Clinical Infectious Diseases

The Need to Improve Antimicrobial Use in Agriculture

Ecological and Human Health Consequences

A Report of the Facts about Antibiotics in Animals and the Impact on Resistance (FAAIR) Project

ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS

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Introduction

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Antimicrobial agents are used widely in agriculture to treat and prevent disease, promote growth, and enhance feed efficiency in food animals. Because their use may contribute significantly to the development of antimicrobial resistance, such uses are not without consequences for human, animal, and environmental health. Outright treatment failure due to antimicrobial resistance is an obvious concern; moreover, exposure to antimicrobials can fundamentally alter microbial ecosystems in humans and animals as well as in the environment. In addition, antimicrobial resistance can potentially increase the number of infections each year and can be associated with the emergence of more virulent bacterial pathogens, leading to more severe infections.

Increasing recognition and understanding of these and other risks have prompted some experts to urge that certain of these agricultural practices be curtailed, both to preserve the effectiveness of antimicrobial agents needed for treatment of infections in humans and animals and to decrease the environmental pool of resistant bacteria. This opinion raises critical public policy questions concerning the appropriate agricultural use of antimicrobial agents.

To address the scientific issues underlying these questions, the Alliance for the Prudent Use of Antibiotics (APUA) initiated a 2-year project, called "Facts about Antimicrobials in Animals and the Impact on Resistance (FAAIR)." APUA convened a Scientific Advisory Panel ("Panel"), whose charge was to gather evidence and draw conclusions about human health impacts of antimicrobial use in agriculture. The Panel was composed of experts from a variety of fields, including human and veterinary medicine, plant pathology, public health, microbiology, and biostatistics and risk analysis. Panel members undertook a comprehensive review of the existing body of scientific and medical evidence, to which they added novel analyses, conclusions, and recommendations intended to influence public policy.

In 1969, the Swann Committee in the United Kingdom published a watershed report recommending more restricted use of antimicrobials in agriculture. Despite this and similar recommendations in reports from national and international health and nongovernmental organizations since then, the debate over the human health risks associated with antimicrobial use in agriculture continues.

Regulatory officials in the European Union, Australia, Japan, New Zealand, and other nations have invoked the "precautionary principle" in restricting some agricultural uses of antimicrobials. US officials are also considering imposing additional restrictions to prevent or postpone the development of resistance in pathogens transmissible from food animals to humans. There has been widespread debate about the risks and benefits of such a policy change. Critical questions relevant to this debate in the United States include the following: (1) How much of total antimicrobial use is directed toward food animal production as opposed to human medicine? and (2) Of the antimicrobials administered to food animals, what proportion is used for treatment and prevention of infections as opposed to nontherapeutic uses? Precise information for these uses is not publicly available.

APUA and the FAAIR Panel intend this report to provide an objective, scientific review and analysis of available data on antimicrobial resistance as it pertains to antimicrobial use in agriculture. While past reports have focused narrowly on clinical studies, the FAAIR Project enlarged the scope of inquiry to include an ecological dimension of antimicrobial resistance. Antimicrobial use in medicine and agriculture affects the general ecology of bacterial communities, including interactions between microorganisms and their environments and mechanisms by which antimicrobial resistance traits spread and persist. This perspective facilitates an understanding of the broad consequences of antimicrobial use.

This ecological framework provides an essential perspective for formulating antimicrobial use policies precisely because it encompasses the root causes of these problems rather than merely their symptoms. Resistant pathogens in the environment may infect people through direct contact or by indirect means, as through

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the food supply. This report also considers the potential importance of resistance acquired by commensal microorganisms in food animals or in humans, as these ordinarily nonpathogenic bacteria may provide a reservoir of resistance genes that can be transferred to human pathogens.

APUA initiated the FAAIR project in accord with its mission, which is to improve control of infectious disease worldwide through more appropriate use of antibiotics and reduction of antibiotic resistance. To this end, the Panel and APUA offer the following evidence, analyses, and recommendations for use in facilitating discussion and improving public policy and practices related to agricultural use of antimicrobials.

Select Findings and Conclusions

FAAIR Scientific Advisory Panel[a]

SELECT FINDINGS

Summarized below are select findings and key pieces of evidence from the articles that comprise the APUA Facts about Antimicrobials in Animals and the Impact on Resistance (FAAIR) Report.

Emergence, Spread, and Environmental Effect of Antimicrobial Resistance (O'Brien)

- Antimicrobial use selects for resistant bacterial strains as well as genetic vectors specifying resistance genes.
- Antimicrobial use anywhere, at any time, can increase resistance in microbes anywhere else. A bacterial isolate may be resistant not only because nearby use of antimicrobials has amplified genetic constructs locally, but also because distant use may have affected the evolution and spread of the construct or its components. Therefore, levels of resistance in a given isolate may, in part, reflect the total number of bacteria in the world ever exposed to antimicrobials.

Generally Overlooked Fundamentals of Bacterial Genetics and Ecology (Summers)

- Propagation of antimicrobial resistance is an ecological problem.
- Ameliorating resistance requires an understanding of the commensal microbiota of mammals as well as genetic vectors involved in the movement of resistance genes and the linkage of resistance genes on these vectors.
- Treatment with any given antimicrobial can result in selection for resistance to not only that specific agent, but also, by genetic linkage of resistance genes, to other antimicrobials.

Antimicrobial Use and Resistance in Animals (McEwen and Fedorka-Cray)

- Most food animals in the United States are exposed to an antimicrobial via feed, water, or injection at some point during their lives. This medication is used to treat or prevent infectious disease, promote growth, or enhance feed efficiency.
- Many antimicrobials used in food animal production are the same as, or closely related to, drugs used in human medicine.
- Precise figures describing the extent and quantity of antimicrobial use in animals are not publicly available, and estimates vary widely.
- There is considerable evidence that antimicrobial use in food animals selects for antimicrobial resistance in commensals and in zoonotic enteropathogens.
- Intended purpose of use, dose, duration, and route of administration can influence the degree to which antimicrobial use exerts a selective pressure for resistance as well as the spread of resistance among bacterial populations.
- Fecal waste from food animals is often composted and spread as fertilizer. Swine operations typically construct lagoons to hold such waste. These practices are implicated in contamination of the environment with resistant bacteria.
- Antimicrobial resistance is also a concern for animal health, but little is known about the magnitude of this problem because surveillance of resistance in exclusively animal pathogens is poor relative to that of zoonotic enteropathogens.

Uses of Antimicrobials in Plant Agriculture (Vidaver)

- Only streptomycin and oxytetracycline are cur-

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rently approved for treatment of bacterial diseases in plant agriculture. Use is primarily prophylactic, and most applications are by spray treatments in orchards.
- Because monitoring and surveillance are not routine, the effect of antimicrobial use in plant agriculture on antimicrobial resistance is unknown.

Human Diseases Caused by Foodborne Pathogens of Animal Origin (Swartz)

- Several lines of evidence may link antimicrobial-resistant pathogens in humans to the use of antimicrobials in food animals. These include the following: (i) direct epidemiological studies, (ii) temporal evidence for emergence of resistance among animal-associated bacteria before related human pathogens, (iii) additional circumstantial evidence, (iv) trends in resistance among *Salmonella; Campylobacter;* and *Escherichia coli* isolates, and (v) studies suggesting that farmers and family members may be more likely than the general population to acquire antimicrobial-resistant bacteria.
- Evidence also suggests a link between enterococci of food animal origin (particularly strains that are vancomycin resistant) and strains found in the human gastrointestinal tract.
- The latent period between the introduction of an antimicrobial and the emergence of resistance varies considerably, but once the prevalence of resistance in a population reaches a certain level, reversal of the problem becomes extremely difficult. The time to act is therefore limited.

Mechanisms of Increased Disease in Humans from Antimicrobial Resistance in Food Animals (Barza)

- There are at least five potential mechanisms by which antimicrobial resistance can have adverse effects on human health: (i) the "attributable fraction," or proportion of infections caused by pathogens that are resistant to antimicrobials taken for unrelated reasons; (ii) linkage of variable traits to resistance traits; (iii) ineffective treatment due to choice of a drug to which pathogens are resistant; (iv) the attributable fraction in food animals, which increases the numbers of resistant foodborne pathogens; and (v) the acquisition of resistance by commensal flora of food animals, which serve as a reservoir of resistance traits that can find their way to commensals and pathogens of people.

Excess Infections Due to Antimicrobial Resistance: The "Attributable Fraction" (Barza and Travers)

- Paradoxically, antimicrobial use can increase vulnerability to infection upon exposure to a resistant foodborne pathogen by up to 3-fold (the "attributable fraction"), because it causes a transient decrease in an individual's resistance to colonization.
- Calculations based on estimates of annual rates of nontyphoidal *Salmonella* and *Campylobacter jejuni* infections suggest that resistance to antimicrobial agents results annually in 29,379 additional nontyphoidal *Salmonella* infections, leading to 342 hospitalizations and 12 deaths, and 17,668 additional *C. jejuni* infections, leading to 95 hospitalizations.

Morbidity of Infections Caused by Antimicrobial-Resistant Bacteria (Travers and Barza)

- Antimicrobial resistance can affect the outcome of infection in 2 ways: (i) virulence of the pathogen may be increased, and (ii) treatment may be less effective as a result of choosing an antimicrobial drug to which the pathogen is resistant.
- Data for *Salmonella* and *Campylobacter* infections suggest that antimicrobial resistance strains are somewhat more virulent than susceptible strains, either via prolonged or more severe illness.
- Fluoroquinolone-resistant infections (stemming from administration of antimicrobials to food animals) lead to an estimated 400,000 excess days of diarrhea per year in the United States relative to fluoroquinolone-susceptible infections.

Review of Assessments of the Human Health Risk Associated with the Use of Antimicrobial Agents in Agriculture (Bailar and Travers)

- Published risk assessments of antimicrobial use in agriculture are likely to underestimate risk to human health because they are subject to multiple limitations in scope.
- Two of the most serious limitations include (i) a tendency to limit the scope of analysis to what has happened in the past, ignoring the potential for cumulative effects in the future; and (ii) a tendency to examine the effect of resistance on only one species of microorganism, ignoring the potential for transfer of resistance.

CONCLUSIONS

On the basis of the scientific evidence in this Report, the FAAIR Scientific Advisory Panel reached these conclusions:

- All uses of antimicrobials in animals, agriculture, and humans contribute to the global pool of antimicrobial resistance genes in the environment.
- Antimicrobial resistance in pathogenic bacteria limits treatment options; raises health care costs; and increases the number, severity, and duration of infections.

- Commensal bacteria also contribute to the antimicrobial resistance problem by serving as reservoirs of resistance genes transferable to pathogenic bacteria.
- It is estimated that, in the United States, the amount of antimicrobials administered to animals is comparable to that used in humans. In contrast to use in humans, much of the antimicrobial use in food animals consists of administration to large groups for nontherapeutic applications, such as growth promotion and disease prevention.
- Antimicrobial use in food animal production selects for resistant strains and amplifies their persistence and dissemination in the environment.
- Transfer of bacteria from food animals to humans is a common occurrence.
- Use of antimicrobials in food animals contributes to the growing problem of antimicrobial resistance in animal and human infections.

Therefore, the committee concludes that the elimination of nontherapeutic use of antimicrobials[1] in food animals and in agriculture will lower the burden of antimicrobial resistance in the environment, with consequent benefits to human and animal health.

[1] Except for ionophores and coccidiostats, for which current evidence indicates no direct or environmentally mediated risk to human health.

