who were known to have been colonized with R-plasmid-containing kanamycin-resistant enterobacteriaceae during a stay in an intensive-care nursery in which kanamycin was extensively used, 89% were still colonized with kanamycin-resistant strains 2 months after their return to the community.[20] Intestinal carriage of such R-plasmid-containing kanamycin-resistant strains gradually decreased, but after 12 months or more, 46% of the infants still harbored the resistant organisms.

EFFECT OF ELIMINATION OF SUBTHERAPEUTIC ANTIBIOTIC USE ON LEVELS OF ANTIBIOTIC RESISTANCE IN FARM ANIMALS

What is the counterpart in animal production of the above-described circumstance of the withdrawal or shift of antibiotic use in humans, and what changes have such shifts made in the prevalence of antibiotic-resistant coliform bacteria in their fecal bacterial population? Results of long-term studies of the responses of the intestinal coliform bacteria of swine to cessation of antibiotic exposure suggest that changes occur slowly (V. Hays, University of Kentucky, personal communication, 1988; also see Langlois et al.[56,57]). In a separated herd of pigs (see additional detail below) maintained on subtherapeutic concentrations of tetracycline (50-100 grams/ton of feed) for 13 years (since 1972), tetracycline resistance averaged over 90% in the fecal coliform population. Such a high level of resistance to tetracycline, almost exclusively R-plasmid-mediated in enterobacteriaceae in this setting, was accompanied by resistance to one or more other antibiotics. Does that high level of resistance indicate such extensive permeation of R plasmids and transposons throughout the intestinal population of *E. coli* and other related coliform species so extensive as to stabilize their predominance and preclude diminution of their major position even if exposure to antimicrobials in feed ceased? On the basis of experience with the use and withdrawal of specific antibiotics in relatively closed populations of hospitalized patients, one might expect a protracted period to elapse before resistance returned to control levels in a comparable population that had not been exposed to antibiotics.

The effects of antibiotic withdrawal on antimicrobial resistance in intestinal coliform organisms in the swine herd

mentioned above has been extensively documented over a 13-year period (V. Hays, 1988, personal communication). This herd of pigs, established in 1963, that received antibiotics routinely as feed additives and in injectable form (when needed for treating sows and pigs), no single antibiotic was used continuously. After 1972, the herd was kept free of any antibacterial agents as feed additives or for therapy. The level of tetracycline resistance among fecal coliform bacteria was over 90% in 1972. The level of resistance subsequently declined, but very slowly. Eight years after cessation of exposure to the tetracyclines and other antimicrobials, the level of tetracycline resistance was still 57%; by 1985, it had dropped to 30%. Those results certainly indicate that an early postwithdrawal "snapshot" should not be the basis for evaluation of the effects of discontinuation of antibiotic use.

Why did reversion to lower levels of antibiotic resistance in the coliform flora take so long in the experiments in swine just described? First, some strains (O-serotypes) of E. coli appear better constituted than others to persist and multiply in the colon, perhaps by virtue of specific plasmid carriage and particular surface antigens.[42] In piglets, the presence of the plasmid K88 confers adhesive properties on some strains of E. coli, facilitating attachment to brush borders of intestinal cells.[49] Similarly, in calves, a comparable plasmid, K99, is important in enteric disease.[113] In a situation where over 90% of coliform organisms start out with R-plasmid-mediated resistance to the tetracyclines (or other antimicrobials), strains with a selective advantage, such as the presence of colonization factors or nutrient sequestration, are preferentially retained. Thus, once R plasmids and transposons are extensively established in the coliform flora, they can be retained by selective advantages other than those provided by antibiotic use.

Second, persistence of R-plasmid-containing resistant strains might be facilitated by environmental factors. In the pigs that showed only very slow loss of antibiotic-resistant intestinal coliform bacteria over 13 years, the isolation of the experimental animals from other animals could have played a role in the slowness of replacement of such bacteria. The quarters of the animals were undoubtedly fouled initially with their own excreta, which contained resistant coliform bacteria. Reintroduction of the same bacterial flora from a constantly soiled environment must have occurred repeatedly over the years. Exposure of the animals to untreated animals whose intestinal flora contains coliform bacteria that colonize efficiently but are susceptible to antibiotics, might be required if the antibiotic-resistant strains are to be "diluted out" more rapidly.

Chickens fed subtherapeutic doses of oxytetracycline rapidly began to excrete an intestinal population in which over 90% of coliform bacteria were tetracycline-resistant; despite frequent cleaning of the cages, the chickens continued to excrete high concentrations of tetracycline-resistant organisms.[64] The resistance determinants might have been plasmids that had established stable relationships with the host bacteria. Insofar as conjugative plasmids help to mediate chromosomal gene recombination and genetic transfer among members of a population, population fitness, rather than individual bacterial fitness might be enhanced by these plasmids.[62] The proportion of resistant coliform bacteria decreased only when the chickens were mixed with other chickens excreting antibiotic-susceptible coliform bacteria or when they were moved to different cages.

Plasmids might acquire multiple resistance determinants when the host bacteria are exposed to a single drug in an animal's intestinal tract. The long-term use of tylosin, a macrolide antibiotic, as a feed additive in pigs resulted in the evolution of an intestinal streptococcal population that was multiple-drug-resistant.[14] Such multiple-drug-resistant plasmids appeared to differ from streptococcal plasmids present in control antibiotic-free pigs only in the presence of added resistance determinants.

The acquisition by conjugative plasmids of individual or multiple resistance transposons from smaller, nonconjugative plasmids under the influence of antibiotic exposure might facilitate subsequent dissemination of the resistance determinants. Whether persistence of antibiotic resistance in intestinal coliform bacteria after cessation of antibiotic exposure indicates the presence of resistance determinants cannot be known, but such persistence could be important in determining the results of cessation. If determinants are present, whether they are on conjugative or non-conjugative plasmids might affect the ultimate rate of spread of resistance. Although the level of resistance to tetracycline remained relatively high after discontinuation of the tetracyclines as feed additives in Great Britain, there was an observable decline.[111]

The prevalence of antimicrobial resistance to one or more drugs in human isolates of salmonellae is 16-31% (8-22% resistance to tetracycline, 5-19% to ampicillin) (see Chapter V), and prevalence of resistance to ampicillin and tetracycline in human isolates of *E. coli* is 23-32% and 25-29%, respectively.[3] These data suggest that the dispersion of resistance genes might not yet have gone so far as to be irreversible.

SPECIFIC MECHANISMS OF RESISTANCE TO ANTIMICROBIAL DRUGS IN PATHOGENS FROM ANIMAL SOURCES

RATIONALE AND EFFICACY OF MOLECULAR GENETIC TECHNIQUES IN RELATING HUMAN BACTERIAL ISOLATES TO FARM ORIGINS

A pivotal issue concerning the potential spread of animal-borne pathogens to humans is the ability to determine that a given pathogen is "the same" in both locations. Because bacteria divide asexually, each parent gives rise to two daughter cells that, assuming no major acquisition or loss of genetic material (e.g., an R plasmid), would be genetically and biochemically identical with the parent. The daughter cells are "clonal" with respect to each other and to the parent. By definition, clonal bacteria have the same recent origin and are therefore identical or nearly identical genetically and biochemically. Clonality cannot be absolutely ensured in the field or in the laboratory, but its existence can be shown with statistical analytic procedures. In this analysis, two bacteria are labeled operationally clonal if the test comparison shows so many similarities that the probability that the bacteria examined are different approaches zero.

The demonstration of clonality, with some degree of statistical certainty, is necessary but not sufficient for proving that a given pathogen has been transferred from an animal source to humans (either through the food chain or by some other means). Also required is evidence that the pathogen in question is not normally a part of the human flora. With consistently pathogenic bacteria, that confounding factor, which would otherwise present substantial background noise, is not usually a problem. Modern epidemiologic techniques for investigating human outbreaks of infectious disease usually make it possible to determine whether a pathogen has entered the human environment from an outside source. The same techniques can be applied to each prior source in the linear chain of contagion, so that, assuming the rarity of a given pathogen in a given setting, the primary source can be determined with reasonable certainty.

The power of the epidemiologic approach rests squarely on the ability to prove clonality. A number of recent detailed reviews have examined the various methods available and have analyzed their resolving strength.[1,32,41,107,131] Other reviews have examined the evolutionary stability of bacterial chromosomes and plasmids, with the greatest attention focused on E. coli and Salmonella spp.[12,43,82,100,105,117,121,125,126] The consensus is that, for large classification projects involving hundreds of strains of a given species, simple, inexpensive techniques, such as isoenzyme analysis, are capable of yielding adequate

discrimination of strains.[1] This biochemical approach has been useful in following the evolution and dissemination of clones of a single species, such as E. coli.[107]

Many of the simple biochemical approaches are not useful for identification, because their discriminatory power is insufficient. When small numbers of isolates are to be identified the use of molecular genetic techniques to "fingerprint" plasmids with DNA-probe technology[122] or restriction-fragment-length polymorphism is extremely useful for exactly the reasons that make these techniques worthless for the large numbers of isolates from population studies gathered over several years. Most plasmids--especially R plasmids, because of their genetic plasticity--are unlikely to be invariant over time. Moreover, because of their conjugative nature, plasmids are spread horizontally between strains (and species) of different clonal origin. Those effects are minimal in short-term outbreaks, so identity of plasmid profiles, particularly in the background of substantial plasmid diversity, is strong evidence of clonality.

In some instances, the use of molecular genetic techniques to analyze plasmids has achieved great certainty of strain identification.[8,43,100,104,117] In situations in which either the bacteria have no plasmids or the plasmids are particularly stable (e.g., those needed to preserve strain virulence), the use of cloned, random chromosomal sequences as probes to identify clones of Salmonella spp. has been valuable in research settings.[125]

R PLASMIDS OF VEGETABLE ORIGIN AND ANIMAL ORIGIN AND THE EMERGENCE OF DRUG RESISTANCE

In evaluations of the origin of drug-resistant bacteria in humans, investigators have analyzed fecal bacterial isolates from meat-eaters and of vegetarians and have compared the flora for resistance. The frequency of drug-resistant bacteria in vegetarians is at least as high as, if not higher than, that in omnivores. No studies have used the molecular epidemiologic techniques described above to demonstrate the origin of the drug-resistant bacteria in vegetarians, but cross-contamination of vegetables, which have been shown to carry high numbers of bacteria,[18,99] presumably occurs in the environment. On the basis of the preceding discussion, increased drug resistance in the environment may be said to reflect the presence of increased amounts of substances with antibacterial activity, probably from any source.

EFFECTS OF RESISTANCE TO ANTIMICROBIAL DRUGS ON BACTERIAL VIRULENCE

Two mechanisms enable bacteria to change from a drug-susceptible to a drug-resistant phenotype: mutation of chromosomal genes or acquisition of new genetic information, typically in the form of R plasmids. The former often involves the alteration of a target of the antibiotic (e.g., the S12 ribosomal protein and streptomycin or the RNA polymerase and rifampin). The antibiotic target is usually required to perform a vital vegetative function of the cell, so the effect of the mutation must be limited specifically to the antibiotic-binding domain. Occasionally, point mutations have pleiotropic effects and can subtly or profoundly alter the ability of the organisms to exist in their environment. Insofar as those vegetative functions are required for the infective stage of the pathogen, the virulence of the pathogen will naturally be attenuated.