Policy Recommendations

FAAIR Scientific Advisory Panel[a]

After an extensive review of the scientific evidence pertaining to the human health effect of antimicrobial use in agriculture, the Facts about Antimicrobials in Animals and the Impact on Resistance (FAAIR) Scientific Advisory Panel makes the following recommendations and urges that policy reforms be implemented in a timely fashion. In some cases, these recommended policies will depend on Congress to enact legislation, whereas other changes may fall within the current regulatory authority of pertinent federal agencies and departments, including the Center for Veterinary Medicine at the Food and Drug Administration, the US Department of Agriculture, and the Environmental Protection Agency.

1. ANTIMICROBIAL AGENTS SHOULD NOT BE USED IN AGRICULTURE IN THE ABSENCE OF DISEASE

- Appropriate use of antimicrobials in food animal production should be limited to therapy for diseased individual animals and prophylaxis when disease is documented in the herd or flock.
- Use of antimicrobials for economic purposes such as growth promotion or enhancement of feed efficiency should be discontinued (with the exception of ionophores and coccidiostats).
- Where possible, alternatives such as changes in management, use of probiotics or competitive exclusion products, and vaccines should be encouraged. Pathogen reduction programs should also be continued or implemented where appropriate.
- Because of their critical role in treating human disease, fluoroquinolones and third-generation or higher cephalosporins should not be used in agriculture except to treat refractory infections in individual animals.

2. ANTIMICROBIALS SHOULD BE ADMINISTERED TO ANIMALS ONLY WHEN PRESCRIBED BY A VETERINARIAN

- Professional societies of veterinarians should develop and revise formularies according to prudent-use guidelines and should consider the likelihood that a given drug will promote resistance. These new formularies should be distributed to all veterinarians.
- Antimicrobials should be prescribed and administered in accordance with established guidelines concerning recommended dosage, interval, and duration to treat disease.
- Economic incentives that promote inappropriate prescription of antimicrobials in animals should be eliminated, and positive incentives should be introduced to encourage prudent use.
- Education efforts should target the elimination of specific areas of antimicrobial misuse.
- Similar guidelines and practices should be established and adhered to by plant pathologists, who guide antimicrobial use in plant agriculture.

3. QUANTITATIVE DATA ON ANTIMICROBIAL USE IN AGRICULTURE SHOULD BE MADE AVAILABLE TO INFORM PUBLIC POLICY

- Pharmaceutical manufacturers and importers should report the quantities of antimicrobials produced, imported, and sold. Reported data

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should include agent, formulation, intended animal species, and route of administration.
- Frequent end-user surveys should be conducted to assess the use of antimicrobials in agriculture.

4. THE ECOLOGY OF ANTIMICROBIAL RESISTANCE SHOULD BE CONSIDERED BY REGULATORY AGENCIES IN ASSESSING HUMAN HEALTH RISK ASSOCIATED WITH ANTIMICROBIAL USE IN AGRICULTURE

- An ecological perspective considers processes by which antimicrobial resistance spreads and persists in bacterial communities and the complex interactions between organisms, including pathogens, commensal bacteria, food animals, humans, and their environments.
- Risk assessment procedures should take into account both direct and environmentally mediated human health effects of agricultural antimicrobial use.
- Regulatory agencies should partner with research organizations to obtain scientifically valid information for use in risk analysis.
- When data are scarce, regulators should invoke the precautionary principle, and regulatory procedures should be revised when more data become available.

5. SURVEILLANCE PROGRAMS FOR ANTIMICROBIAL RESISTANCE SHOULD BE IMPROVED AND EXPANDED

- Surveillance programs should be harmonized to allow for linkage and joint analysis of data, particularly between animal and human data.
- Specific improvements should include standardization of sampling, culture, identification, and susceptibility testing methods. Protocols should be available to interested parties.
- Surveillance systems, including the National Antimicrobial Resistance Monitoring System, should be expanded to obtain greater numbers and more geographically diverse isolates of both commensal and pathogenic bacteria of humans, animals, and plants.
- Results should be published frequently and archived in databases.

6. THE ECOLOGY OF ANTIMICROBIAL RESISTANCE IN AGRICULTURE SHOULD BE A RESEARCH PRIORITY

More funding should be allocated for the purpose of antimicrobial resistance in agriculture. Priorities for research funding should include the following:

- Understanding the effects of antimicrobials as environmental contaminants, especially in areas adjacent to farms where antimicrobials are used.
- Increasing the understanding of the genesis and flow of resistance elements among bacterial populations and communities, including the role of commensal microorganisms as reservoirs of resistance.
- Increasing knowledge of the transfer of resistance among bacteria associated with humans, animals, and plants.
- Developing new approaches to infection control and growth promotion, such as vaccines, probiotics, improved management practices, and growth-promoting feed supplements without antimicrobials.
- Developing more accurate, cost-effective, and rapid laboratory techniques that will enable characterization of microbial isolates by serotype and strain.
- Developing and testing new risk-assessment models.

Regulatory agencies should provide for rapid review of alternatives to antimicrobials.

SUPPLEMENT ARTICLE

Emergence, Spread, and Environmental Effect of Antimicrobial Resistance: How Use of an Antimicrobial Anywhere Can Increase Resistance to Any Antimicrobial Anywhere Else

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Use of an antimicrobial agent selects for overgrowth of a bacterial strain that has a gene expressing resistance to the agent. It also selects for the assembly and evolution of complex genetic vectors encoding, expressing, linking, and spreading that and other resistance genes. Once evolved, a competitive construct of such genetic elements may spread widely through the world's bacterial populations. A bacterial isolate at any place may thus be resistant—not only because nearby use of antimicrobials had amplified such a genetic construct locally, but also because distant use had caused the construct or its components to evolve in the first place and spread there. The levels of resistance at any time and place may therefore reflect in part the total number of bacteria in the world exposed to antimicrobials up until then. Tracing the evolution and spread of such genetic elements through bacterial populations far from one another, such as those of animals and humans, can be facilitated by newer genetic methods.

Estimates of the costs to human health of antimicrobial use in animals are often based on the direct infection of humans through contact with animals or animal food products by an epizootic pathogen such as *Salmonella* or *Campylobacter*. There is growing evidence, however, that antimicrobial-resistance genes and their genetic vectors, once evolved in bacteria of any kind anywhere, can spread indirectly through the world's interconnecting commensal, environmental, and pathogenic bacterial populations to other kinds of bacteria anywhere else.

The evidence for widespread indirect dissemination of antimicrobial resistance comes from varied sources, such as microbial population biology, microbial genetics, and clinical and epidemiological observations. The purpose of this article is to sketch how evidence from such sources fits into a picture of widespread indirect dissemination.

DIVERSITY, INTERCONNECTIVITY, AND PERIODIC SELECTION IN BACTERIAL POPULATIONS

More than a billion trillion bacteria of diverse types live and compete on the world's people and animals and in the environment. Hypothetically, any one of them might replicate every half hour to generate a billion progeny overnight; however, in reality, any given bacterium has only a 50-50 chance of replicating successfully.

Each germ competes in a niche somewhere. A mutation in one of its thousands of enzymes might enable it to better use or tolerate something in the complex environment of that niche. By thus outgrowing its competitors even slightly, it could greatly amplify its prog-

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eny. Atwood et al. [1] noticed 50 years ago that mutants in a continuous culture of *Escherichia coli* would persist for hundreds of generations but abruptly disappear when a new advantageous mutation arose. They called this recurrent purging of diversity "periodic selection." Periodic selection is limited, however, by niche diversity. A new strain with even a small advantage in one of many available ecological niches might sweep through that niche, as in the flask, but lack that same advantage in the different conditions of the next niche. Niche diversity thus tends to limit periodic selection in the real world.

A strain of antimicrobial-resistant bacteria is a special case, however, because antimicrobial exposure affects all niches. In the presence of the agent, the strain has not a slight local advantage but rather a near-universal overwhelming advantage. Its competitors in all niches die, and its advantage overrides lesser niche-to-niche differences, enabling the strain to disseminate through many or all of the niches exposed to the agent. By thus diminishing effective niche compartmentalization, an antimicrobial agent has the potential to make a treated human, animal, or portion of the environment (or a group, as in an intensive care unit or feedlot)—or all antimicrobial treated hosts everywhere—more like a single flask.

RESISTANCE GENES AND THE EMERGENCE OF RESISTANCE

A strain of bacteria resistant to an antimicrobial agent usually differs from susceptible strains by being able to make a specific protein that inactivates the agent or otherwise circumvents the agent's damaging effect on bacteria. That specific protein is expressed by a resistance gene. Some resistance genes (e.g., those expressing resistance to quinolones or resistance in *Mycobacterium tuberculosis*) arise from mutations in genes native to the chromosome of the bacterial species in which they are found. Most resistance genes, however, differ from any genes in susceptible strains of the same species and are imported from outside of it via genetic vectors such as plasmids.

Before patients were first treated with antimicrobial agents, only 65 years ago, bacteria isolated from them had almost no resistance genes [2]. However, after each new agent became widely used, a gene expressing resistance to it ultimately emerged. Emergence means here that the resistance gene, wherever its origin, had spread enough to get into a strain of a species that was isolated and noticed as resistant by a clinical laboratory somewhere.

We can infer from molecular homology what the remote ancestors of some resistance genes may have been. We know little, however, of the events that occurred during the years that elapsed between the time an agent became widely used and the time that the first gene expressing resistance to it emerged. After each antimicrobial agent had become widely used, it presumably eventually encountered a strain of bacteria somewhere that expressed at least some slight level of resistance to the agent. Antimicrobial agents are dosed to attain high concentrations at sites of tissue infection, but gradients down to trace levels in nearby niches can give advantage to strains just resistant enough to survive such trace levels [3–5]. If this occurred in a strain of an obscure species, the evolved resistance gene might transfer on genetic vectors, perhaps repeatedly, before reaching a species that would ultimately be isolated in a clinical laboratory. Whatever the molecular details of the emergence of each resistance gene, and wherever they went on, the time elapsed and the amounts of agent use before emergence have usually been great. They suggest that an enormous number of encounters between agent and germs have been needed to produce the first emergence of most resistance genes.

Eventually, many different genes emerged to express resistance to some agents, such as trimethoprim, while only two have been described to express resistance to the oldest agent, sulfonamide. Some resistance genes may reemerge repeatedly from different origins; others appear to have spread widely from a few emergences—or perhaps only one [6].

GENETIC VECTORS OF RESISTANCE GENES

Resistance genes are most often encoded in extrachromosomal genetic elements or in segments that appear to have been recombined into the chromosome from other genomes. The largest of the extrachromosomal elements are the plasmids, which are self-replicating, double-stranded circles of DNA, some of which express mechanisms that transfer the plasmid to another bacterial cell. Bacteria isolated from patients 70 years ago or more, before antimicrobials were first used, had plasmids similar to those seen now, but then, the plasmids had no resistance genes [2].

Resistance genes encoded in plasmids are often located within segments called transposons. Functioning transposons include transposases that enable the transposon to recombine into other genomes; defective transposons have lost that capability. Such recombination can be demonstrated in vitro; evidence in vivo is provided by transposons with identical nucleotide sequences on a variety of different plasmids [7].

Resistance genes are often further clustered within elements called integrons, which are frequently found within transposons and plasmids but also found in bacterial chromosomes [8]. Each resistance gene in an integron is encoded in a mobile gene cassette that can be excised and then incorporated into another integron on another genome. Multiple cassettes with different resistance genes are commonly lined up, one after another, in an integron and expressed as a group from one upstream promoter [9].