Perhaps in part for that reason, nearly all drug resistance in bacteria, particularly in pathogenic species, is due to R-plasmid acquisition, as reviewed above. Most drug-resistance genes act either by inactivating an antibiotic or by excluding it from its target site.[22,23,29,87] As opposed to the mutation of chromosomal genes, the acquisition of new genes (on the R plasmid) might be expected to have little if any effect on bacterial virulence. Presumably, no vegetative function has been altered. Any pleiotropic consequences to the bacterial cell might be expected to be limited to the effect of the small additional burden of replicating new genes and synthesizing new gene products. To balance that theoretical deleterious effect on cell physiology, it should be pointed out that R plasmids can carry genes in addition to those essential for drug resistance. Thus, the selection of R plasmids that also contain toxin genes or other virulence factors would indirectly select for the acquisition of new virulence factors.[25,28,31,37,39,40,70,71,73,77,85,130]

Two approaches have been used to analyze the effects of R-plasmid acquisition on the virulence of a bacterial strain. Some investigators have begun with a strain known to be virulent in an experimental infection model, genetically transferred a given R plasmid into the strain, and then compared the parent and the recipient for virulence in the model.[5,10,19,50,54,85,112,123] Others have surveyed isolates in the field (e.g., a hospital setting), determined which strains were drug-susceptible and which drug-resistant, and then evaluated the amount of disease that the two classes of bacterial strains were capable of producing.[44,47]

Studies comparing bacterial strains that differ only in whether they contain an R plasmid have generally revealed little difference in virulence, although a few exceptions are

notable. Two early reports of the effects of an R plasmid on S. typhimurium[112,123] showed decreased virulence in the strain that had acquired the R plasmid by conjugative transfer. The molecular basis of those results was not pursued; but they might be explained by more recent studies that have shown many clinical isolates to be relatively poor recipients in conjugative crosses. The efficiency of mating, though, can be overcome with a mutation in the smooth lipopolysaccharide (LPS), which concomitantly reduces bacterial virulence.[72] A single mutation is sufficient for that effect, so a single back-mutation to the virulent wild type readily appears in an appropriate setting, such as the animal gastrointestinal tract.

The consequences of R-plasmid acquisition can be dramatic, in contrast, when the R plasmid contains, in addition to drug-resistance determinants and RTF, a virulence gene, such as enterotoxin[25,37,40] or hemolysin,[130] or when the R plasmid is capable of mobilizing[85] or recombining with[77] a virulence-encoding plasmid. Thus, in special cases in which R plasmids are linked with virulence genes, selection by antibacterial agents might promote spread of virulent strains. For example, aerobactin plasmids in E. coli strains isolated are commonly associated with resistance to one or more antimicrobials, such as the tetracyclines and ampicillin.[48] The broad epidemiologic studies suggest that R plasmids do not interfere with bacterial pathogenicity and might significantly increase the severity of disease. The best examples include cases of plasmid coinheritance of drug-resistance factors and various enterotoxins, as in clinical isolates of Staphylococcus aureus[73] and E. coli.[5,25,37,69] Even without specific evidence of a coinherited virulence factor, it is clear that drug-resistant bacteria are culprits in major outbreaks of disease.[47] A recent review examined 175 published and unpublished reports evaluating the effects of resistance to specific antibiotics on the outcome of bacterial infections in both community and nosocomial settings.[44] Regardless of the setting, the drug-resistant bacteria, compared with drug-susceptible bacteria, were found to be associated with illness that was significantly more severe; in particular, the mortality, the likelihood of hospitalization, and the length of hospitalization were at least twice as great. Further analysis determined that the underlying causes of the worse outcome in hospitalized patients infected with a variety of drug-resistant bacteria were twofold: drug resistance led to a high incidence of antibiotic failure, and drug-resistant pathogens emerged in the superinfection that followed prior antibiotic treatment for a different disease.

In summary, R plasmids have been shown repeatedly to increase the virulence of bacteria, both when specific mechanisms can be elucidated[28] and when they cannot.[44] The

few experimental situations in which reduced virulence after R-plasmid acquisition has been demonstrated are unlikely to reflect the importance of selective pressures in nature. Any environmental factors that select for spread of R plasmids in bacterial populations (such as use of antimicrobial drugs) are likely to coselect for spread of coinherited virulence factors.

THE SPREAD OF ANTIMICROBIAL-RESISTANCE GENES THROUGH BACTERIAL POPULATIONS IN STAGES

Bacteria live in the world not in pure culture, but as mixed strains and species competing in ecosystems, many of which are carried by animals or humans.[129]

Bacteria isolated from humans a half-century ago had resistance plasmids, but not the resistance genes found in comparable isolates today.[21] The use of each new antibacterial agent since than has commonly led eventually to the emergence and spread on plasmids of genes that encode resistance to the agent.[93] The recent appearance of new resistance genes and of older ones in pathogens that had been free of them indicates that the process of emergence and spread is continuing.[26,80,83,86]

We review here evidence that the use of antimicrobial agents advances the emergence and spread of antimicrobial-resistance genes through bacterial populations in discrete stages triggered by specific events than are increasingly identifiable.

A previously unknown resistance gene can emerge by becoming mobilized from an obscure strain or by evolving from an ancestral gene. Use of an antimicrobial agent could mobilize a resistance gene from an obscure strain by selecting for the strain's overgrowth and thus increasing its contact with other strains. Use would similarly favor evolution by selecting for increasingly resistant mutants of the ancestral gene. Mobilization and evolution would each be likely to progress through stages, and some resistance genes might progress through stages of both.[36,127]

Broad-spectrum β-lactamases able to hydrolyze newer β-lactam antibiotics, for example, have recently emerged apparently by two-step mutations of β-lactamases (SHV-1 or TEM-2) that had emerged earlier.[52,116] Use of the older β-lactams had presumably mobilized the older resistance genes to stages of sufficient prevalence for their very rare mutants that were resistant to the new β-lactams to occur often for selection by use of the new agents and thus the beginning of the new stage.

Antibacterial agents can affect bacterial populations directly only by inhibiting susceptible strains. A bacterial ecosystem so depleted by an antimicrobial agent can be

repopulated by the progeny of a single bacterium that has a gene that encodes resistance to the agent. Overgrowth of a clone in the face of such selection can generate overnight a billionfold amplification of the number of copies of the resistance gene. That amplification might be transitory, however, because the isogeneic bacteria that carry these copies might prove less fit for the diverse niches of the ecosystem than would their heterogenic predecessors or successors.

SIGNIFICANCE OF PLASMIDS

Once inserted on a plasmid, a resistance gene can replicate and persist in the niches of all the strains to which the plasmid can be transferred. Recombination by various mechanisms, such as transposition or site-specific recombination, moveover, can move the resistance gene to other plasmids capable of transfer to additional strains and thus to additional niches, beyond the host range of the first plasmid.[6,75]

LINKAGE AND DISSEMINATION OF RESISTANCE GENES

Recombination of a resistance gene with additional plasmids and coresidence of the recombinant plasmids with additional plasmids and chromosomes in new strains would also tend to link or associate the resistance gene with other genes in the presence of different selection. Such other genes would include other resistance genes in the presence of selection by other antimicrobials, other adaptive genes on chromosomes, and genes that were maintained on plasmids and had unknown survival values before antibacterials began to be used.[21,109] Selection for any of these would preserve or amplify the resistance gene in the absence of the agents to which it encodes resistance.

Spread of plague and cholera across continents showed long ago that the world's bacterial ecosystems interconnect.[38] Accordingly, dissemination of an emerged resistance gene through them would await only sufficient amplification, recombination and transfer of the gene to get it into intercolonizing strains. Studies of resistance-gene phenotypes and more recently of their nucleotide sequences are, in fact, revealing few examples of parochial resistance genes confined to one area, and those examples may prove to have been premature.[46,79]

In recent years, moreover, new technology has made possible the detection, not just of distinctive resistance genes, but of distinctive plasmids carrying them that have

become widely distributed among bacterial genera, animal and human hosts, and widely separated geographic areas.

Two small multicopy plasmids that encode resistance to sulfonamide and streptomycin and that share homology over half their length were found in escherichia and salmonellae from animals and humans in many parts of the world.[4,128] Three distinctive multiple-resistant plasmids were found in collections of salmonella isolates from both animals and humans in the United States; one of them was endemic in cattle in 20 states and sporadic in humans in at least two other states.[95] A plasmid in an isolate from Venezuela was found also to carry one of the earliest gentamicin resistance genes in seven genera of enterobacteriaceae from eight widely separated medical centers in the United States.[94] A plasmid that encodes resistance to trimethoprim and other agents was in isolates of E. coli from pigs on a farm and from patients in a medical center 40 miles away.[13]

It has become possible to recognize identical or closely related specific transposons in plasmids from bacteria isolated in different parts of the world.[101,118] One large (20-kilobase) transposon, Tn 21, has been found in plasmids from some of the earliest multiple-resistance shigellae isolated in Japan and in a remarkable number of different-looking plasmids from other genera in different parts of the world. They include nearly all of the varied plasmids that first brought gentamicin resistance to 20 medical centers in Germany and a number of plasmids from various locations that carry different β-lactamase genes.[61,106]

A lineage has been proposed for the evolution of Tn 21 and its progressive acquisition of different resistance genes.[120] Tn 21 appears to have specific recombination sites that permit exchange of new resistance genes between its progeny.[11] If Tn 21 continues to be found in additional resistance plasmids, it will raise the possibility that resistance might have progressed in the world quite differently if it had not evolved.

SPREAD OF RESISTANCE GENES IN STAGES

The preceding observations indicate that resistance genes emerge and spread in successive stages.[90] The initial mobilization or evolution of the gene, insertion into a plasmid, transfer to strains within the plasmid host range, recombination with other plasmids, and insertion into other transposons and consequent linkage with other genes in the presence of different antibiotic selection, as well as the parallel evolution of the plasmids and transposons themselves (e.g., Tn 21) toward fitness in more niches, are all discrete steps in the dissemination of the gene.

Each of the stages sketched above can be seen to have the qualities that characterize the steps in an enzymatic cascade: amplification and irreversibility. At any stage, a given amount of antibacterial use would select more copies of the resistance gene than it would have at the preceding stage. Similarly, the use needed to maintain a new stage would be less than the threshold amount needed to initiate it.[76]

Each new stage begins with a mutation, a chance encounter of rare strains in vast populations, a conjugative transfer, or a recombinational event. Each such event has a low probability of occurrence, and the ultimate prevalence of a resistance gene depends on the product of the probabilities of the events that inaugurate each of its stages of spread.

We know, for example, that recombination of DNA segments that encode the TEM-1 β-lactamase with plasmids resident in strains of Hemophilus influenzae and Neisseria gonorrheae was the final step in the disastrous emergence of resistance to penicillins in these two pathogens 15 years ago. We can only guess, however, at the chain of preceding events that required 30 years of penicillin use before those final stages could occur.[9,133]

A resistance gene is an essential participant in each of the improbable events cited above, therefore, the chance that any of them will occur is a function of the prevalence of the resistance gene in the preceding stage.

EFFECT OF ANTIMICROBIAL USE ON SPREAD OF RESISTANCE GENES

Because use of antimicrobial agents can greatly amplify the prevalence of genes that encode resistance to them at each stage, such use can be seen as the main force driving the progression of resistance genes through their stages of spread.

If resistant bacteria arose only through frequent single-stage mutation of susceptible strains, as was once thought the genesis of a resistant strain isolated from any patient might be due entirely to antimicrobial use in that patient or a neighbor. If, however, the resistance in each such isolate is encoded on a complex genetic element that has been assembled in sequential stages, each triggered by a rare event, the lineage of that resistance might be traced back to almost anywhere. Antimicrobial use by the patient might have produced overgrowth and manifestation of the resistant strain in that patient, but the evolution of the strain's resistance genome and its spread to the patient required much greater use elsewhere.

The emerging connection between the molecular evolution of the genomes that carry a resistance gene and its stage of

spread suggests that even very little interchange between bacterial populations might be sufficient for wide dispersion through them of a resistance gene that had been selected to an advanced stage by intensive antimicrobial use in the first population. The analogy would be to a virus that evolves to high contagiousness on one continent and becomes epidemic on a second continent when taken there by a single tourist.

The growing information reviewed above is enough to show that there is a global epidemiology of antimicrobial-resistance genes and genomes, but not enough to measure them. A range of models can be considered.

At one extreme, a model would propose that antimicrobial use in bacterial populations in one region would have no effect on later resistance-gene prevalence in distant bacterial populations. At the opposite extreme is a model that proposes that the prevalence of resistance genes in a bacterial population is a function of total antibacterial use in all bacterial populations, however remote.

Neither of those extreme models appears to be appropriate. All the above evidence indicates some effect of use in one population on prevalence of resistance in others. For a more specific example, we have good reason to believe that current prevalence of penicillinase-producing Neisseria gonorrheae (PPNG) in a number of American cities is a consequence of antibiotic use in areas of the Far East 15 years ago.[29]

There appears to be (see Chapter V) gradients of prevalence of resistance from high to moderate to low as one moves from populations of intense to moderate to no use of antimicrobials. The critical question is: How much greater is the prevalence of resistance (and how much sooner is it attained) in populations of moderate or no use than would be the case if there had been no (or fewer) populations of intense use.

IMPLICATIONS FOR ANTIMICROBIAL USE

The models indicate the potential effect of the use of antimicrobial agents in animal bacterial populations on the prevalence of resistance genes in human bacterial populations. Nearly half the antimicrobial use in the United States is in animals. The pool of resistance genes in animal flora in the United States may be estimated (see Chapter V) to be 10 times that in the total human flora. Animal bacterial populations are not distant from human bacterial populations, in as much as continuous samples of the animal populations flow on slaughtered carcasses through food distribution chains to most households in the United States.

Although recent work with new technology has elaborated many of the observations presented above, the global effect

of antibacterial use on bacterial resistance has long been grasped intuitively by workers studying resistance in the laboratory and by clinicians coping with resistance in their patients.[29,134] Many have urged that antimicrobial use be minimized whenever possible and have identified use of antimicrobials in animal feed as the largest use category that could be reduced in the United States.[67]

Their concerns and recommendations, however, were based on indirect evidence. The complexity of the processes outlined above and the hugeness of the bacterial populations in which they operate had precluded reconstructing the entire chain of use that led to the presence of a particular resistance gene in the isolate from a particular patient.[68]

DUAL ROLES OF SALMONELLAE AS PATHOGENS AND TRACERS

The first direct evidence that antimicrobial use in animals led to resistance in bacteria in humans came from an epidemic of multiple-resistant salmonellae among calves in Britain, apparently augmented by the antimicrobial use, that spread to infect humans. It became the occasion to enact legislation banning routine addition of antibiotics to animal feed in Britain and countries of the European Common Market (see Chapter V).[2]

Some observers felt that the epidemic in Britain had been exceptional, ascribable to peculiar husbandry practices there, and not pertinent for the United States.[51] When the National Research Council Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds met in 1980, it could still be argued that in the United States animal and human bacterial resistance genes were in separate pools that did not interchange.[88] The committee judged that hazard to human health associated with the subtherapeutic use of antimicrobials in animal feeds was neither proved nor disproved.[88]

A report published two years later found three different examples in which isolates of salmonellae from animals and humans in the United States carried resistance plasmids that had the same distinctive restriction endonuclease fragments. Several later studies used the same method to trace spread of resistant strains of salmonellae from animals to humans in recognized food-borne outbreaks in the United States (see Chapter V).

All the direct evidence comes from salmonellae, because they are peculiarly traceable. Relatively rare in human flora, they signal their presence by producing a conspicuous illness. Laboratories routinely find them with selective media even when they are sparse in stool flora and send them to reference laboratories, where their serotypes are discriminated from more than a 1,000 possible types. More

than 40,000 strains are winnowed out of human flora in the United States by that elaborate system each year, and their serotypes are recorded. A parallel system exists for salmonellae isolates from animals.

Although their relative rarity in human flora helps to make salmonellae traceable, it also leaves them a very small part of the antimicrobial-resistance problem. It is probably reasonable to estimate that, of all the courses of antimicrobial therapy administered to humans in the United States, including all the expensive and sometimes toxic second and third generation agents used to circumvent resistance to older agents, much less than 1% are directed against infection by salmonellae.

DUAL LINES OF EVIDENCE OF FEED-ADDITIVE HAZARD

Two lines of evidence of hazard associated with antimicrobial use in animal feed additives have been developing in the last several years.

One line of evidence, as outlined here, arises from the growing understanding that the resistance genomes in bacterial isolates of humans are the products of extensive evolutionary development that required long exposure of vase bacterial populations to antibacterial agents. Where the populations might not be critical, given the growing evidence of their interconnection. Which antibacterials were used might also not be critical, given the close linkages observed between resistance genes and the ease with which some plasmids, once made prevalent by use of an antimicrobial, appear to acquire new resistance genes by site-specific recombination.[97]

The second line of evidence arises from epidemiologic observations, supported by molecular fingerprinting of plasmids, of outbreaks and other surveys of salmonella infection in the United States in recent years. They provide valuable examples for the first line of evidence, but also direct evidence of the effect of antibacterial use in animals on the outcome of specific types of human infection.

The first line of evidence addresses all resistance in human bacterial flora, but still cannot yield enough direct evidence for development of a quantitative risk assessment model. The second line of evidence addresses a subset of human bacterial infections, but can yield direct evidence for a risk assessment model, as developed in Chapter VII.

SUMMARY OF THE BIOLOGIC IMPACT OF DRUG RESISTANCE

Although present at low incidence in the preantibiotic era, drug resistance has burgeoned since the wide use of

antimicrobial drugs began 4 decades ago. Most drug resistance is due to the presence of transposable genetic elements, called transposons, that are found on R plasmids, which are usually conjugative. Because of the flexibility of this mechanism of genetic expression, bacteria can acquire resistance to a given antimicrobial agent quite easily and at minimal cost in terms of growth rate and general hardiness. Because of the ability of R plasmids to add additional transposons in modular fashion, the conjugative acquisition of an R plasmid typically confers drug-resistance phenotypes in addition to that specifically selected by the presence of antibiotic in the environment.

R factors make a direct contribution to the ability of bacteria to cause disease. Unfortunately, however, R factors occasionally carry virulence factors as well. The mere presence of the drug-resistance phenotype has been shown, in epidemiologic surveys, to increase severity of illness. The mechanism of the latter is twofold: antimicrobial resistance leads to delays in the selection of an appropriate therapeutic agent and permits the differential outgrowth of the resistant organism during antimicrobial therapy or some other infection or presumed infection.

With greater understanding of the molecular biology of drug resistance, R-plasmid passage and strain dissemination have become better understood as well. R plasmids are genetically plastic and in many cases are transmissible to a wide variety of strains and species. Therefore, the outbreak of disease due to a clonally expanded strain can often be followed with great precision by following its plasmid molecular profile, as deduced from restriction fragment-length polymorphism. With this sophisticated technology, R-plasmid-containing strains of salmonella have been traced unequivocally from the farm through the food chain to clusters of human patients. Outbreaks have provided strong evidence of the deleterious role that antimicrobial use in the environment has played in the selection and propagation of antimicrobial resistant bacterial pathogens of humans.

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IV

QUANTITATION OF ANTIBACTERIAL AGENTS USED IN LIVESTOCK AND POULTRY FEEDS

Exposing large numbers of bacteria to an antibacterial agent at concentrations that inhibit growth is the most effective means of selecting antimicrobial-resistant strains (or species, if the initial population comprises a mixture of species). The concentration of the antimicrobial chemical to which bacteria are exposed determines both the number of resistant organisms that may be isolated initially and the magnitude of resistance to that antimicrobial chemical. The duration of exposure to an antimicrobial compound has an important role in the elimination of susceptible strains or species in mixed populations, e.g., in the lower gastrointestinal tracts of humans and animals.

Antibacterials are used in livestock and poultry in several dosages: at high (therapeutic) concentrations to treat established infectious diseases and at low (subtherapeutic) concentrations for enhancement of growth and for disease prevention (prophylaxis). The Food and Drug Administration (FDA) approves separately each label claim for efficacy for each dose and animal species. The Feed Additive Compendium summarizes all FDA-approved feed additives, label claims, doses, animal types, and federal regulations. It is estimated that approximately 75% of dairy calves, 60% of beef cattle, 75% of swine, and 80% of the poultry marketed have received one or more antimicrobial drugs in their feed at some time.[1,2]

Twenty antimicrobial drugs have been approved by FDA as feed additives (Table IV-1). Penicillin and chlortetracycline were approved in 1951 and oxytetracycline in 1953. Nonantibiotic antimicrobial drugs, such as nitrofurans and sulfonamides, are used in the same fashion. In addition, ionophores, such as monensin, which have little antibacterial activity, are extensively used as coccidiostats in the poultry industry. Combinations of antimicrobials such as chlortetracycline and hygromycin or chlortetracycline, penicillin, and sulfathiazole--are approved as feed additives.