In some species of bacteria, such as *Bacteroides,* a chromosomal

resistance gene may be within a conjugative transposon. The conjugative transposon may be excised to form an intermediate that may transfer and regenerate a double-stranded circle in another bacteria cell and integrate into its chromosome [10].

THE NEED FOR RESISTANCE ELEMENTS TO EVOLVE FOR COMPETITIVE SUCCESS

The wealth of gene recombination and transfer mechanisms might predict a near-infinite diversity of genetic resistance elements in clinical isolates. Whenever looked for in molecular detail, however, as seen in examples presented later, certain genetic elements, constructs, plasmids, and bacterial clones predominate in parts of the world or even throughout the whole world. Such observations remind us that resistant bacteria compete not only with susceptible bacteria but also with one another, both in the presence and in the absence of antimicrobial agents.

These considerations suggest the need for ongoing intricate evolutionary adjustments for competitive success. For example, plasmids vary in their host ranges and in the types of bacteria that they can infect; their stability within any type is affected by a variety of different mechanisms. Plasmids also appear to vary in the costs they impose on their host strains, and they (or the strains, or both) may evolve over time to reduce those costs [11]. A transposon made defective by an insertion into its transposase gene may still offer enough homology with another for homologous recombination. A resistance gene inserted in the first promoter-proximal cassette of an integron is expressed more effectively than it would be in a more distal cassette, resulting in a higher level of resistance to the antimicrobial agent [9]. Recombination frees resistance genes from the limitations of their original genetic backgrounds [12].

STEPS IN THE SPREAD OF AN EMERGED RESISTANCE GENE

If a resistance gene has emerged on the chromosome of a bacterial strain, its spread may depend mostly on that strain and thus be restricted by the fitness of that particular strain for various niches. However, the mechanisms described above can mobilize the resistance gene into another strain or species (e.g., genes for penicillin-resistant penicillin-binding proteins recombined from other streptococci into *Streptococcus pneumoniae* [13]) or into a mobile genetic element.

A resistance gene that has emerged on a plasmid or become inserted into one later may be transferred to other strains and species fit for niches not accessible to its original host strain. The resistance gene might also be within a transposon, an integron, or both, which could mobilize it to a different plasmid able to transfer to additional strains and species. Insertion of a resistance gene into progressively more plasmids carried by more strains and species extends its range and enables it to penetrate into more niches and persist longer after each antimicrobial exposure. Such insertions may put the resistance gene on plasmids already carrying genes expressing resistance to other agents, or even into integrons sharing promoters with such genes. Any of those agents will thereafter select for all of

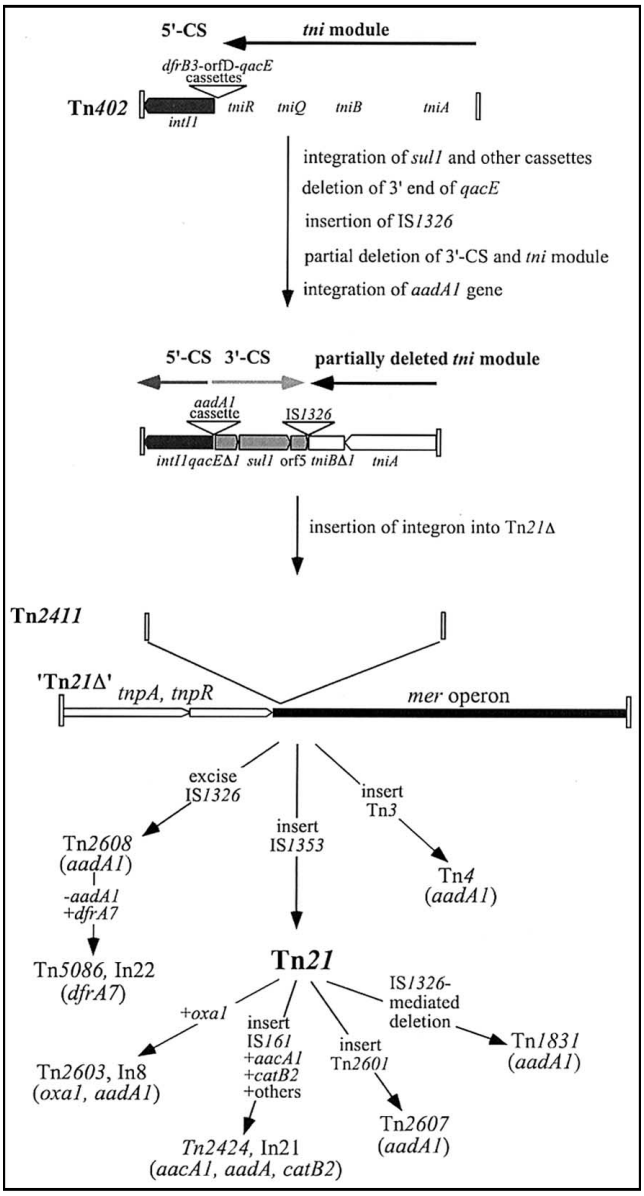

Figure 1. Reconstruction from sequence and other data of the evolution and diverging lineages of the widespread transposon *Tn*21. Within the expanded segments showing integrons, the 5′ conserved segments (CS) are represented by dark gray and the 3′ CS by light gray. Cassette insertion is indicated by a plus sign and excision by a minus sign. Presented here as an example of the complex evolution of a genetic vector, the figure is reproduced from *Microbiology and Molecular Biology Reviews* with the kind permission of the authors [8].

these now-linked resistance genes. The complexity of these processes may be exemplified with the evolution of Tn*21*, shown in figure 1 [8].

A rare mutational or recombinational event is required for each step in the evolution of genetic vectors encoding resistance genes. The chance of each such event occurring depends on the prevalence of the construct produced by the preceding event. This multistep assembly of increasingly fit, antimicrobial-resistant genetic constructs would thus be expected to occur mostly where large populations of bacteria are kept under intense antimicrobial selection to amplify and concentrate the elements being assembled. Such places may serve as unintended recombinant DNA "laboratories" for the development and export of more competitive resistance vectors.

SELECTION IN THE EMERGENCE, PACKAGING, AND DISSEMINATION OF RESISTANCE GENES

Some rough estimate of the selection necessary for the emergence of a new resistance gene might be made from the time elapsed between the onset of selection and the emergence of the gene or its product, as well as the total amount of selecting agents used. Similarly, an estimate for the amount of selection needed for the evolution of the "packaging" of the antimicrobial-resistance gene could come from measuring the time between the first detection of a gene or its product and its detection in the genetic constructs in which it is most widely spread. An estimate for the amount of selection needed for dissemination of the resistance constructs would incorporate the time period from then until the time those constructs actually became widespread. Although conceptually helpful, such estimations are difficult because the stages from emergence through wide dissemination are often not distinct. At the time the resistance genes emerged—and probably as part of the prolonged process of emergence—some resistance genes were already encoded in genetic constructs that succeeded at dissemination [14]. To improve our understanding of these processes, we need both wider searches for resistance genes from early in the use of each new agent and systematic, detailed tracing of the molecular lineage of successful genetic vector constructs.

The basic event in selection is simple. Enough molecules of the antimicrobial agent impinge on a bacterial cell that is about to divide to stop it from doing so, while in its place another cell divides that would not otherwise have divided. The second cell divides either because it was not inhibited by the same exposure (i.e., had some level of resistance) or because it did not quite get that same exposure (e.g., by being a bit away and coming into the space later). We may try to estimate how many times that basic event occurs at any place as a measure of the magnitude of antimicrobial selection there. This measure would reflect both the size of the bacterial populations being exposed to antimicrobials and the duration of that exposure. Such a measure might, for example, show the magnitude of selection in the intestines and the environment of all of the cattle, pigs, and poultry in the United States to equal or exceed that in humans, even if the tonnage of antimicrobials given to animals were less than that given to humans.

Beyond this basic event, other variables may supervene. Exposure to low concentrations of antimicrobials may select for some types of resistance that progress by small increments and would be obliterated by higher concentrations [7]. Genetic vectors of resistance that were concentrated in certain bacterial populations by prolonged exposure to antimicrobial agents might have a greater chance to recombine and evolve to greater efficiency than those allowed to become sparse during intervals of no exposure. Certain agents may drive the evolution and spread of resistance vector constructs more effectively in certain populations of bacteria because of the previous deployment of particular resistance genes and vectors.

SPREAD OF RESISTANCE

A resistant strain made prevalent by selection in the bacterial populations of one host is more likely to be among the strains that the host transfers to a second host [15]. Similar selection in the second host would boost the strain's chances of becoming established, amplified, and then transferred to a third host. These considerations would predict that resistant strains travel the world selectively through networks of hosts being treated with antimicrobial agents. The experiences of intensive care units, day care centers, and feedlots tend to confirm this prediction.

Relieved of competition from susceptible strains, resistant strains spreading through networks of antimicrobial-treated hosts would compete more directly with one another. So would strains carrying copies of a resistance vector construct at different stages of its evolution. A vector with an additional resistance gene could prevail in hosts treated with that agent over a vector that had not. A vector with improved stability in a strain or strains carrying it could similarly confer an advantage to those strain or strains. In such ways, the resistance vector construct emerging from a long chain of transmission through treated hosts could be more competitive and more persistent than either the original vector or the other vectors that it had marginalized along the way.

Examples of emerged resistance constructs spreading throughout the world. The history of antimicrobial resistance, in the examples where it can be delineated, has often been that of a successful resistance construct evolving under selection somewhere, emerging under further selection, and then spreading nearly everywhere. Strains of *Staphylococcus aureus* belonging to a few phage types that possessed an inducible

penicillinase gradually spread throughout the world's hospitals in the 1950s and throughout communities everywhere in the 1960s [16]. A few clones with intricate constructs expressing the *mec* gene then spread methicillin resistance through the world's hospitals [17, 18].

In the 1970s, a single plasmid carried gentamicin resistance to several genera of enteric bacteria in a number of hospitals in different parts of the United States and in one hospital in Venezuela, none of which had seen any gentamicin-resistant enteric bacteria until then [19]. A particular transposon, Tn*1331,* was first noted to encode amikacin resistance in Argentina and Chile but was later seen in other parts of the world [10]. Resistance to sulfonamides, possibly the most prevalent type of resistance, has been found throughout the world encoded by only 2 resistance genes [20]. One of them was found to be virtually ubiquitous on a small multicopy plasmid [21] also carrying a streptomycin-resistance gene; this may contribute to the persistence of resistance to streptomycin decades after its use in human therapy has almost completely ceased. Several multidrug-resistant plasmids found to be endemic in several *Salmonella* serotypes isolated from animals in some US states were found in clinical isolates of the same serotypes from humans infected in distant states [22].

After decades of penicillin use, certain strains of *Streptococcus pneumoniae* acquired long, contiguous nucleotide sequences expressing foreign penicillin-binding proteins that made the strains resistant to penicillin [13]. Once arisen, these new chromosomal genetic constructs spread clonally in strains of pneumococci belonging to serotypes that could be traced through countries and continents [23]. One such construct in serotype 6B, originally prevalent in Spain, was later found in other countries in the Western Hemisphere; its eventual incursion into Iceland accounted for nearly all penicillin-resistant pneumococci there [24].

New example of vancomycin-resistant enterococci. The emergence over the last decade of vancomycin-resistant enterococci (VRE) is especially relevant to discussions of the spread of resistance. Vancomycin had been in use for >20 years before anyone saw resistance to it acquired in a species of bacteria that had not always been resistant to it. This history may be explained by the finding that not only the resistance-carrying plasmid and transposon but also the resistance genes themselves comprised an elaborate genetic construct. Nine genes, with codon use indicating varied origins, were arranged in an operon expressing 9 products that interacted to produce and regulate the resistance; this implies enormous selection [14, 25].