POPULATION OF LIVESTOCK AND POULTRY

It is useful to know the population of each species of animal for calculating the amounts of penicillin or the

TABLE IV-1

COMMON ANTIBACTERIAL FEED ADDITIVES
APPROVED BY U.S. FOOD AND DRUG ADMINISTRATION

Additive	Year Approved
Sulfaquinoxaline (sulfonamide)	1947
Roxarsone (arsenical)	1951
Chlortetracycline	1951
Penicillin	1951
Bacitracin	1953
Sodium arsenate (arsenical)	1953
Furazolidone	1953
Oxytetracycline	1953
Erythromycin	1955
Hygromycin B	1957
Neomycin	a
Novobiocin	1961
Tylosin	1961
Sulfamethazine[b]	1963
Sulfamerazine[c]	1967
Oleandomycin	1968
Lincomycin	1970
Sulfathiazole[b]	1971
Bambermycin	1973
Virginiamycin	1974

Source: U.S. Food and Drug Administration, Division of Animal Feeds, Center for Veterinary Medicine, December 1987 (personal communication).

[a] Never officially approved; has been marketed since before 1958.

[b] For use in combination only.

[c] For use in fish.

tetracyclines used to medicate them. The food-animal population in the United States is very large--more than 20 times the human population (Table IV-2). In 1971 and 1985, for example, the total U.S. food-animal population was 3,522 and 5,122 million head, respectively (Table IV-2). The number of head of livestock (exclusive of poultry) for the same 2 years was 237 and 206 million. In the intervening 14 years, production of red meat declined somewhat, and poultry production increased. The relationship of the amount of red- to white-meat food produced is important in considering the magnitude of human exposure to meat or poultry products contaminated with pathogenic bacteria of farm-animal origin. An understanding of the magnitude can be had by inspection of per-capita consumption figures for meat and poultry in this country. The consumption of red meat per capita ranged from a high of 168 lb in 1971 to a low of 153.2 lb in 1985. In the same period, the amount of poultry consumed increased from 49.0 to 69.7 lb.[6]

ANIMAL CONSUMPTION OF FEED AND BACTERIAL CONTENT OF GASTROINTESTINAL WASTE

To understand the number of coliform organisms potentially exposed to antimicrobials in animal feed, one need only examine Table IV-3, which shows the large amounts of feed consumed per head of livestock or poultry annually. (The amounts of medicated feed consumed are discussed later.) Multiplying the amounts of feed by numbers of animals in production in the United States (Table IV-2) yields an estimate of the large amounts of manure produced. Animal manure is a direct source and a vector of bacterial contamination of the farm, of farm animals, of forage crops and feed, of farmers, of slaughterhouse workers, and of meat or food by products (such as milk and eggs) consumed by humans.[3] The spread of bacteria can be enhanced by the coprophagic habits of some animal species. As an example of the magnitude of the potential bacterial contamination problem associated with farm and feedlot exposure of animals, the numbers of coliform organisms and the large amount of manure produced by beef cattle can be cited. A single 900-lb feedlot steer produces about 9 lb (4.1 kg) of manure solids daily; each gram contains approximately 10^7 coliform organisms, for a total of 4.14×10^{10} such organisms per day.[5]

TOTAL ANTIBIOTIC PRODUCTION

Accurate analysis of the impact of antimicrobials on farm animals (on their infectious bacterial flora, health, or

TABLE IV-2

ANIMAL PRODUCTION IN UNITED STATES
(Thousands of Head Marketed or Produced for Marketing)

Year	Cattle	Calves	Sheep	Lambs	Chickens	Broilers	Turkeys	Hogs	Hogs/pigs[a]
1987	49,900	10,564	-	-	-	-	-	-	-
1986	49,995	10,477	-	-	216,938	4,646,312	207,216	-	-
1985	48,739	10,488	1,610	6,456	251,957	4,478,749	185,282	86,583	52,298
1984	50,682	10,253	1,821	7,007	234,769	4,282,391	171,296	87,344	54,073
1983	48,089	10,443	1,820	7,104	231,821	4,183,660	170,723	89,129	56,694
1981	46,647	10,383	1,510	7,013	223,721	4,147,521	170,875	95,986	58,698
1979	48,358	10,151	1,347	6,336	225,066	3,951,291	156,457	92,499	67,318
1977	56,378	12,621	1,450	7,361	235,856	3,939,897	136,890	80,939	56,539
1975	54,315	12,239	1,812	9,039	238,576	2,950,099	124,165	73,627	49,267
1973	48,369	11,652	2,198	10,879	236,710	3,008,667	132,231	82,410	60,614
1971	49,143	12,086	2,202	12,627	220,195	2,945,348	119,657	98,644	62,412

Source: U.S. Department of Agriculture.[6a]

[a] Combination of pigs and overweight market hogs.

TABLE IV-3

FEED CONSUMED BY LIVESTOCK AND POULTRY 1971-1983
(Feed Consumed Per Head, Pounds, Equivalent Feeding Value of Corn)

Year (Beginning October)	Dairy Cattle		Beef Cattle[a]	Sheep and Lambs	Poultry				Hogs (per 100 lb)
	Milk Cows	Other			Hens and Pullets	Chickens	Broilers	Turkeys	
1971	11,370	5,859	9,198	1,008	99	32	9.3	96	954
1973	11,570	5,774	7,708	2,021	100	32	9.7	94	660
1975	11,540	6,303	7,593	1,260	101	26	7.7	71	566
1977	12,129	8,864	6,524	1,442	103	24	8.6	79	633
1979	12,978	6,800	8,122	1,373	107	26	9.4	76	688
1981	12,181	6,248	8,759	1,409	108	26	10.0	84	620
1983	12,648	5,795	7,848	1,723	105	28	9.3	82	605

Source: Adapted from U.S. Department of Agriculture.[6a]

[a] Feed consumed divided by the number of cattle on feed January 1.

rates of growth) requires reliable data on the total amounts of penicillin and the tetracyclines used annually in animals (as feed additives, for growth promotion, for prophylaxis, and for treatment of infections) and medical use in humans. The same information on other common feed-additive drugs would be useful for purposes of comparison, but such detailed information is not available. Although gross estimates of production and use have been made, there is not good agreement between the figures from different sources. However, the figures that are available to the committee provide a general basis for estimating the breadth of use of antibiotics in animals reared for human food.

The U.S. International Trade Commission provides information on the annual production of antibiotics in the United States (Table IV-4). The data show total pounds produced for medicinal and nonmedicinal use in humans and animals. From 1950 to 1986, the total annual antibiotic production in the U.S. increased by a factor of about 49 (from 0.9 to 44.4 million pounds). Production figures for 1986 show an atypical annual increase (39%) over the preceding year and may represent an aberration. However, the figure for 1985 (31.9 million pounds) is within the range for the previous 4 years (30.4-32.5 million pounds) and represents an increase by a factor of about 35 over production in 1950.

The data in Table IV-4 show that the percentage of total production directed to animal feed and other uses increased from 16% in 1951 to 38% in 1959. In the 1960s, an average of about 40% of the total antibiotic production was directed to animal feed and other uses. By the late 1970s, 42-48% of antibiotic production was directed to animal feed and other uses. Although information on the actual amount of antimicrobial production used in animal feed rather than other use is not available, an assumption can be based on information presented later in this chapter--that the nonmedicinal represented the predominant use. In Table IV-5, for 1981-1986, the total production figures are available for classes of antimicrobials, but a direct breakdown into medicinal and nonmedicinal uses is not available. Although Tables IV-4 and IV-5 are not directly comparable, they do show that large amounts of antimicrobials were used as feed additives. In 1983, for example, 31.9 million pounds of antibiotics was produced, of which 22.5 million pounds (71%) was tetracyclines plus other non-β-lactam antibiotics. In the same year, 75% of the antibiotics (other than penicillins, cephalosporins and tetracyclines) were directed to feed additive and other uses (Table IV-4). Thus, 36% of the entire antibiotic production for 1983 consisted of antibiotics (other than β-lactams and tetracyclines) that were directed to feed additive and other uses.[7] Considerable amounts of tetracycline and penicillin go into animal feed,

TABLE IV-4

U.S. ANTIBIOTIC PRODUCTION 1950-1986[a]

	Total		Medicinal Use	Nonmedicinal Use		
Year	U.S. Production, 10^6 lb	Annual Change, %	In Humans and Animals, 10^6 lb	Added to Animal Feed and for Other Uses, 10^6 lb	Annual Change, %	Portion of Antibiotic Production Added to Animal Feed and Other Uses, %
1986	44.4	+39	b	b	b	b
1985	31.9	+ 5	b	b	b	b
1984	30.4	- 5	b	b	b	b
1983	31.9	- 2	b	b	b	b
1982	32.5	+ 6	b	b	b	b
1981	30.6	+24	b	b	b	b
1980	24.6	- 2	b	b	b	b
1979	25.2	- 2	14.6	10.7	-13	42
1978	25.7	+11	13.4	12.3	+22	48
1977	23.1	+13	14.0	10.1	+ 1	43
1976	20.5	+12	10.4	10.0	+12	48
1975	18.3	-11	9.4	8.9	+20	48
1974	20.5	- 1	13.2	7.4	-10	36
1973	20.8	+25	12.6	8.2	+21	39
1972	16.6	- 7	9.8	6.8	- 4	41
1971	17.9	+ 6	10.8	7.1	- 3	40
1970	16.9	+28	9.6	7.3	+26	43
1969	13.2	+28	7.4	5.8	+35	43
1968	10.3	+ 8	6.0	4.3	+ 2	42
1967	9.5	- 2	5.2	4.2	0	45
1966	9.7	29	5.4	4.2	+50	43
1965	7.5	+15	4.7	2.8	+ 8	37
1964	6.5	- 3	3.9	2.6	+ 4	40
1963	6.7	+ 6	4.2	2.5	+ 9	37
1962	6.3	+24	4.0	2.3	+28	36
1961	5.1	+ 9	3.3	1.8	+ 6	35
1960	4.7	+27	3.0	1.7	+21	36
1959	3.7	+ 6	2.3	1.4	+56	38
1958	3.5	+ 9	2.6	0.9	0	26
1957	3.2	+19	2.4	0.9	+12	26
1956	2.7	+29	2.0	0.8	+60	28
1955	2.1	- 9	1.6	0.5	0	25
1954	2.3	+10	1.8	0.5	+25	21
1953	2.1	+24	1.6	0.4	+33	21
1952	1.7	+13	1.5	0.3	+50	15
1951	1.5	+67	1.3	0.2	-	16
1950	0.9	-	0.9	b	-	-

TABLE IV-4 FOOTNOTES

a 1950-1978 data from National Research Council (NRC) report, The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds[4a] (NRC data is based on reports of the U.S. International Trade Commission [ITC][7]) 1979-1986 data culled from ITC reports[7] (animal-feed antibiotic data breakdown not presented by ITC for 1980-1986).

Amounts of sulfonamides produced not included. Only chemicals included in ITC production figures; not included are finished pharmaceutical preparations and products in form of pills, tablets, and capsules.

In ITC data, amounts of antibiotics produced differ from reported amounts sold; e.g., in 1986, 44.4 million pounds of antibiotics produced and 11.3 million pounds sold. Difference between reported production and sales attributed to inventory changes, losses in processing, and captive consumption converted into ethical and proprietary pharmaceutical products by primary manufacturer. Many pharmaceutical manufacturers not included in ITC reports if not primary producers of medicinal chemical (i.e., their drug requirements are met by purchases from U.S. or foreign producers).