Within a few years of its emergence, VRE had spread to become a growing nosocomial problem in US hospitals, where the further shift of plasmids carrying it into already multidrug-resistant *Enterococcus faecium* produced a nearly untreatable antimicrobial-resistance construct [26, 27]. It may be too early to say whether US hospitals are a special niche for VRE or just happen to be early in the path of their spread, just as those hospitals happened to be late in the global spread of methicillin-resistant *S. aureus.*

A high degree of genomic heterogeneity in the plasmids of VRE isolates has impaired the tracing of their lineage to date [28]. An important issue arose when it was found that in Europe, unlike the United States, VRE are widely distributed in the community, in farm and pet animals, and in meat and meat products, but are still uncommon in hospitals. Concern that this was due to selection by the use for growth promotion in animals in Europe of a vancomycin analog, avoparcin, led to its being banned there in 1997 [29–32]. The possibility remains that VRE evolved in the vast bacterial populations of animals fed avoparcin in Europe and were then exported to a niche in US hospitals, in which they can now flourish but might not have been able to evolve. If further evidence for this develops, it would provide a special example of the concepts discussed here.

***Resistance in* E. coli.** Numerous published reports of common-source food outbreaks in which the implicated food product had an animal source have established that *Salmonella* and *Campylobacter* infecting humans in developed countries come mostly from animal or other agricultural sources [33–36]. *Salmonella* have been especially conspicuous, not just because they are epizootic pathogens causing illness in both animals and humans, but also because wide availability of an elaborate serotyping system makes them easy to trace. However, although the relative rarity of *Salmonella,* as compared with *E. coli,* makes *Salmonella* especially useful for tracing the spread of resistance, it limits their ability to measure the magnitude of that spread. For every *Salmonella* in the colons of humans or animals, there are thousands or more *E. coli,* subject to the same antimicrobial selection and capable of carrying and spreading the same or similar genetic resistance elements.

The abundance of *E. coli* implicates them as the likely predominant vehicles for the spread of resistance genes and vectors, as opposed to the spread of infection, between the bacterial populations of animals and humans; however, their abundance also makes such spread difficult to trace. A new strain is unlikely to be noticed, especially if the strain does not cause illness, and there is little typing of *E. coli* strains. However, there is a screening and serotyping program to detect *E. coli* strain O157:H7, an antimicrobial-susceptible but pathogenic strain, and there are now many examples of this strain spreading from animals to humans [36]. If antimicrobial-resistant *E. coli* from animals also flow to humans in similar proportion, they would be the major route for such spread of resistance.

Two recent lines of evidence further indicate a role for *E. coli* in the spread of resistance between animals and humans. Studies from Spain and Taiwan, where quinolones are used in

commercial poultry production, have found that a large proportion of retail chicken carcasses now carry strains of *E. coli* with reduced susceptibility to quinolones; a rising percentage of isolates from humans, including children, also have reduced susceptibility. Because quinolones are not indicated for treatment of children, the resistance presumably did not originate within the *E. coli* bacteria while the children were carrying them [37, 38].

Another recent report found the same multidrug-resistant clone of *E. coli* to be causing urinary tract infections in multiple patients at widely separated locations in 3 different states in the United States; a similar report from the United Kingdom a decade ago was referenced. Neither directly identified a food source. The authors of the report did, however, question how a clone could gain such wide distribution and noted the similarity of that distribution to that of multistate *Salmonella* outbreaks due to a contaminated food product distributed through the food chain [39].

MANAGING THE GLOBAL MARKETPLACE FOR COMPETITIVE RESISTANCE CONSTRUCTS

On the basis of what we know of the genetic elements that carry resistance and what we have observed of the real-world spread of resistance, we can describe a model that appears to have analogies in economics. If we could imagine that a city had been completely isolated from the rest of the world for the past century but had used antimicrobials exactly as it actually did use them during that time, we would expect it to have less resistance. That city's use of antimicrobials would not have been enough to select the constructs, the methicillin-resistant *S. aureus,* the VRE, and so on, that the whole world's use in reality has selected and delivered. This is analogous to the fact that the city alone could not have invented and produced all the products it uses. A superior product developed anywhere may come to prevail everywhere.

Management of such systems necessitates restraint and understanding. The global interdependence of antimicrobial resistance requires that we restrain antimicrobial use to its essential minimum—not just locally, but everywhere in the world. The intricacy of the processes involved in the spread of resistance suggests that we might control them better if we understood them better.

The total genome of a resistant strain of bacteria and of its accessory genetic elements accumulates evidence of their lineages, including mutations, recombinations, acquisitions, and deletions (figure 1) [4]. Being able to read these would help greatly in tracing, understanding, and controlling the spread of antimicrobial resistance. Rapid and inexpensive nucleotide sequencing can now facilitate this task but will have to be focused by all other analyses on those elements that can provide the most epidemiological information [40]. The goal would be to define in detail the predominant resistance constructs in the bacterial populations of animals and humans and to identify their reservoirs, the sequences of their assembly and evolution, paths of spread, relationships to antimicrobial use, and the optimal means for their containment.

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Generally Overlooked Fundamentals of Bacterial Genetics and Ecology

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Several important aspects of the antimicrobial resistance problem have not been treated extensively in previous monographs on this subject. This section very briefly updates information on these topics and suggests how this information is of value in assessing the contributions of human and agricultural use of antimicrobial agents on the problem of increasing antimicrobial resistance. The overall themes are (1) that propagation of resistance is an ecological problem, and thus (2) that ameliorating this problem requires recognition of long-established information on the commensal microbiota of mammals, as well as that of recent molecular understanding of the genetic agents involved in the movement of resistance genes.

THE ECOLOGY OF ANTIMICROBIAL RESISTANCE

Although antimicrobial resistance has traditionally been viewed as a problem of the treatment (or treatment failure) of an individual patient in a given clinical setting, it is actually an ecological problem. The science of ecology (the study of how living systems interact with each other and with their nonliving environment) is a relatively new one and has only recently begun to impinge on the practices of Western medicine and agriculture, the settings that have given rise to the antimicrobial resistance problem.

One aspect of ecology, the relationship between a host macroorganism and its commensal microorganisms, is especially significant in this context but has also received only scant attention compared with extensive work on individual pathogenic bacteria. Growing evidence indicates that, with respect to the resistance problem, the 2 most important aspects of the host-commensal ecosystem are (1) that it can serve as a relatively stable reservoir of resistant microorganisms (including potentially pathogenic ones) long after cessation of antimicrobial treatment, and (2) that host macroorganisms are continually being reinoculated by microorganisms from their environments. How do these 2 phenomena impinge on the occurrence and spread of antimicrobial resistance?

THE RESISTANCE RESERVOIR

In general, pathogenic bacteria differ little in their basic cellular biology from commensal bacteria. For example, a pathogen may have the ability to make toxins or invasion factors, but in the fundamental cellular processes targeted by all classical antimicrobials, pathogenic bacteria do not differ significantly from the benign commensal bacteria. This similarity makes sense because a pathogen also needs to be able to grow in or on the host that it invades. The consequences of this are 3-fold: (1) elimination of both benign commensal microbiota and pathogens by antimicrobials, (2) genetic exchange of antimicrobial-resistance genes in the commensal ecosystem, and (3) reinoculation of commensal ecosystems.

Elimination of susceptible commensals and pathogens by broad-spectrum antimicrobials. Administration of an antimicrobial agent may not only kill the pathogen but will also change the composition of the

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commensal ecosystem. Benign commensal bacteria that lack the relevant resistance gene will die, and those that, by chance, have the relevant resistance gene will proliferate and expand into the niche abandoned by the exterminated antimicrobial-susceptible bacteria. This is true for humans, animals, and plants, all of which have their own type of commensal microbiota (see also Barza, Swartz, this supplement).

For example, a class of antimicrobial agents that can affect the commensal flora is the ionophores (such as monensin). Ionophores are deemed acceptable to use in animals because they are not used in human medicine and because resistance to them in pathogens of interest has not been observed. Ionophores target almost exclusively gram-positive bacteria, which constitute a large proportion of the intestinal tract of humans (table 1) [1] and animals (including ruminants). Administration of ionophores causes a shift in the microbial populations colonizing the animal intestinal tract [2, 3] with a concomitant potential for more facile establishment of pathogens.

It has been assumed that after the antimicrobial treatment is completed, antimicrobial-resistant commensal bacteria will not have any selective advantage and will lose out in competition with the antimicrobial-susceptible commensal bacteria. Although declines in the incidence of bacteria with resistance to a specific antimicrobial agent have been noted in individual hospitals [4] or farms [5] after use of the specific agent has been restricted or discontinued, a return to the preterm level of antimicrobial resistance does not usually occur. The widespread dissemination of antimicrobial resistance genes in nonhospitalized humans, most of whom are not undergoing antimicrobial treatment, strongly suggests that resistant strains can persist in the commensal microbiota in the absence of selection by any one antimicrobial agent [6–10]. Possible molecular bases for this phenomenon are considered below.

Genetic exchange in commensal ecosystems. Here again, there is limited information on the rates and extent of such exchanges, although existing data do show that it can occur [11–14]. The high numbers of bacteria and the rich nutritional resources of most commensal niches make them ideal settings for gene exchange. Thus, even without colonizing, an entering pathogen might obtain resistance genes from the commensal microbiota. Alternatively, a transient benign microbe carrying a resistance plasmid might transfer the plasmid to another commensal bacterium during passage through the human intestinal tract or while the bacteria reside on the skin or the mucosal surfaces of the upper respiratory tract or vagina.

Reinoculation of commensal ecosystems. Although animals and plants are most obviously reinoculated by environmental sources, humans are also continuously exposed to exogenous bacteria, benign and pathogenic, from other humans, animals, fomites, and food [15–17]. Nonspecific host defenses, including secreted lytic enzymes and stomach acidity in animals (including humans), limit colonization by these exogenous bacteria. However, one of the most potent factors involved in preventing colonization by pathogens is the competitive effect of the autochthonous microbiota (see also Barza, this supplement). For example, the intestinal tracts of most mammals are colonized by ~20 or so different genera, with the majority of commensal bacteria belonging to as few as 6–8 genera of bacteria [18, 19]. However, there may be as many as 400 different species that colonize this niche even in humans; many strains

Table 1. Typical numbers of prokaryotes in human feces.

		Log_{10} no. of cultivatable organisms per gram of feces (dry weight)	
Genus or group	Gram stain group	Mean	Range
Bacteroides	Negative	11.3	9.2–13.5
Eubacterium	Positive, low G + C	10.7	5.0–13.3
Anaerobic cocci	Positive, low G + C	10.7	4.0–13.4
Bifidobacterium	Positive, high G + C	10.2	4.9–13.4
Clostridium	Positive, low G + C	9.8	3.8–13.1
Lactobacillus	Positive, low G + C	9.6	3.6–12.5
Actinomyces	Positive, high G + C	9.2	5.7–11.1
Propionibacterium	Positive, high G + C	8.9	4.3–12.0
Streptococcus	Positive, low G + C	8.9	3.9–12.9
Enterobacteriaceae	Negative	8.7	4.0–12.4
Fusobacterium	Negative	8.4	5.1–11.0
Other facultative anaerobes	Negative	6.8	0.7–12.7

NOTE. From Finegold et al. [1]. C + G, content of guanine and cytosine in microbe's chromosome.

of these species are distinct from those of the same genus that colonize nonhuman mammals. Moreover, in any individual, the ensemble of strains can be quite idiosyncratic [20].

With such extensive and specific barriers to colonization by exogenous bacteria, it is hard to see how colonization by transient benign or pathogenic microbes could take place at all. Recent research indicates that, for unknown reasons, the intestinal microbiota may be quite dynamic and, in some people, readily subject to turnover of species and strains [20]. Such individuals, as well as those whose commensal bacteria had been recently eliminated by antimicrobial treatment [21], might be especially subject to recolonization with allochthonous commensals or even pathogens.

GENETIC LINKAGE OF ANTIMICROBIAL RESISTANCES

Most antimicrobial-resistance genes in bacteria occur in genetically linked arrays. Largely overlooked in the epidemiological literature on the spread of antimicrobial resistance is explicit consideration of the well-established fact that the multiple antimicrobial resistances in most clinically isolated Enterobacteriaceae are not the result of single, sequential, chance spontaneous mutations of the target genes of the antimicrobial agents occurring in all of these strains. Rather, in most cases, especially in the last 2 decades, such multiresistant strains result from acquisition of tandem arrays of genetically linked resistance genes borne by integrons or other transposons that can reside in the chromosome and on conjugative or mobilizable plasmids [22]. Although single point mutations in target genes can give rise to the resistance genes [23, 24], mutations are rare events (10^{-9} to 10^{-8} per cell per generation) [25] in comparison with the transfer of resistance genes (previously derived from such mutant chromosomal genes) now carried by mobile plasmids and transposons in many bacteria in humans, animals, plants, and the environment (10^{-5} to 10^{-4} per cell per generation). Indeed, in the case of the conjugative transposons that can operate in both gram-positive and gram-negative bacteria, transfer of the resistance element from cell to cell can be induced by antimicrobial exposure [26].

Genetically linked transmissible resistance was first reported in hospital isolates in the early 1960s, just 20 or so years after the widespread introduction of antimicrobial therapy for infectious diseases [27–31]. Transmissible resistance has been reported with increasing frequency in clinical isolates and in agricultural and environmental isolates [32–41] from the mid-1970s to the present. There was ample evidence as early as 1978 that such plasmids were distributed worldwide in hospital and environmental settings [42]. In many cases, the plasmids carrying the multidrug-resistant gene cassettes have been extensively documented and even sequenced [43].

Implications of genetic linkage for spread of multiresistance. Each gene cassette can provide resistance to a chemically distinct class of antimicrobial agents. The first ominous aspect of multiple-resistance transfer agents, such as plasmids, transposons, and integrons, is that they can collect and recombine extant resistance gene cassettes in almost any combination [44, 45]. Consequently, treatment with any given antimicrobial agent can result in selection for bacteria resistant not only to that specific agent, but by genetic linkage of resistance genes, to other unrelated antimicrobial agents. The consequence of this "mix and match" gene cassette transfer is not realized by most clinicians, who, in treating with an aminoglycoside, assume they will only be selecting for strains resistant to that antimicrobial agent (or to a narrow cross-resistant set of related aminoglycosides). This would be true if the above-noted assumption about spontaneous point mutations were actually the basis for most nosocomial or community-acquired resistance; indeed, 60 years ago, this might have been true. However, multiresistant isolates can now be readily isolated from all populations of contemporary human-associated bacteria (commensals or pathogens), as well as many related bacteria associated with domestic animals or with commercial horticultural crops, and in bodies of water experiencing any human, urban, or agricultural effects [35, 37, 38, 40, 46]. Selecting (by treating) with one antimicrobial agent will enrich the population for strains resistant to all antimicrobial agents whose resistance genes are genetically linked to the one for the antimicrobial agent used.

Moreover, nonantimicrobial environmental toxins such as heavy metals can also select for multidrug-resistant plasmids. Copper is frequently used in horticulture [47], and arsenicals are frequently used in animal feed [48, 49] (McEwen and Fedorka-Cray, this supplement) and horticulture [50] (W. K. Vencill, personal communication; T. Murphy, personal communication). Humans have used heavy metals, including arsenic, silver, and mercury, as medications from ancient times and now continuously ingest mercury from dental restorations [51]. Resistances to all these metals and many others that occur in the environment, including cadmium, lead, cobalt, nickel, and tin, are found on plasmids of both clinical and environmental gram-positive and gram-negative bacteria [47, 52, 53], in many cases along with antimicrobial-resistance genes [54, 55].

Thus, use of any antimicrobial or other selective agent selects for all the resistance genes in these arrays as well as for the plasmids where they reside. Regardless of the fact that they confer resistance to distinct chemical classes of antimicrobial agents, these genes are coselected because they are physically linked to each other. The reality of this genetic relatedness (i.e., actual physical linkage of genes) trumps control efforts that are based only on the more limited concept of chemical relatedness. This may be why, despite periodic cycling of antimicrobial

agents in hospitals or agricultural settings (see below), the prevalence of multiresistant bacteria not only does not diminish but even continues to increase [6, 56]. Unfortunately, recognition of the importance of physical linkage of resistance genes still escapes policy makers, who continue to promulgate guidelines for animal use solely on the basis of whether an antimicrobial agent is used (or is similar to one used) in human medicine [57].

Implications of linked resistance for the usable "lifetime" of newly introduced antimicrobial agents. The second ominous aspect of these multiple resistance transfer agents is that they appear to be quite ancient, widely distributed among human-, animal-, and plant-associated eubacteria, capable of moving readily among all members of the eubacteria, and capable of accumulating and disseminating new resistance genes as they arise by spontaneous point mutations under strong selection or as they occur in antimicrobial-producing bacteria [57, 58]. Such mobile elements are the engines of the ubiquitous horizontal transfer of all kinds of genes; they antedated by millennia the widespread use of antimicrobials by humans. The resistance-bearing versions of these mobile elements were probably originally selected by exposure to antimicrobial agents or heavy metals in the environment. As noted above, the origin of some of the genes currently spread by plasmids and transposons was a spontaneous mutation in the chromosomally encoded target gene. Where did the other resistance genes come from originally? As Julian Davies postulated [57], and as has since been proven in many cases, many were recruited from the chromosomal genes of antibiotic-producing soil bacteria (e.g., the aminoglycoside resistances). Others are adapted versions of the ubiquitous efflux pumps present in the genomes of most eubacteria (e.g., the quaternary ammonium resistance gene, *qac*) [59, 60]. Regardless of their sources, the key concept here is that these genes were "recruited" from their original chromosomes and assembled into tandem arrays on transmissible genetic elements. The "recruiting agent" may be one or more other genes carried by the mobile elements (e.g., the integrases and insertion sites of the integrons) or other genes yet to be identified.

Although as yet there are no biochemical data on the agents of initial recruitment, it is worth noting that variant integrase genes are found in every eubacterial genomic sequence yet reported [61, 62], often in huge arrays [63]. All sequenced eubacterial chromosomes also show evidence of considerable horizontal gene exchange, suggesting that when a spontaneous point mutation to resistance arises in the target gene of a novel antimicrobial agent, there is a finite (but unknown) chance for it to be picked up by ubiquitous transmissible plasmids, transposons, and bacteriophages and spread to other bacteria.

Although the existence of linked multiple resistance genes on bacterial plasmids and transposons has been known for >2 decades, most of the extant literature on antimicrobial resistance is based on epidemiologic prevalence studies, usually reporting assessment of a single resistance gene in a single organism of interest to the investigator (or to those supporting the study) [23, 64–70]. Thus, information on the degree to which linked resistances allow for selection (and thus persistence) of unrelated resistance genes is difficult to discern. However, some recognition of these well-established, underlying molecular processes is beginning to appear in studies of antimicrobial resistance [9, 71].

BASIS FOR THE PERSISTENCE OF ANTIMICROBIAL RESISTANCE

Early studies of plasmids were plagued by high spontaneous losses of these elements from laboratory strains unless selective pressure was exerted. This gave rise to a reasonable assumption that later became unexamined dogma: only selective pressure by antimicrobials kept plasmids in a population; lacking selective pressure, bacteria carrying these genes would be at a disadvantage and would be lost from any ecosystem. However, during the last 40 years, many studies have demonstrated the persistence of antimicrobial-resistant bacteria even after antimicrobial use was discontinued in hospital [72, 73], community [74], and agricultural settings [5, 56, 75]. More recent studies have also demonstrated the presence of antimicrobial-resistant bacteria in contexts where antimicrobial agents have not been used [6, 76]. Likely this persistence is based in part on the phenomenon of linkage described above; periodic exposure to any antimicrobial will maintain a multiresistance array in a bacterial population. However, there are several additional reasons why early assumptions about ready loss of resistance in the absence of selection are not correct.

PLASMID-ADDICTION SYSTEMS

Bacterial plasmids carry genes that kill a daughter cell if it fails to get a copy of the plasmid upon division. These genes, referred to as "plasmid addiction" [77] or "postsegregational killing" [78] systems, come in various forms, but all result in a kind of bacterial apoptosis, destroying any daughter cell that chances to "give up" its plasmid. Such addiction systems are ubiquitous among large transmissible plasmids of gram-negative bacteria. At equilibrium, the concentration (prevalence, in this context) of any component in a system (e.g., a resistant bacterium) is determined equally by its rate of synthesis (acquisition of resistance genes) and its rate of decay (loss of resistance genes). Plasmids can not only control their own acquisition via conjugation or mobilization but can also prevent their loss. As a result, periodic selection for any plasmidborne resistance gene

allows that plasmid and all the genes it carries to become a fixed component of the population.

ADAPTIVE MUTATIONS IN CHROMOSOMAL GENES

Drug efflux pumps. Among the largest class of functions found in many prokaryotic genomes are membrane-mounted protein pumps for ridding the cell of toxic substances ranging from metal ions to disinfectants to antimicrobials [60, 79]. Often similar in structure, and usually dependent on ATP or the proton motive force as an energy source, these ubiquitous P- or ABC-type transporters are homologs to the multidrug transporters of higher eukaryotes. Most of these pumps are indiscriminate in their substrate range, frequently handling a wide variety of either hydrophobic or ionic substrates. The resistance they provide to any particular antimicrobial agent is less than the resistance provided by a mutation in the target gene, conferring intrinsic resistance, or even the resistances provided by the various plasmidborne genes. They can also readily mutate to provide slightly higher resistance, often as a result of increased expression [79]. Occurrence of such variants among clinical isolates suggests that these pumps do play a real-world role in multidrug resistance. Several such pumps, including those providing resistance to tetracycline, quaternary ammonium compounds, and a variety of toxic metal ions often used as disinfectants, have moved from the chromosome to plasmids and enjoy worldwide distribution in human- animal-, and plant-associated bacteria as well as in bacteria in fresh and estuarine waters.

Compensatory chromosomal mutations. Because there is often a cost of any alteration in the target gene as it becomes resistant to an antimicrobial agent, cells have ways of adapting to offset this evolutionary cost. The periodic selection of chromosomal mutations has been shown in laboratory strains to compensate [80] for loss of fitness engendered either by plasmid carriage or spontaneous chromosomal resistance mutations. It is not known to what degree such spontaneous adaptations contribute to persistence of resistance in field isolates, although *Pseudomonas* strains colonizing patients with cystic fibrosis often have an enhanced rate of mutation [81].

EFFORTS AT CONTROL BASED ON SWITCHING THE ANTIMICROBIAL AGENT USED

The strategy of changing the antimicrobial agent used is most often referred to as "antibiotic cycling" and involves the replacement in a human medical or agricultural setting of an antimicrobial agent to which many bacteria in the community exhibit resistance with a different antimicrobial for which there is less resistance in the particular environment at the moment [4]. In the United States, when this is done in human medicine, it is limited to hospital rather than community practice; in countries such as Denmark [82], managed care also allows such regimens to be implemented via outpatient therapy. In the United States, cycling regimes in clinical practice demonstrate highly varied, but generally limited, success [73].