Amounts of antibiotics produced annually assumed to provide better estimate of amounts used than ITC estimates of amounts sold, because finished dosage-form products made under captive use not included in estimates of amounts sold.

b Figures not available.

TABLE IV-5

U.S. PRODUCTION OF INDIVIDUAL CLASSES OF ANTIBIOTICS, 1981-1986

	Production, Millions of Pounds					
	1981	1982	1983	1984	1985	1986
Cephalosporins	1.1	1.1	1.4	1.4	a	1.7
Penicillins	7.4[b]	7.4[b]	6.1[b]	a	6.8[c]	7.7[c]
Semisynthetic penicillins[d]	2.3	2.1	1.9	2.0	-	-
Tetracyclines	6.8	7.2	7.2	a	a	a
Others	13.0	14.7	15.3	27.0	25.1[e]	35.0[f]
Total sulfonamides	3.9	3.1	2.8	a	a	a
Total	30.6	32.5	31.9	30.4	31.9	44.4

Source: Adapted by the committee from U.S. International Trade Commission (ITC) Reports.[7]

[a] Figures not available.

[b] Penicillins other than semisynthetic penicillins.

[c] Includes all penicillins.

[d] Includes ampicillin, amoxicillin, dicloxacillin, cloxacillin, and oxacillin.

[e] Includes tetracyclines and cephalosporins.

[f] Includes tetracyclines.

so it is clear that, on the basis of production data, the true figure for the percentage of antibiotic production going into feed additives probably lies somewhere between 42% and 48% (Table IV-4).

Penicillins and tetracyclines together make up 42% of the total 1983 antibiotic production. Of the other antibiotics, which account for 58% of the total production for the same year, only a few are approved for use as feed additives (see Table IV-1). Total sulfonamide production of 2.8 million pounds in 1983 was not included in the previously cited antimicrobial-use data. Information is not available as to what portion of sulfonamide production was directed to animal feed uses.

Although precise figures on the amounts of individual antibiotics used in feed are not available from the ITC data, it appears that a large percentage (42-48%) of the total antibiotic production in the United States is used in animal feed; penicillins and tetracyclines represent a sizable fraction thereof. FDA, in using 1979 data from ITC, has estimated that approximately 55-60% of the penicillin and tetracycline consumed in the United States is used in animal feed in subtherapeutic dosages; an additional percentage is used for therapeutic purposes (FDA, Personal Correspondence, Office of Planning and Evaluation, 21 May 1986).

ANTIBIOTIC USE IN FARMS AND FEEDLOTS

Actual percentages of each type of antibiotic used in farms and feedlots would be more informative than the foregoing calculations of total antibiotics used.

A summary analysis of amounts used in farms and feedlots for the period 1980-1985 has been attempted (see Table IV-6) with data from IMA America, Ltd., private organizations (on purchases of feed antibacterials by feed manufacturers, livestock supply stores and distributors, etc.), the Animal Health Institute (AHI), ITC, researchers, trade associations, and the companies that manufacture drugs for use in animals. The figures in Table IV-6, the best available to this committee, show little variation in this period in total feed use (amount sold to the feed trade) of antibacterials: 9.7-11.7 million lbs/yr. Tetracycline accounted for 57% of this volume in 1980 and 49% in 1984 and 1985. Penicillin accounted for only 5-8% of the total. The total use of tetracyclines and penicillin in feed in this period gradually declined. The foregoing figures on annual sales of antibacterials for livestock and poultry feeds can be related to the total annual production of all antibiotics (for all uses) and to the total annual production of the tetracyclines and penicillin. For example, in 1983, 31.9 million pounds of antibiotics was produced and 9.9 million pounds was sold to

TABLE IV-6

ANNUAL SALES OF ANTIBACTERIALS FOR LIVESTOCK AND POULTRY FEEDS, 1980-1985

Year	Production for all Uses[a]	Annual Sales for Livestock and Poultry Feeds											
		Total			Tetracyclines			Penicillin			Others		
	10^6 lb	10^6 lb	10^6 kg	%[b]	10^6 lb	10^6 kg	%[c]	10^6 lb	10^6 kg	%[c]	10^6 lb	10^6 kg	%[c]
1980	24.6	11.2	5.1	46	6.4	2.9	57	0.9	0.4	8	3.9	1.8	35
1981	30.6	9.7	4.4	32	5.2	2.4	54	0.7	0.3	7	3.8	1.7	39
1982	32.5	10.8	4.9	33	5.3	2.4	49	0.7	0.3	6	4.9	2.2	45
1983	31.9	9.9	4.5	31	5.1	2.3	52	0.6	0.3	6	4.2	1.9	42
1984	30.4	11.7	5.3	38	5.7	2.6	49	0.7	0.3	6	5.3	2.4	45
1985	31.9	11.0	5.0	34	5.4	2.4	49	0.6	0.3	5	5.1	2.3	46

Source: Modified from a presentation (unpublished) to the committee by H. W. Jamison of the Animal Health Institute's Antibacterial Research Criteria Task Force, March 21, 1988, entitled "Estimating the Volume of Antibacterials Used in Livestock and Poultry Feeds."

[a] From Table IV-4.

[b] Percent of total antibiotic production.

[c] Percent of total annual sales for livestock and poultry feed.

the feed trade for use in livestock and poultry feeds. Tetracyclines and penicillin accounted for 58% of the 9.9 million pounds. In the same year, of the 7.2 million pounds of tetracyclines produced, 5.1 million pounds (71%) was sold for use in livestock and poultry feeds (Tables IV-5 and IV-6).

SUBTHERAPEUTIC USE OF ANTIBACTERIAL DRUGS IN ANIMAL FEED

The current FDA-approved uses of the tetracyclines and penicillin in animal feeds are for growth enhancement, disease prevention, and treatment of disease. In the first two of these categories (commonly designated "subtherapeutic"), these antibiotics are used at 200 grams or less per ton for 2 weeks or more.[4] Some combinations with other antibacterials (tetracycline at 100 g/ton [g/ton = grams drug per ton of feed], sulfamethazine at 100 g/ton, and penicillin at 50 g/ton) have been approved by FDA for use in pigs that weigh up to 75 lbs. The Center for Veterinary Medicine (see Footnote #1, page 1 of Preface) considers any extended use of antibiotics in feed at 200 g/ton or less beyond 2 weeks as "subtherapeutic use," whether it is for growth enhancement or disease prevention. "Use levels are generally 200 g/ton or less of penicillin or tetracycline, but dosage units will vary by species. Levels approved for claims of growth promotion and disease prophylaxis are usually lower than those approved for disease treatment; however, there is some overlap in the claims for dose levels of 200 g/ton or less." There is more concern in the agency with the length of time the antibiotic is used in feed than in the level of drug. (FDA, personal correspondence, April 26, 1988.) Although the need for such information is critical, there are no actual data (only estimates) on the amounts of penicillin or tetracycline used subtherapeutically in animal feed.

Experts in veterinary medicine from the Animal Health Institute and elsewhere testified to this committee that use of the prophylactic dosage of antibacterial agents is common and important for successful and profitable rearing of livestock. It appears that hog farmers use antibacterial feed additives rather consistently during the early periods in raising swine. That involves such use of antibacterial additives for about 10 weeks in feeder pigs (approximately one half of their life span), divided into several stages. The first stage often consists of the tetracycline-sulfamethazine-penicillin combination or a tylosin-sulfamethazine combination. After that stage, tetracycline (25-50 g/ton), tylosin alone, bacitracin, or bambermycin is used. In cattle it is common to feed a tetracycline-sulfamethazine combination per head (350 mg of each drug to

each animal) per day for 4-6 weeks as the cattle go into feedlots. The intent of such administration is primarily "prophylactic." Although penicillin and the tetracyclines are approved by FDA for use in several classes of poultry, they are not used commonly in broilers.

In its efforts to obtain broader and more up-to-date information about the use of antibiotics by the different animal industries, the committee made inquiries to the National Broiler Council (NBC), the Texas Cattle Feeders Association (TCFA), and the National Pork Producers Association (NPPA). NBC in 1984 surveyed 30 companies representing 77.8% of the industry's broiler production for that year. None of the companies reported using penicillin or the tetracyclines to increase rate of growth and feed efficiency. However, they did indicate that those antibiotics were widely used in disease prevention and treatment programs. Of the 30 companies surveyed, 18 (60%) reported using penicillin in disease prevention or treatment programs, 28 (93%) reported using chlortetracycline, 23 (77%) reported using oxytetracycline, and 10 (33%) reported using tetracycline. The use of the antibiotics for disease prevention is summarized in Table IV-7.

A survey by the TCFA in 1986 involved 102 feedyards that produce 75% of the feed cattle in the TCFA area (New Mexico, Oklahoma, Texas). Responses from nutritionists responsible for more than 11 million head of cattle (approximately 42% of all the feed cattle produced annually in the United States, assuming a total feed cattle production of 26 million a year) indicated that none of the animals receives continuous low-dose tetracyclines in feed (penicillin is not approved for such use). However, the survey did not address the use of subtherapeutic concentrations for disease prevention.

The NPPA has not conducted a recent survey on the use of penicillin or tetracyclines in swine feed. However, Virgil Hays (personal communication, 1988) has calculated the amount of tetracycline used in swine feed for the committee (Table IV-8). According to U.S. Department of Agriculture figures, approximately 86.5 million pigs (see Table IV-2) with an average weight of 110 kg were marketed in 1985. On the basis of the numbers in Table IV-8 and the survey estimate of 6.6 g of tetracyclines per pig, it is possible to derive a figure for the total amount of tetracyclines used in the rearing of swine in that year: 86.5 million (head of swine) x 6.6 g (tetracyclines per pig) = 0.57 million kilograms (1.25 million pounds) of tetracyclines. If all swine feed were medicated with tetracyclines, the total would be 1.7 million kilograms (3.7 million pounds). Because 5.4 million pounds of tetracyclines were estimated to have been used in livestock and poultry feeds in 1985 (Table IV-6), the figure of 1.25 million pounds used in pig production represents approximately 23% of the total tetracyclines used in animal

TABLE IV-7

USE OF PENICILLIN OR TETRACYCLINES FOR DISEASE PREVENTION IN POULTRY, 1984

Drug	No. Companies[a]	Route	Dosage	Duration of use, days
Penicillin	3/14	Feed	50-100 g/ton	3-14
Penicillin	4/18	Water	100-160 KU/gal[b]	3-14
Chlortetracycline	9/28	Feed	100-500 g/ton	2-7
Chlortetracycline	9/28	Water	100-400 mg/gal	2-7
Oxytetracycline	6/23	Feed	100-200 g/ton	3-14
Oxytetracycline	8/23	Water	100-400 mg/gal	3-14
Tetracycline	5/10	Water	200-1,000 mg/gal	3-7

Source: Data from J. P. Pretanik, National Broiler Council, 1988 (personal communication).