Missing in reports of such cycling practices is any ecological perspective—that is, long-term data that might reveal any general trends in the background level even in a single treatment unit or throughout the entire hospital or farm after repeated rounds of such cycling. Is it ever possible to achieve a "pre-antibiotic" prevalence of susceptibility, even for a single antimicrobial agent? Does subsequent reintroduction of the replaced antimicrobial result in a faster increase in resistance during the second "cycle" of its use? Also generally missing are data on the effect of replacement on the prevalence of resistances to other antimicrobial agents, especially those that might be genetically linked to the resistance locus whose reduction is desired. Moreover, owing to the stochastic occurrence of the need as well as ethical requirements to implement such interventions, carefully matched replications of such experiments are lacking [73]. Metastudies, as well as encouragement to report unsuccessful cycling attempts, would contribute considerably to evaluating the actual utility of this practice, the basis of which is questionable in light of linkage of unrelated resistance genes.

Finally, the implicit assumption that a decline in the prevalence of a resistant bacterium in a single hospital or farm unit as a result of antimicrobial withdrawal can be mapped precisely onto the behavior of these bacteria in the individual human or animal treated is erroneous. Notable reduction in resistance prevalence often takes weeks or months to occur (if it does so at all) [6, 56, 83], and the effect of antimicrobial withdrawal is rarely deconvoluted from replacement by antimicrobial-naive patients or animals (who simply lack the resistance locus in question) entering the hospital or farm. Reports of even modestly "successful" (e.g., 2-fold) reduction in the numbers of bacteria resistant to a single antimicrobial agent in a single hospital have thus given rise to the narrow view among physicians that it is possible, by these practices, to control the spread of resistance. This perspective overlooks the fact that discharged patients spread their resistant microbes to their home communities, as do outpatients.

ANTIMICROBIAL RESIDUES IN SURFACE WATERS

A final ecological issue that is gaining prominence is the presence and possible effect of myriad pharmaceutical residues, including antimicrobials, in sources of potable water [35, 84–87]. When detected, most antimicrobial agents appear intact, rather than as metabolites. The exception is erythromycin,

several metabolites of which are often found. Most antimicrobials are below detectable limits; only erythromycin (and its derivatives) are routinely found at detectable levels. Various sulfa derivatives are also more frequently found, whereas tetracycline and penicillin are almost never observed. In all cases, the amounts observed are in the parts per billion range, ~1000-fold below what would select for enrichment of resistant bacteria.

Thus, it is reasonable to conclude that resistant bacteria found in surface waters have not been selected by the vanishingly small amounts of antimicrobial agents in those waters but have traveled there via animal or insect vectors, in airborne dusts [88, 89], or simply in the flow of the waters after being released from some antimicrobial-rich setting. However, as noted above, given the stability of plasmids and other resistance replicons, thousands of bacterial generations may have taken place since that selective exposure.

CONCLUSION

The continuous exchange of bacteria between humans and their environment and among the genetic elements of these bacteria means that imposition of selection on any microbial ecosystem will result in proliferation of highly resistant bacteria. Long-established molecular mechanisms inherent in the bacteria themselves ensure that, once acquired, these resistance genes will be lost very slowly (and maybe not at all) from their large and ubiquitous populations. Discovering new antibiotics will buy us time, but the same ancient molecular mechanisms will ensure their eventual loss of efficacy as well. All sectors that use antibiotics—human medical, veterinary, and horticultural—need to cooperate in devising novel methods to minimize proliferation of resistant bacteria while meeting their respective therapeutic and economic needs. Such cooperative efforts must be based on a thorough grasp of the population biology of commensal as well as pathogenic bacteria, the mechanisms of gene exchange among them, and the simple ecological principle that everything is connected to everything else.

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Antimicrobial Use and Resistance in Animals

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Food animals in the United States are often exposed to antimicrobials to treat and prevent infectious disease or to promote growth. Many of these antimicrobials are identical to or closely resemble drugs used in humans. Precise figures for the quantity of antimicrobials used in animals are not publicly available in the United States, and estimates vary widely. Antimicrobial resistance has emerged in zoonotic enteropathogens (e.g., *Salmonella* spp., *Campylobacter* spp.), commensal bacteria (e.g., *Escherichia coli*, enterococci), and bacterial pathogens of animals (e.g., *Pasteurella, Actinobacillus* spp.), but the prevalence of resistance varies. Antimicrobial resistance emerges from the use of antimicrobials in animals and the subsequent transfer of resistance genes and bacteria among animals and animal products and the environment. To slow the development of resistance, some countries have restricted antimicrobial use in feed, and some groups advocate similar measures in the United States. Alternatives to growth-promoting and prophylactic uses of antimicrobials in agriculture include improved management practices, wider use of vaccines, and introduction of probiotics. Monitoring programs, prudent use guidelines, and educational campaigns provide approaches to minimize the further development of antimicrobial resistance.

INDICATIONS FOR ANTIMICROBIAL USE IN FOOD ANIMALS

Antimicrobials are used in food animals to treat or prevent disease and also to promote growth (table 1). Various sources provide data on such uses of antimicrobials in animals, including dosing schedules, contraindications, and withdrawal times [1–3].

Therapeutic treatments are intended for animals that are diseased. In food animal production, individual animals may be treated, but it is often more efficient to treat entire groups by medicating feed or water. For some animals, such as poultry and fish, mass medication is the only feasible means of treatment. Certain mass-medication procedures, called metaphylaxis, aim to treat sick animals while medicating others in the group to prevent disease. Other prophylactic antimicrobial treatments are typically used during high-risk periods for infectious disease (e.g., after weaning or transport). Terminology is not uniform. For example, the American Veterinary Medical Association defines "therapeutic" as including treatment, control, and prevention of bacterial disease [4]. Typically, metaphylaxis involves administering drugs at therapeutic levels for short periods of time.

Some antimicrobials, described as coccidiostats (e.g., ionophores, sulfonamides), prevent coccidiosis, a common parasitic disease of poultry. Some coccidiostats, which are administered in feed at strategic intervals, also have antibacterial properties. Withdrawal times for antimicrobials are intended to prevent harmful drug residues in meat, milk, and eggs. These waiting periods, which are indicated on labels, must be observed between treatment and slaughter [2, 3]. Meat and meat products that contain antimicrobial residues exceeding a certain level at the end of the withdrawal period may be banned from human consumption [1].

Producers may also administer antimicrobials to food animals (except farmed fish) to promote growth and to enhance feed efficiency. The distinction between

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disease prophylaxis and growth promotion is less clear than between prophylaxis and therapy. In North America, certain antimicrobial drugs may be approved for both purposes, and some growth promoters may help to prevent disease, even at subtherapeutic doses [5]. This is an important point because administration of antimicrobials, at least for limited time periods, can almost always be justified on the grounds of disease prevention. Growth promoters are usually administered in relatively low concentrations, ranging from 2.5 to 125 mg/kg (ppm), depending on the drug and species treated [5–9]. In the United States, "subtherapeutic" means uses of antimicrobials in feeds at concentrations <200 g per ton for >2 weeks [10]. However, the term "nontherapeutic," which seems more precise [11], could include both growth-promotion and disease-prophylactic uses. In practice, nontherapeutic treatment often occurs early in production and is typically discontinued as the animals mature.

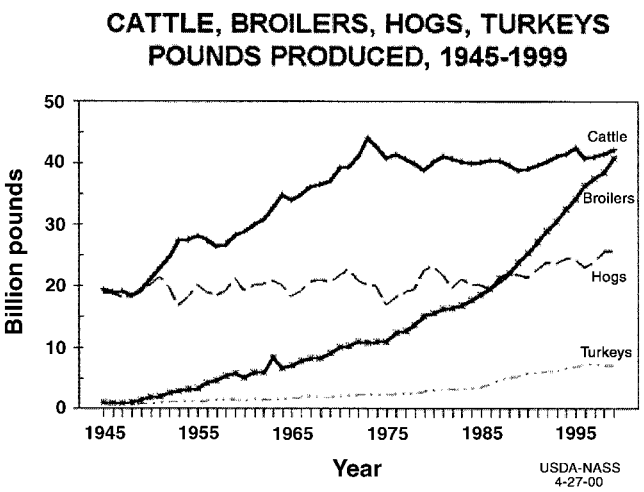

Figure 1. US meat production, 1945–1999. From US Department of Agriculture (http://www.usda.gov/nass/aggraphs/lbspr.htm).

FOOD ANIMAL PRODUCTION AND ANTIMICROBIAL USE PRACTICES

Since World War II, food animal production in the United States has been characterized by greater intensity (i.e., fewer but larger farms) and scale of production (figure 1), improved infectious disease management, and better nutrition [5]. Many antimicrobials are approved for treatment or growth promotion in the United States (table 2).

Beef. After weaning at ~7 months, beef calves typically are shipped to stock or backgrounder farms and then to feedlots, where they are maintained in large groups and fed high-energy rations. Beef cattle feedlot sizes (animals per feedlot) have been increasing: in 2000, ~35% of cattle were fed on farms of 32,000 head or more [12]. Pneumonia and diarrhea are major causes of calf mortality, and calves are often treated with individual or group medication [13]. A variety of important viral infections contribute to pneumonia and diarrhea, but bacterial agents (e.g., *Escherichia coli,* pasteurellae, *Haemophilus* spp., and *Salmonella* spp.) may also be involved. Shipping fever complex (pneumonia) is a major feedlot health problem and an important determinant of antimicrobial use [1, 14]. Comparatively little antimicrobial use occurs in cow-calf production [15].

Various antimicrobials (table 2) are administered to cattle on feedlots for a variety of reasons, including control of liver abscesses, acceleration of weight gain, and prevention or treatment of respiratory disease outbreaks. According to a 1999 US Department of Agriculture survey of antimicrobial treatment practices [14], ~83% of feedlots administered at least one antimicrobial to cattle in feed or water for prophylaxis or growth promotion. Monensin and lasalocid were commonly used for growth promotion, whereas some producers used drugs such as neomycin and virginiamycin. Chlortetracycline was administered on 51.9% of feedlots, chlortetracycline-sulfamethazine combination on 16.8%, oxytetracycline on 19.3%, and tylosin (a macrolide antimicrobial) on 20.3%. On average, tetracyclines were administered for 4–12 days and tylosin for 138–145 days.

Table 1. Types of antimicrobials use in food animals.

Type of antimicrobial use	Purpose	Route or vehicle of administration	Administration to individuals or groups[a]	Diseased animals
Therapeutic	Therapy	Injection, feed, water	Individual or group	Diseased individuals; in groups, may include some animals that are not diseased or are subclinical
"Metaphylactic"	Disease prophylaxis, therapy	Injection (feedlot calves), feed, water	Group	Some
Prophylactic	Disease prevention	Feed	Group	None evident, although some animals may be subclinical
"Subtherapeutic"	Growth promotion	Feed	Group	None
	Feed efficiency	Feed	Group	None
	Disease prophylaxis	Feed	Group	None

[a] Food animals are usually grouped by pen, flock, pond, barn, or other aggregate.

Table 2. Examples of antimicrobials approved for use in the United States in food animals.

Purpose	Cattle	Swine	Poultry	Fish
Treatment of various infections	Amoxicillin	Amoxicillin	Erythromycin	Ormetoprim
	Cephapirin	Ampicillin	Fluoroquinolone	Sulfonamide
	Erythromycin	Chlortetracycline	Gentamicin	Oxytetracycline
	Fluoroquinolone	Gentamicin	Neomycin	
	Gentamicin	Lincomycin	Penicillin	
	Novobiocin	Sulfamethazine	Spectinomycin	
	Penicillin	Tiamulin	Tetracyclines	
	Sulfonamides	Tylosin	Tylosin	
	Tilmicosin		Virginiamycin	
	Tylosin			
Growth and feed efficiency	Bacitracin	Asanilic acid	Bambermycin	
	Chlortetracycline	Bacitracin	Bacitracin	
	Lasalocid	Bambermycin	Chlortetracycline	
	Monensin	Chlortetracycline	Penicillin	
	Oxytetracycline	Erythromycin	Tylosin	
		Penicillin	Virginiamycin	
		Tiamulin		
		Tylosin		
		Virginiamycin		

NOTE. Adapted from [5].

For individual animal therapy, ~50% of feedlots used tilmicosin, florfenicol, tetracyclines, or some combination of these drugs. Feedlots also used cephalosporins (38.1%), penicillins (31.1%), macrolides (17.4%), and fluoroquinolones (32.1%) for individual animal therapy. Approximately 41% of feedlots administered antimicrobials such as tilmicosin, florfenicol, and oxytetracylcines for metaphylaxis [14].

Veal. Typically, culled dairy bull calves in the veal industry are fed an iron-limited diet to produce pale muscle from shortly after birth until they reach 400–500 pounds [5]. Although many antimicrobials are available to treat respiratory and enteric diseases in such calves, little information is available describing which of these drugs are being used and at what frequency. Milk replacers for calves can contain antimicrobials for disease prophylaxis.

Dairy. On dairy farms, most calves are removed from dams within a day of birth, housed separately to control infection, fed milk or milk replacer (which may contain tetracycline) for 6–8 weeks, weaned, and then housed in groups. Antimicrobials (e.g., tetracyclines, penicillins, sulfonamides) may be administered orally or by injection (e.g., ceftiofur) to treat or prevent diarrhea and pneumonia, both of which are important diseases of dairy calves [16]. Although lactating dairy cows receive few antimicrobials in feed, antimicrobials (penicillins, cephalosporins, erythromycin, and oxtetracyclines) are administered through intramammary infusion to treat mastitis, an important disease caused by a variety of gram-positive and gram-negative bacteria [1, 5, 17, 18]. Such drugs are often routinely administered to entire herds to prevent mastitis during nonlactating periods [18].

Poultry. During 1945–1999, broiler chicken production increased from ~5 billion to nearly 40 billion pounds per year [19]; the industry grew to be highly integrated, with fewer companies controlling most sources of birds, feed mills, farms, and slaughter and processing facilities. Broilers are typically raised under confinement in pens containing 10,000–20,000 birds, and turkeys are raised in groups of 5000–10,000 [5]. Integration led to standardized management practices, including drug treatment policies and procedures, and to many successes in the prevention and control of infectious diseases. Many problematic infectious diseases are controlled with antimicrobials (table 2). For instance, broiler rations usually contain a coccidiostat, several of which are broader antimicrobials (e.g., ionophores, sulfonamides). Other antimicrobials (e.g., bacitracin, bambermycin, chlortetracycline, penicillin, virginiamycin, arsenical compounds) are approved for growth promotion and feed efficiency in broilers, turkeys, and egg layers (table 2). Bacitracin is used mainly for growth promotion and to control necrotic enteritis, an intestinal infection caused by *Clostridium perfringens,* with virginiamycin used to a lesser extent for these same purposes. Because older drugs such as the tetracyclines are considered ineffective (presumably because of the emergence of resistance), newer drugs such as the fluoroquinolones are used to treat *E. coli* infections, a major disease problem in poultry [20].

Fluoroquinolones are currently approved only for treatment

of certain infections in poultry (e.g., *E. coli*) to control mortality (table 2), although the US Food and Drug Administration (FDA) proposed to withdraw this approval as a result of concerns about fluoroquinolone resistance in *Campylobacter*. Treatment entails administration of the antimicrobial in water to an entire flock (usually thousands of birds contained within a single barn) because single-bird treatment is not practical.

Hatching eggs may be dipped in gentamicin to reduce mycoplasma or bacterial contamination (sarafloxacin, a fluoroquinolone, was formerly approved for in ovo injection but was withdrawn recently by its sponsor). Because of the risk of yolk sac infections (omphalitis) and vaccine-injection-site abscesses, day-old chicks may be injected with gentamicin, ceftiofur, or other drugs [20].

Swine. Swine are usually raised in confinement, either from birth through slaughter (farrow-finish) or in age-segregated management systems (e.g., nursery, grower, finishing) [21], with many farms of both types practicing all-in, all-out management to control infectious diseases. Average herd size is increasing; in 1995, ~60% of pigs were raised on farms of >1000 head [22]. Antimicrobial use is predominantly in feed, at relatively low concentrations, for growth promotion or disease prophylaxis [23], with antimicrobials typically removed at the finishing stages of production to avoid residues. Therapeutic treatments are also administered in feed, although producers also treat individual swine. Most pigs receive antimicrobials in feed after weaning ("starter rations") [24, 25], when they are most vulnerable to infectious disease.

Several antimicrobials (e.g., ceftiofur, sulfonamides, tetracyclines, tiamulin) are used to treat and prevent pneumonia, an important problem among swine [1]. Gentamicin, apramicin, and neomycin are used to treat bacterial diarrhea, another important problem, caused by organisms such as *E. coli* and *Clostridium perfringens.* Swine dysentery *(Serpulina hyodysenteriae)* and ileitis *(Lawsonia intracellularis)* are other important diseases that may be treated with antimicrobials such as lincomycin, tiamulin, or macrolides [26]. Overall, the antimicrobials used most frequently in swine are tetracyclines, tylosin, and sulfamethazine or other sulfas.

Aquaculture. Catfish, rainbow trout, salmon, tilapia, striped bass, shrimp, crawfish, and a variety of shellfish are the main species cultivated in the United States. No antimicrobials are approved for growth promotion in the United States, and only ormetoprim-sulfadiazine and oxytetracyline are approved for treatment of bacterial infections (e.g., bacterial hemorrhagic septicemia, furunculosis, enteric septicemia) in salmonids and catfish. Drugs are usually administered in feed to the entire group, although broodstock may be treated individually [27].

Organic food animal production. Organic foods account for ~1%–2% of total US food sales but are expected to increase 20%–30% annually [27]. US Department of Agriculture rules require that animals raised organically not receive antimicrobials. If sick, these animals must be removed from the organic operation.

ANTIMICROBIAL APPROVAL AND AVAILABILITY

National regulatory authorities, including the FDA [28], evaluate antimicrobials for use in animals on the basis of safety for humans consuming the foods, animal safety, efficacy, and effect on production. The FDA emphasized possible effects on humans of residues in edible products, although the agency also evaluates microbial effects of drugs intended for subtherapeutic administration [1, 29].

In 1998, the FDA proposed a "framework" for evaluating antimicrobials used in food animals and minimizing their adverse human health effects, including development of resistance [30]. That framework, which categorizes drugs according to their importance to human health, would establish "human health thresholds" for antimicrobial resistance [31]. This framework would help the agency comply with the Food, Drug, and Cosmetic Act, which specifies a "reasonable certainty of no harm" standard to regulations concerning human safety [31, p. 3].

Primary decision making about antimicrobial use ideally rests with veterinarians, who can diagnose diseases on the bases of symptoms and appropriate laboratory tests, including culture and susceptibility testing as they pertain to individual animals or groups. Other criteria, including herd production goals and animal welfare, should also be considered. Veterinarians can then recommend the most appropriate therapeutic regimen by use of the optimal drug, dosage, and duration of treatment.

In reality, however, antimicrobials are often used in food animal production with little or no veterinary consultation. In a 1995 US survey, for example, ~42% of pig farms used the services of a veterinarian [21], although a survey indicates this figure is up to 78% [22]. Producers have access to over-the-counter antimicrobials from retail outlets as well as in feeds containing nonprescription drugs. Various over-the-counter antimicrobials are made available to producers for purely practical reasons—for instance, they lack convenient access to veterinary services—and because the FDA deemed certain drugs safe for over-the-counter use [28]. In 1988, the FDA mandated that all new antimicrobials be prescription only.

Pharmaceutical companies, importers, pharmacies, and other retailers have financial incentives to market antimicrobials to animal producers. Some veterinarians also derive income from such sales. No published data demonstrate conclusively that profit motives routinely affect the antimicrobial-prescribing practices of veterinarians. Denmark placed restrictions on the

degree to which veterinarians can profit from antimicrobial prescriptions [32].

In the United States, the Animal Medicinal Drug Use Clarification Act enables veterinarians to prescribe approved drugs for extralabel use (additional uses not described in the product label). Veterinarians may prescribe extralabel antimicrobials when there is no suitable product approved for a specific species and indication, or when the approved product is ineffective, provided there is a valid veterinarian-client-patient relationship [28]. Extralabel use in food animals is not permitted in feed, by direction of a layperson, or at all for certain drugs such as fluoroquinolones or glycopeptides [28].

Several national veterinary organizations have developed judicious (or prudent) antimicrobial use principles and programs (e.g., American Veterinary Medical Association [4], American Association of Swine Veterinarians [33]). Moreover, the American Association of Avian Pathologists prepared guidelines for drug use in treating poultry diseases that are based in part on the importance of antimicrobials in human medicine [34]. It is too soon to evaluate the effect of these programs; however, if widely adopted, they could benefit both animal and human health.

Swine and cattle producer groups have also developed a variety of food animal quality assurance programs to enhance domestic and export markets. Until recently, these programs tended to focus on preventing antimicrobial residues as a result of consuming contaminated meat. Because concerns about resistance are receiving increased attention, some producers are changing antimicrobial use practices. For example, the Minnesota Certified Pork program requires member farms to use antimicrobials for therapeutic purposes only (University of Minnesota College of Veterinary Medicine, http://www.cvm.umn.edu/anhlth_foodsafety/MinnCERT.html).

QUANTITY OF ANTIMICROBIAL USE IN FOOD ANIMAL PRODUCTION

Reliable antimicrobial use data for animals are not publicly available, making it difficult to determine which drugs are used in what quantities and for what purposes. However, several organizations have published estimates. The most widely quoted of these is the 1989 report from the Institute of Medicine [10], which cited data from the National Research Council and the US International Trade Commission. The Institute of Medicine estimated that total US production of antimicrobials increased from ~1 million pounds in 1950 to ~44 million pounds in 1986.

More recently, a report from the Union of Concerned Scientists [11] estimated that ~50 million courses of treatment, or ~3 million pounds, are administered to humans annually; it also estimated that an additional 1.5 million pounds of antimicrobials are used in topical creams, soaps, and disinfectants, contributing to a total of 4.5 million pounds being used annually in humans. The report further estimated that 27.5 million pounds of antimicrobials are used for "nontherapeutic" purposes (growth promotion and disease prophylaxis), and another 2 million pounds are used for therapeutic purposes in animals. All these figures were based on extrapolations and indirect methods [11].

In February 2000, according to a survey of the members of the Animal Health Institute, 17.8 million pounds of antimicrobials were used in animal production in 1998—14.7 million pounds (83%) for prevention and treatment of disease, and 3.1 million pounds (17%) for growth promotion [35].