[a] No. Companies = (number of companies reporting this use)/(number of companies reporting).

[b] KU = thousand units.

TABLE IV-8

ESTIMATES OF TETRACYCLINE USED IN REARING SWINE

Use	Feed, kg	Tetracycline, g[a]	
		All Diets	Survey Estimate
In sow breeding and gestation: estimate used 21-day breeding period	44	2.7	0.17
In lactation: assumption of 100 g/ton	15	1.6	0.17
Starter: assumption of 100 g/ton	23	2.5	0.47
Grower: assumption of 50 g/ton	96	5.3	1.92
Finisher: assumption of 25 g/ton	280	7.7	3.87
Total per pig	458	19.8	6.60

Source: Data from Virgil Hays, University of Kentucky, 1988, (personal communication).

[a] As baseline for comparison, amount of tetracycline is calculated for medication of all diets. On basis of surveys done in the state of Illinois and those done by University of Nebraska in 1983, and more recently by Hays (1988) of seven major suppliers of feed for swine, figures have been calculated in right-hand column. The committee recognizes that accuracy of figures cannot be validated in practice. However, in absence of recent comprehensive survey data, the committee views these as best estimates of amounts of tetracycline used in swine.

and poultry feed. According to Virgil Hayes (personal communication, 1988), triple-drug medication that includes the tetracyclines in the feed of growing swine is used routinely, thus subtherapeutic dosing is frequent and widespread.

Estimates of antimicrobial consumption indicate their use in farm animals is predominantly in subtherapeutic concentrations. In December 1984, Gustafson testified before the Subcommittee on Investigations and Oversight of the U.S. House of Representatives Committee on Science and Technology. Gustafson presented estimates (Table IV-9) derived primarily from industry sources, that, of the 8,316,000 kg (18.3 million pounds) of antibiotics used in animal production, 88% was used in subtherapeutic concentrations; of the latter amount, 28% was for growth promotion. Of the 2,640,000 kg of the tetracyclines administered to farm animals (Table IV-9), 2,403,000 kg (91%) was used in subtherapeutic concentrations. Only 7% and 2%, respectively, of all the tetracyclines administered to cattle and swine were for therapeutic purposes, but 85% of the tetracyclines given to poultry was used therapeutically. In addition to reviewing the above estimates made by Gustafson in 1984, the committee sought and received estimates made by AHI that would have involved data from the same sources as were available to Gustafson (Table IV-6). In those estimates, the total sales of antibacterials for livestock and poultry feeds was 5.0 million kilograms in 1985, contrasting with the figure of 8.3 million kilograms reported by Gustafson (Table IV-9). The difference is substantial and is not readily explicable. However, it should be noted that the total figures for annual use (or sales) of tetracyclines in livestock and poultry are very similar: 2.6 million kilograms in the Gustafson estimates and 2.4 million kilograms in the AHI estimates, prorated for total U.S. antibiotic production in 1985 (Tables IV-6 and IV-9). The AHI estimates are related to "antibacterials" and the Gustafson estimates to "antibiotics," so the differences might be due to inclusion in the latter category of antibiotics that are coccidiostats (e.g., monensin), rather than antibacterial agents. As a result of the aforementioned differences, the committee is uncertain as to which estimates should be used as a basis for comparison of trends in annual use.

The estimates of tetracycline use in feed for individual animal and avian species show some possible discordances. The estimates by Hays of tetracycline use in swine indicate that 1.7 million kilograms would be used if all swine feed were medicated with this drug. This figure would be close to the 1.65 million kilograms in the 1985 estimate of Gustafson. However, not all swine are medicated with tetracyclines during growth. In addition, the previously noted NBC and TCFA surveys both indicate recent marked reductions in the

TABLE IV-9

ESTIMATED 1985 ANNUAL ANTIBIOTIC USE IN THERAPY, DISEASE PREVENTION, AND GROWTH PROMOTION

All Antibiotics, thousands of kilograms

		Subtherapeutic Use		
Therapeutic Use		Disease Prevention	Growth Promotion	Total
Cattle	458	1,100	340	1,898
Swine	250	3,578	1,391	5,219
Poultry	304	580	315	1,199
Total	1,012	5,258	2,046	8,316

Tetracyclines (Chlortetracycline and Oxytetracycline), thousands of kilograms

		Subtherapeutic Use		
Therapeutic Use		Disease Prevention	Growth Promotion	Total
Cattle	50	589	130	769
Swine	30	950	701	1,651
Poultry	187	33		220
Total	267	1,572	831	2,640

Source: Correspondence from R. H. Gustafson (American Cyanamid Company) to E. Eastman (Subcommittee on Investigations and Oversight, House Committee on Science and Technology), January 24, 1985 (personal communication).

use of tetracyclines for growth promotion. However, the committee believes that the tetracyclines are widely used in poultry production for disease prevention. In cattle-raising, data on the extent of use of prophylaxis are not reported. Although use of tetracycline for growth promotion in poultry and cattle has reportedly decreased, it is unknown whether the prophylactic use of subtherapeutic concentrations has remained constant or has increased correspondingly. Thus, it is very difficult to quantify current subtherapeutic use of tetracyclines (and penicillin) on U.S. farms and feedlots.

In summary, exact data on antibiotic use in animal feed are not obtainable. The best available estimates indicate that 31-46% of the total annual production of antibiotics (31.9 million pounds) in the United States is used in animals for all purposes. (Even higher figures have been suggested). A range of 9.7-11.7 million pounds (4.4-5.3 million kilograms) was used annually from 1980 through 1985 (Table IV-6). In the same years, tetracycline use in livestock and poultry totaled 5.1-6.4 million pounds, and penicillin use 0.6-0.9 million pounds. Although data are limited, it appears that about twice the amount of antibacterials is used for disease prevention as for growth promotion.

The committee is aware that strict compliance with the regulations in the United States governing subtherapeutic use of antimicrobials is not always achieved in common practice. On the farm, planned brief periods of antimicrobial use and the amount of medication and duration of its administration might sometimes be below or exceeded the specifications. For example, concentrations of antimicrobials actually achieved in feed might vary widely from the specified regulatory limits, because of miscalculation or improper mixing of drug and feed either on the farm or by the feedmill. The true period of medication might be longer or shorter due to the time taken to consume the amount of feed in storage or for other reasons. For purposes of analysis, the committee has considered all use of antimicrobials as specified for both growth enhancement and disease prevention as being in the category of subtherapeutic use. That is also the view taken by FDA (M. F. Lowe, 1988, personal communication). The approach seems reasonable, in that, with the exception of the report of Gustafson, there is little quantitative information to distinguish use for growth promotion from use for disease prevention. Gustafson's estimates indicated that 88% of all antibiotic use in livestock and poultry was subtherapeutic.

Tetracyclines account for almost 50% of total antibacterial use in livestock and poultry feeds (Table IV-6). In the estimates of Gustafson (Table IV-9), subtherapeutic use of tetracyclines accounts for 29% of all antibiotic use in cattle, swine, and poultry for all purposes. If data in Tables IV-4, IV-5, and IV-9 are

combined, it appears that subtherapeutic use of tetracyclines (2.4 million kilograms) accounts for 17% of total annual production of all antibiotics (approximately 14 million kilograms in 1983) and 73% of all tetracyclines produced for all purposes (3.3 million kilograms in 1983). Data on subtherapeutic use of tetracyclines for the last 3 years are not available, and it is not possible to calculate the percentages of total antibiotic production or of total farm and feedlot use that they account for today.

Antibiotic use in animals, in quantitative terms, is an important pressure for selection of antibiotic resistance in enteric bacteria on the farm and in feedlots. Subtherapeutic use in animal feed appears to be greater in quantative terms in this regard than antibiotic use for therapy, and tetracycline accounts for about half the amount of antibacterials used in this fashion. Although distinction between the growth-promotion and disease-prevention uses of subtherapeutic concentrations would be helpful, no accurate data is available. Furthermore, disease-prevention uses apparently are routine when there is suspicion of disease in some animals in a group or the durations of medication might be prolonged beyond specified regulatory time periods in severe cases or simply because of mistakes.

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V

ANTIMICROBIAL RESISTANCE IN HUMANS AND ANIMALS

FREQUENCY OF DRUG RESISTANCE IN CLINICAL ISOLATES OF SALMONELLA SPP. FROM HUMANS AND ANIMALS IN THE UNITED STATES

Thousands of clinical laboratories throughout the United States isolate occasional strains of salmonellae from humans, test their susceptibility to antimicrobial agents, and send the isolates--but not the susceptibility test results--to state reference laboratories for serotyping. Similarly, a parallel set of veterinary service and reference laboratories process animal isolates of salmonellae similarly. This whole elaborate salmonella reporting apparatus lists about 45,000 total isolates per year. These salmonella test results, however, are scattered through the files of thousands of laboratories, and even the reference laboratory files rarely have both susceptibility and serotype results. Thus, an expensive system obscures a major part of its only product, epidemiologic information.

To survey the prevalence of resistance in salmonellae, it is therefore necessary to repeat work that has already been done, to collect serotyped isolates from reference laboratories to retest their susceptibility and to file the results together. Table V-1 summarizes an example of such work carried out 8 years ago as background for a study of resistance plasmids in salmonellae. Susceptibility testing was performed on several thousand human isolates of salmonellae collected and serotyped by the Massachusetts State Laboratory and several thousand animal isolates collected and serotyped from all parts of the United States by the National Veterinary Laboratory, in Ames, Iowa.

In this chapter and throughout the report the committee has included data on the antibiotic resistance profile of bacterial isolates collected from various veterinary laboratories throughout the United States over several years. The history of antibiotic exposure was not available in most cases. The committee has made the assumption that the antibiotic resistance profile of the isolates is indicative of exposure of the animal host to the specific antibiotics listed in the profile. There is an inherent problem in this assumption that is clearly recognized; the antibiotic resistance profile of an isolate cannot be used with absolute certainty to determine direct exposure of a

TABLE V-1

RESISTANCE TO ANTIMICROBIAL AGENTS IN SALMONELLA ISOLATES (1979-1980)

HOST	SALMONELLA SEROTYPE	NO. (%) ISOLATES	NO. (%) ISOLATES RESISTANT TO: tetracycline	chloramphenicol	streptomycin	sulfonamide	ampicillin	any
	agona	141 (5)	19 (13)	0 (0)	26 (18)	6 (4)	4 (3)	34 (24)
	anatum	24 (1)	6 (25)	0 (0)	6 (25)	1 (4)	0 (0)	9 (37.5)
	blockley	55 (2)	0 (0)	0 (0)	5 (9)	0 (0)	3 (5)	7 (13)
HUMAN	enteritidis	884 (31)	15 (2)	0 (0)	12 (1)	2 (0)	12 (1)	28 (3)
(A)	heidelberg	121 (4)	20 (17)	2 (2)	53 (44)	26 (21)	28 (23)	68 (56)
	infantis	127 (4)	3 (2)	0 (0)	12 (9)	2 (2)	0 (0)	13 (10)
(MASS.	montevideo	61 (2)	6 (10)	0 (0)	2 (3)	2 (3)	0 (0)	8 (13)
STATE	newport	84 (3)	8 (10)	4 (5)	10 (12)	8 (10)	7 (8)	10 (12)
LABORATORY)	oranienburg	34 (1)	2 (6)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6)
	St. paul	75 (3)	17 (23)	3 (4)	15 (20)	8 (11)	14 (19)	22 (29)
	typhimurium	959 (34)	119 (12)	3 (0)	134 (14)	86 (9)	52 (5)	188 (20)
	" v.copen.	261 (9)	32 (12)	1 (0)	41 (16)	23 (9)	23 (9)	48 (18)
		2826	247 (8.7)	13 (0.5)	316 (11.2)	164 (5.8)	143 (5.1)	437 (15.5)
	agona	197 (11)	52 (26)	30 (15)	67 (34)	43 (22)	38 (19)	79 (40)
	anatum	167 (10)	81 (49)	2 (1)	102 (61)	79 (47)	9 (5)	130 (78)
ANIMAL	blockley	36 (2)	0 (0)	0 (0)	10 (28)	0 (0)	1 (3)	9 (25)
(B)	enteritidis	55 (3)	1 (2)	0 (0)	2 (4)	1 (2)	1 (2)	2 (4)
	heidelberg	240 (14)	110 (46)	0 (0)	178 (74)	110 (46)	40 (17)	183 (76)
(NATIONAL	infantis	68 (4)	15 (22)	3 (4)	24 (35)	9 (13)	5 (7)	29 (43)
VETERINARY	montevideo	56 (3)	5 (9)	0 (0)	19 (34)	15 (27)	12 (21)	21 (37.5)
LABORATORY)	newport	45 (3)	8 (18)	1 (2)	13 (29)	10 (22)	10 (22)	14 (31)
	oranienburg	42 (2)	0 (0)	0 (0)	7 (17)	2 (5)	2 (5)	7 (17)
	st. paul	131 (8)	84 (64)	2 (2)	49 (37)	86 (66)	7 (5)	108 (82)
	typhimurium	502 (29)	345 (69)	32 (6)	357 (71)	275 (55)	243 (48)	391 (78)
	" v.copen.	206 (12)	143 (69)	1 (0)	158 (77)	142 (69)	108 (52)	162 (79)
		1745	844 (48.4)	71 (4.1)	986 (56.5)	772 (44.2)	476 (27.3)	1135 (65)
OTHER SEROTYPES	choleraesuis	290 (41)	37 (13)	1 (0)	289 (100)	285 (98)	37 (13)	289 (100)
IN ANIMALS	derby	95 (13)	22 (23)	2 (2)	31 (33)	16 (17)	6 (6)	40 (42)
(C)	dublin	125 (18)	100 (80)	3 (2)	106 (85)	25 (20)	93 (74)	116 (93)
(NAT. VETERINARY	pullorum	95 (13)	12 (13)	0 (0)	56 (59)	8 (8)	11 (12)	60 (63)
LABORATORY)	san diego	106 (15)	63 (59)	0 (0)	69 (65)	42 (40)	15 (14)	79 (75)
		711	234 (32.9)	6 (0.8)	551 (77.5)	376 (52.9)	162 (22.8)	584 (79)
B+C TOTAL		2456	1078 (43.9)	77 (3.1)	1537 (62.6)	1148 (46.7)	638 (26.0)	1697 (69)

Source: Adapted from data compiled by the committee.

human or an animal to any specific antibiotic, nor to the dosage, nor to the route of administration (oral or parenteral). This could result in errors in judgement, e.g., the inability to differentiate bacterial resistance to an antibiotic administered either parenterally or via the feed (per os). (Note: Penicillin/ampicillin is used as terminology to include penicillin G to reflect the fact that, although this is the form of the β-lactam administered parenterally to livestock, susceptibility testing of salmonellae isolates from humans is performed with ampicillin as the β-lactam drug.)

The prevalence of several serotypes can be seen to differ greatly between the human and animal isolate collections (See Table V-1). Strains of S. enteritidis constitutes 31% of human but only 3% of animal isolates; S. choleraesuis, S. derby, S. dublin, S. pullorum, and S. san diego were found mostly in the animal isolate collection.

Overall, resistance to each of the antibacterial agents tested or to any of the agents was more frequent in the animal than in the human collections. Resistance was also disproportionately prevalent in isolates of several serotypes (e.g., S. typhimurium, S. heidelberg, S. anatum, S. agona, and S. st. paul) in both animal and human isolates. The frequency of resistance to the different antibacterial agents had the same rank order of resistance in both animal and human isolates as follows:

(streptomycin>tetracycline>sulfonamide>ampicillin> chloramphenicol).

Table V-2 arranges animal isolate data similar to that in Table V-1 by serotype and antibiotype. Each antibiotype that was found in 2% or more of the isolates of any serotype is shown in Table V-2. The table shows that much of the resistance in the isolates of a given serotype is clustered into a small number of antibiotypes, and that these prevalent antibiotypes vary greatly from serotype to serotype. When collections of isolates representing several of the prevalent serotype-antibiotype combinations were tested (e.g., the antibiotype TSKUHA for S. typhimurium var. copenhagen) it was found that resistance in many of the isolates was due to a common resistance plasmid which they shared, even when the isolates came from different states or from both animals and humans.[15]

The results shown in Table V-2 also illustrate a problem encountered in surveys of the prevalence of resistance in Salmonella spp. The resistance tends to be discontinuous, that is, clustered in groupings of multiple resistance in particular serotypes. Any survey that includes, for unsuspected epidemiologic reasons, a disproportionate number of isolates belonging to one of these clusters will not

TABLE V-2

PERCENTAGES[+] OF ANIMAL ISOLATES OF EACH COMMON SEROTYPE OF SALMONELLA THAT TESTED RESISTANT TO VARIOUS COMBINATIONS OF ANTIMICROBIAL AGENTS

ANTIBIOTYPE*	SEROTYPE																									
TCSKUHAGX	Agona	Anatum	Blockley	Bredeney	Cerro	C-suis Kzf.	Derby	Dublin	Elmsbuettel	Enteritidis	Havana	Heidelberg	Infantis	Johannesburg	London	Montevideo	Newport	Oranienburg	Pullorum	Reading	St. Paul	San Diego	Senftenburg	Thompson	Typhimurium	Typh. Copenhag.	Worthington
none	52	22	70	61	74		60	11	14	96	72	19	52	86	57	62	64	78	34	40	17	22	42	72	23	18	42
T	4	5			5		5								4												21
C																											
S	8	15	26	12	13		10	4	9		14	22	25		9	4	9	14	49	9	6	5	13	17	10	9	12
K					3											3					3						
U		3									3				2				2	5	10	8					
H																											
A																											3
G																											
X																											
T S	4	5					6					5	9								5	35	9	3	3		3
SK		5										2			2												3
T U		4																			25				2		
S U		7				84					3								4	4	4		3	3			
S G																											3
U A														3													
TCS									3																		
T SK								3				2			2					7	3						6
T S U		12		16		3	3					7			2					5	8	7	4		4	4	
T S A								8							11					14					3		
SKU												5															
S U A					3									7													
T SKU		17		4			4					10				3					11	6	4		6	8	3
T SK A								39											3					3	3		
T S U A						3		4									3					5			3		
T UHA																	3										
T S HA								3							2												
SKU G									9																		
SK HA																							6				
S UHA																											3
T SKU A					3	2		9						3	6		13		4	4		3			16	14	
T SK HA								3																			
T S UHA								4														3			6		
SKUHA															2												
T SKUHA																									6	37	
SKU AG																22		3									
SKUHAG									6																		
T SKUHAG									60			9															
TCSKU AG											7																
T SKU G												4										2					
TCSKUHAG	20																										
TOTAL PATTERNS	28	26	11	15	12	24	20	26	14	12	12	40	19	13	17	17	18	13	15	22	26	21	28	13	48	28	16
NUMBER ISOLATES	282	232	43	69	38	473	111	158	35	76	29	337	112	29	47	74	67	59	125	57	167	134	78	29	717	287	33

*AGENTS TO WHICH RESISTANT

T = tetracycline
C = chloramphenicol
S = streptomycin
K = kanamycin
U = sulfonamides
H = cephalothin
A = ampicillin
G = gentamicin
X = sulfatrimethoprim

[+]Percentages less than 2 not shown

Source: Adapted from O'Brien et al.[15]

accurately represent the prevalence that might be found in a larger or more broadly-based sample.

Table V-3 summarizes in chronologic order the prevalence of resistance in collections of salmonella isolates from humans and from animals in the United States. Among the human isolates, the first two reports, from the late 1960s, show similar prevalence of resistant strains at 21-22%.[20,29] Four reports of studies done in the 1970s were based on reference laboratory collections--those of Cherubin et al.,[5] Saad and Farrar,[19] O'Brien et al.,[16] and MacDonald et al.[14] --the results in these surveys were in close agreement; percentage of resistant strains ranged from 15.5 to 17.4. The higher percentage reported during this period was the survey of Bissett et al.,[2] that showed 31% resistant strains.

The report of Lorian,[13] covering the decade from 1975 through 1984, was produced by a different method of surveillance than those mentioned above. This method was not based on collections of reference laboratories, but on analysis of computer-data files from several hundred hospital laboratories. Although these computer files did not give serotype identification, there was broad geographic representation in this very large data file. Lorian's analysis of these data did not attempt to determine the percentage of isolates resistance to at least one of the tested antibacterial agents. However, we can project that the percentage of isolates resistant to any of the agents would be around 30%, since the resistance to either tetracycline or to ampicillin alone was 22% and 18%, respectively, assuming a distribution of groupings of resistance similar to that seen in the other studies. Thus, we are left with the puzzle of why these hospital-based survey data showed approximately twice the percentage resistance as the reference-laboratory-based surveys of approximately the same time period.

One possible explanation is that the different reporting method for the data in the Lorian survey[13] might have accounted for some of the observed difference in prevalence of resistance. These data did not include the primary measurements of the extent of resistance (inhibition zone diameter or MIC), but only reported susceptibility rather than resistance, and this has been projected here to make the results comparable with those of the other studies. In Lorian's method the percentages in the intermediate range, which are not reported here, were reported as resistant, while in the other studies, they would have been reported as nonresistant. The intermediate percentages for the antibacterial agents reported in the other studies, are too small to account for the difference between them and the Lorian data, also there was no information on quality control

TABLE V-3

RESISTANCE TO ANTIMICROBIAL AGENTS AMONG ISOLATES OF SALMONELLAE FROM HUMANS AND ANIMALS IN THE UNITED STATES

HOST	PERIOD	REGION	NO. ISOLATES	PERCENT RESISTANT TO tetra-cycline	chloram-phenicol	strepto-mycin	sulfon-amide	ampi-cillin	any	INVESTIGATOR
HUMAN	1967	NATIONAL	400	12.5	0	14.2		8	22.2	SCHROEDER ET AL[20]
"	1968-1969	NORTHEAST	292	8.2	0	15	11.5	13.5	21.6	WINSHELL ET AL[29]
"	1970	NORTHEAST	315	11.7	0	14.9		9.5	17.4	CHERUBIN ET AL[5]
"	1971-1972	CALIFORNIA	2246	20.3	1.5	26	19.5	18.5	31	BISSETT ET AL[2]
"	1973-1974	GA AND SC	305	10	0.3	11	6	8	16	SAAD AND FARRAR[19]
"	1975-1984	NATIONAL	20708	22	4			18	730	LORIAN[13]
"	1979-1980	MA	2826	8.7	0.4	11.2	5.8	5.1	15.5	O'BRIEN ET AL[16]
"	1979-1980	NATIONAL	511	8.6	0.8	12	8	8	16	MACDONALD ET AL[14]
"	1984-1985	NATIONAL	485	13	2	12.2	7	9	24	" " "
		TOTAL	28088							
ANIMAL	1973-1974	GA AND SC	152	10	0	16	4	4	21	SAAD AND FARRAR[19]
"	1979-1980	NATIONAL	2456	43.8	3.1	62.5	46.7	25.9	69.09	O'BRIEN ET AL[16]
"	1980-1981	NATIONAL	3500	45	12	66	57	31	80	BLACKBURN ET AL[3]
BOVINE	1981-1987	S. DAKOTA	207	80				62		PERSONAL COMMUNICATION
"	1985-1986	TEXAS	621	47				39		FROM VETERINARY
"	1981-1987	S. DAKOTA	596	81				42		LABORATORIES
PORCINE	1985-1986	TEXAS	51	37				23		(COLE, THAYER,
"	1986-1987	GEORGIA	223	69				72		LIBAL, WHITFORD)
POULTRY	1981-1987	S. DAKOTA	124	43				15		" " "
		TOTAL	7930							

Source: Adapted by the committee from data by Blackburn et al.[3], Bissett et al.[2], Cherubin et al.[5], Lorian[13], MacDonald et al.[14], O'Brien et al.[16], Saad and Farrar[19], Schroeder et al.[20], Winshell et al.[29], and personal communications (Cole, Libal, Thayer and Whitford, Veterinary Laboratories).

in the hundreds of laboratories that generated the data reported in Lorian's large survey.[13]

The survey results reported by MacDonald et al.[14] showed a rise from 16 to 24% from 1980 to 1985 for human isolates of salmonellae resistant to any of the tested antimicrobial agents. These percentages are important, because they indicate that the antibacterial resistance found in human isolates of salmonellae has increased. Accordingly, the latter value (~24%) would be the more nearly contemporary percentage to use in the risk assessment model.

The validity of the results of the 1985 survey by MacDonald et al.[14] is supported by the geographic representation incorporated in its design and by its comparability to the survey performed 5 years earlier. Also, the percentage of resistance (16%) found in the earlier (1980) survey reported by MacDonald agrees closely with the percentages mentioned in the three other surveys of that time period. Given the complexity of the problem, however, a sample of 485 isolates is not large. This illustrates the committee's earlier concern about the inadequacy of surveillance of resistance in isolates of salmonellae in the United States; when we consider that the 485 isolates on which this survey was based were from nearly 200,000 human isolates of salmonellae serotyped by reference laboratories in the United States since the earlier survey.

The report by Saad and Farrar[19] of the percentage of resistant salmonellae in animal isolates from Georgia and South Carolina during 1973 and 1974 shows a relatively low percentage of resistance, in contrast with the two later studies of animal isolates (Table V-3). This probably is due to regional differences and small-sized samples, and thus does not represent a true national secular trend. Studies by O'Brien et al.[16] covering the period 1979-1980 and by Blackburn et al.[3] covering 1980-1981--were both based on the same data source, namely, the large collection of animal isolates at the National Veterinary Laboratory in Ames, Iowa. Although the results of the Blackburn et al.[3] study showed somewhat higher percentage of resistance than the O'Brien study, more recent data would be needed to determine whether these results represents a trend. In addition to these three surveys, veterinary laboratories in several states have provided results of susceptibility tests done on salmonellae isolated in recent years. Yearly values for percentages of resistance showed no clear trends over time, so values for all years were averaged for each state and host species, as shown in the lowest six lines of Table V-3.

Table V-3 essentially amplifies the observations of Table V-1. The percentage of isolates resistant to any antibacterial agent for which data are available in any of the nine collections of animal-isolates, except for the small group reported by Saad et al., exceeds the percentage

reported in any of the human isolate collections. The percentages reported for resistance to tetracycline are greater than those resistant to ampicillin in eight of the nine collections of human isolates and in all nine collections of animal isolates. The average percentage of resistance of salmonellae isolates to ampicillin or to tetracycline in the animal collections is 3 to 4 times greater than in the human collections.

FREQUENCY OF DRUG RESISTANCE IN CLINICAL ISOLATES OF E. COLI FROM HUMANS AND ANIMALS IN THE UNITED STATES

The data in Table V-4 shows the percentages of isolates resistant to antimicrobial agents in collections of isolates of E. coli from humans and animals in the United States. The data in the first row, reported by O'Brien, is from all isolates tested during 1986 by the laboratory of a large general hospital in Boston (O'Brien, 1988, personal communication). It is the committee's belief that these percentages are similar to those commonly found in United States hospital laboratories, because they are comparable to the percentages in the second row reported by Atkinson and Lorian from isolates tested over a decade in several hundreds hospitals in the United States.[1] The percentages in the latter large study by Atkinson might be slightly inflated in comparison with the percentages in other collections, because they were reported as "percent nonsusceptible"--that is the total of resistant isolates plus isolates in the intermediate resistant category.

The data in the first two rows of Table V-4, reported by O'Brien, Atkinson, and Lorian, are representative of data that are commonly reported by hospital laboratories. Data from this source are considered biased, because most of the isolates are from patients who are hospitalized presumably due to an illness, thus constituting a small group having more isolates that are resistant than the human population at large. The E. coli isolates in the third row reported by Lester et al. from the general human population probable give a better representation of the resistance profile from the human population at large. These data are from a collection of 10 random colonies of E. coli from stool cultures from each of 38 healthy children who had not been treated with any antimicrobial agents for at least 4 months before culture. The percentages of resistance reported in these community-based isolates of E. coli are about one-third of those reported for hospital-laboratory isolates.

The lower part of Table V-4 shows percentages of resistant isolates in various collections of animal isolates of E. coli. The first three rows are from earlier studies by

TABLE V-4

RESISTANCE TO ANTIMICROBIALS AMONG ISOLATES OF E. COLI FROM HUMANS AND ANIMALS IN THE UNITED STATES

HOST	SOURCE	PERIOD	REGION	NO. OF ISOLATES	% RESISTANT TO: tetracycline	chloramphenicol	streptomycin	sulfonamide	ampicillin	INVESTIGATOR
HUMAN	HOSPITAL	1986	BOSTON	3757	26	8		27	29	O'BRIEN ET AL[16]
"	" "	1971-1982	U.S.	1.8 mil	28	5		29	28	ATKINSON AND LORIAN[1]
"	COMMUNITY	1985	BOSTON	388	12	1		9	10	LESTER ET AL[12]
ANIMAL	PORCINE	1974	ILLINOIS	530	90	1.7	93	83	53	SIEGAL ET AL[21]
"	BOVINE	1974	" "	106	49	0	50	29	13	" " "
"	" "	1974	MONTANA	431	0	2	1	1	1	" " "
"	ANIMAL	1980	U.S.	100	80	6	72	70	29	O'BRIEN ET AL[15]
"	BOVINE	1981-1987	SOUTH DAKOTA	366	76		69		38	PERSONAL COMMUNICATION
"	" "	1985-1986	TEXAS	405	71				49	FROM VETERINARY
"	" "	1986-1987	GEORGIA	265	57		55		35	LABORATORIES
"	PORCINE	1981-1987	SOUTH DAKOTA	1015	96		93		44	(COLE, THAYER,
"	" "	1985-1986	TEXAS	107	93				77	LIBAL, WHITFORD)
"	" "	1986-1987	GEORGIA	405	93				62	" " "
"	POULTRY	1981-1987	SOUTH DAKOTA	30	83		80		31	" " "

Source: Adapted by the committee from data by Atkinson and Lorian[1], Lester et al.[12], O'Brien et al.[15,16], Siegal et al.[21], and personal communications (Cole, Libal, Thayer and Whitford, Veterinary Laboratories).

Siegal et al. of E. coli in fecal samples from pigs and cattle on farms in Illinois and from range cattle in Montana.[21] The isolates from range cattle were remarkable, in that scarcely any of them were resistant to any of the antimicrobial agents tested.

Isolates from animal hosts reported in the fourth row of Table V-4 shows the resistance in 100 E. coli strains chosen randomly from among the large collection of animal isolates sent to the National Veterinary Reference Laboratory for serotyping. The data in the other seven rows show the percentage resistance in the collections of E. coli isolates from different animal host species tested by the same veterinary laboratories that provided the data on salmonellae isolates in Table V-3. In all collections of E. coli isolates resistance to tetracycline was more frequent than resistance to ampicillin, with the exception of those from range cattle.

In addition to the data in Table V-4, data from other studies provide information about the resistance to antibacterials in salmonellae and E. coli isolated from animals in the United States. Fagerberg and her associates[6] surveyed fecal samples from production cattle, broilers, and swine at various slaughter plants in the United States. Salmonellae were isolated from 5% of the broilers, 5% of the production swine, 9% of the beef units, and 60% of the swine at the slaughter plants. The resulting 199 salmonella isolates were of 27 serotypes; 82% of the strains were drug resistant. The survey results showed 1,563 strains of E. coli, of which 95% were drug-resistant: 72% resistant to tetracycline, 60% resistant to streptomycin, 84% resistant to sulfadiazine, and 13% resistant to ampicillin.

Gustafson et al.[7] found differing rates (10 to 84%) for the isolation of Salmonella from healthy market hogs taken at slaughter plants in Pennsylvania, Iowa and Georgia and different percentages (0-24%) of drug resistance among the isolates, although only 24 isolates out of 1491 from 658 swine were resistant to multiple antibacterials.

A study by Søgaard[22] involved pigs without clinical signs of illness which had not been fed any antibiotics in their feed but were given therapeutic dosages of antibiotics. A prevalence of 74% resistance was reported to one or more antibiotics. The incidence of resistance to sulfonamides, tetracyclines, and streptomycin was high. Most strains were susceptible to ampicillin and chloramphenicol. Pigs never given antibiotics either subtherapeutically or therapeutically showed a prevalence of resistance among E. coli of 53%. Thus, the therapeutic use of antibiotics in swine accounted for an absolute 21% increase (from 53% to 74%) of organisms resistance to various antibiotics. Langlois et al.[10] found tetracycline resistance in 76% of the fecal coliform organisms in swine fed no subtherapeutic