Having access to accurate values will be essential for overcoming the marked discrepancies among estimates and would help to put these issues into perspective. Accurate estimates of use are needed for each drug by animal species, purpose (e.g., therapy, growth promotion), route of administration, and duration of treatment. Figures related to human use are also needed. To date, few countries possess information at this level of detail, although some European countries have established veterinary databases that come close. For example, the Danish VETSTAT program is designed to monitor the use of antimicrobials on all food animal herds in the country, the species and age class of animals treated, and reasons for treatment [36].

Volume estimates and other simple comparisons between antimicrobials used for animals and humans can give only a very rough idea of the potential effect of those uses on the development of antimicrobial resistance and human health. Total volume figures do not account for differences in drug potencies or resistance selection pressures. For example, ionophores, which are counted among the Union of Concerned Scientists' antimicrobial totals, are widely used in food animal production but not in human medicine and presumably do not contribute significantly to the development of resistance in clinically useful drugs. On the other hand, drugs such as the fluoroquinolones are used extensively to treat diseases in humans, and their agricultural uses may exert considerable selection pressure for pathogens to develop resistance.

EFFECTS OF WITHDRAWAL OF GROWTH PROMOTERS OR OTHER ANTIMICROBIALS

Members of the agricultural and allied industries are concerned over the possibility that restrictions may be placed on the use of therapeutic or nontherapeutic antimicrobials in food animal production [5]. If restrictions were to be imposed, they would most likely include limitations on new drug approvals or elimination of antimicrobial growth promoters. Possible consequences of such restrictions include the following: (1) decreased incentive for new drug development, (2) poorer production

efficiency, (3) compensatory increases in prophylaxis or therapy, (4) increases in the incidence of infectious disease in animals, and/or (5) limitations on the ability of veterinarians and farmers to treat and prevent disease. Alternatively, restrictions could also result in little or no change in animal health or production efficiency.

How antimicrobials improve growth or feed efficiencies in farm animals is not fully understood [1]. One possibility is that antimicrobials dampen the effects of subclinical disease on growth and also suppress certain sensitive bacteria that compete with host animals for nutrients [8, 9, 37, 38]. Another possibility is that growth promoters enhance the immune system of recipient animals by affecting hormones, cytokines, and other immune factors [39–42]. Antimicrobials at subtherapeutic levels may also modulate the metabolic activity of bacteria in the gut or shift the balance among microbial species, resulting in weight-gain benefits.

Although some reports indicate that such uses yield 1%–11% weight-gain improvements [8], these benefits may not be realized amid other modern production practices. Moreover, such benefits tend to be greater when hygiene is poor [7]. With improvements in hygiene and other measures in place to control disease (e.g., biosecurity, vaccination, improved management), questions are being raised as to whether intensive animal husbandry practices eliminate the benefits of growth promoters. For example, according to a Danish study [43], removal of antimicrobial growth promoters reduces broiler chicken feed efficiency by <1% without affecting other measures of production efficiency. Despite an increase in the rate of necrotic enteritis infections, death rates did not change, and there was no decrease in kilogram broilers produced per square meter [43].

Danish scientists also evaluated how a 1999 ban on the use of growth promoters in pigs and broilers affected antimicrobial use and resistance in fecal enterococci [44]. In 1994, farmers used 206,000 kg of antimicrobials for growth promotion and therapy in Denmark. After the elimination of growth promoters, overall antimicrobial use levels dropped to 80,900 kg in 2000 [44], although there has been some increase in use of therapeutic antimicrobials [32]. Decreases in use of virginiamycin and avilamycin were also accompanied by decreases in resistance to these drugs [44]. However, since the ban, *Lawsonia intracellularis,* an intestinal pathogen that infects pigs, has become a problem [32]. Meanwhile, the 1995 ban on avoparcin use in broilers in Denmark was followed by a substantial decrease (72.7% to 5.8%) in glycopeptide-resistant *Enterococcus faecium* in commercial flocks. A substantial resistance decrease was not observed in pig enterococci until after the decrease in use of tylosin in 1998–1999. Subsequently, it was shown that the genes encoding macrolide (tylosin) and glycopeptide resistance were genetically linked. Decreases in use of virginiamycin and avilamycin were also accompanied by decreases in resistance to these drugs [44]. These studies offer evidence that the prevalence of resistance can be reversed, even if not eliminated, suggesting that unidentified environmental factors may help in sustaining resistant microbial populations (see Summers, this supplement). Avoparcin has never been used in animal agriculture in the United States.

In 1986, Sweden banned the use of growth promoters in animal production [45] and began monitoring antimicrobials sold for use in animals. Shortly after the ban, there were some increases in morbidity and mortality among farm animals (e.g., postweaning diarrhea in piglets, necrotic enteritis in chickens); those increases were counteracted by administration of antimicrobials for prophylaxis during high-risk periods and by adoption of other management improvements. In the early 1990s, zinc oxide replaced antimicrobials as prophylactics for piglets, but by 1998, Swedish officials designated this product as prescription-only, leading to a 90% decline in its use. Total sales of all antimicrobials for animals also decreased by a substantial 60% [46]. Whether this ban affected resistance prevalence is not known.

The economic effects from banning subtherapeutic antimicrobial uses in US agriculture were estimated in a 1999 report from the National Academy of Sciences [5]. According to that report, nearly 100% of chickens and turkeys, 90% of swine and veal calves, and 60% of beef cattle were fed rations medicated with antimicrobials. Even so, according to the report, meat producers following good management practices would not be greatly affected by such a ban, in part because antimicrobial growth promotants are not particularly effective unless animals are living under stress and suboptimal sanitation conditions. In economic terms, such a ban of subtherapeutic drug use would cost, on a per capita basis, $4.84 to $9.72 per year ($1.2–$2.5 billion overall). Estimated increases in cost per pound were lowest for chicken ($0.013–$0.016) and highest for beef and pork ($0.03–$0.06) [5].

Hayes et al. [47] estimated the economic effect in the United States of a ban on the use of over-the-counter antimicrobials in pork production, basing their analysis on figures from the Swedish pork industry. A comparable US ban would increase production costs by $6.05 initially per animal, dropping to $5.24 per animal after 10 years. Higher pork prices would be anticipated because of reduced supply (as a result of anticipated increased feed costs, changes in sow productivity, and piglet loss), and net profits would decline by $0.79 per head, increasing the retail price of pork by $0.05 per pound. Some projected costs include addition of space and troughs to allow restricted feeding. Another estimate of the effects of discontinuing antimicrobial use in hog production suggests that feed efficiency would decrease, feed costs would rise, and production would decrease, leading to higher prices for consumers [48].

ANTIMICROBIAL USE IN ANIMALS AND EMERGENCE AND SPREAD OF RESISTANT BACTERIA

Several recent reviews survey antimicrobial resistance across many animal species [49–52]. In animals, antimicrobial resistance in zoonotic enteropathogens (e.g., *Salmonella, Campylobacter, Yersinia,* and some strains of *E. coli,* such as serotype O157:H7) and commensals (e.g., enterococci, most generic *E. coli*) is of special concern to human health because these bacteria are most likely to be transferred through the food chain to humans, or resistance genes in commensal bacteria may be transferred to the zoonotic enteropathogens [53]. There is considerable evidence that antimicrobial use in animals selects for resistance in commensals [54–58] and in zoonotic enteropathogens [59–61].

However, other studies (on-farm and experimental) failed to show an association between antimicrobial use and resistance [62, 63], suggesting that the development of resistance is a complex process, and perhaps easier to acquire and maintain for some species of bacteria than others. Nonetheless, antimicrobial use in animals apparently contributes to the selection and spread of resistance among populations of bacteria in animals; other forces also contribute to its spread in animal populations. Examples include the movement of carrier animals between herds or between countries, the assembly of susceptible animals in close confinement, and the movement of resistance determinants throughout the ecosystem (figure 2) by means of vectors such as rodents, insects, and birds. Moreover, some bacteria cause disease regardless of resistance status, meaning we need to maintain surveillance programs while trying to reduce both resistant and susceptible zoonotic pathogens.

Some antimicrobial animal-treatment practices may exert greater selective pressures for resistance than others. For example, feeding animals growth promoters, which entails exposing bacteria to sublethal concentrations of drugs over long periods, would appear conducive to selecting and maintaining resistant organisms [1]. This practice in effect corresponds to the general principle in which fit microorganisms able to withstand the effects of antimicrobial agents survive and flourish, whereas those that are not resistant do not survive. Many infeed medications are administered at comparatively low concentrations to animals for weeks and often for years in successive generations of animals.

Although not everyone agrees that such uses of subtherapeutic drugs lead to the development of resistance, considerable selection pressure may be applied when animals are treated in this way. Moreover, not all mass medication is administered at subtherapeutic doses. For instance, many antimicrobials are administered at therapeutic doses in feed or water, or by injection to all or a substantial proportion of individuals in herds or flocks for prophylactic or metaphylactic purposes. Fluoroquinolone resistance in *Campylobacter* and gentamicin resistance in some serotypes of salmonellae of poultry appears to have been amplified, at least in part, by this practice [64]. However, there are differences among drugs in the rate at which resistance occurs. Thus, when assessing resistance risks from uses of antimicrobials in animals and attempting to reduce those risks, it is important to consider other factors that may contribute to selection and spread of resistance among animals. These factors may include species of animal, dose, duration of treatment, numbers of animals treated, animal husbandry practices, animal movement, and potential for environmental spread.

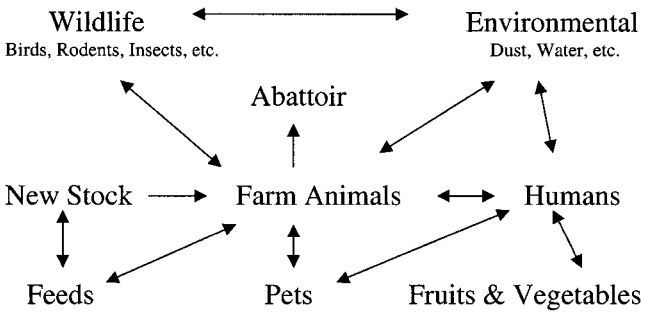

Figure 2. Complexity of the problem and interaction between groups

The fecal waste from thousands of animals reared under intensive conditions often is spread as fertilizer or spread on pasturelands, sometimes after composting. Alternatively, swine operations typically construct lagoons to hold such wastes, and they are implicated in the contamination of the environment with resistant bacteria [65]. Groundwater, streams, and other waterways contaminated with these wastes also may facilitate the spread of bacteria carrying antimicrobial resistance traits.

Food animal production is by no means the sole contributor to this problem. Human wastes from homes, offices, and especially hospitals frequently spill into rivers and waterways from defective septic or municipal systems [66]. Pharmaceutical compounds have been detected in low levels throughout waterways in Europe [67]. How these environmentally borne antimicrobials might affect resistance patterns among microorganisms is not well understood [68] (see also Summers, this supplement). Resistant organisms may also spread between farms by means of infected carrier animals [69], contaminated feedstuffs, wildlife vectors, or on humans wearing pathogen-contaminated clothing. A few studies document the role of antimicrobial treatment in spread of resistance [56], although other studies indicate that such use may select for resistance in individuals (e.g., nosocomial *Salmonella* infections in horses) [70], groups (*E. coli* in pigs or poultry) [57], or in regional populations (e.g., temporal relations between quinolone use in the United Kingdom and the emergence of reduced susceptibility in salmonellae) [71].

Food animal production in North America is becoming progressively more intensive, especially in poultry, swine, and beef feedlot production: the number of farms is steadily decreasing while total production is increasing. Grouping large numbers of susceptible animals in close confinement no doubt facilitates the spread of resistant bacteria, much as occurs in human hospital settings. Improvements in animal disease control and disease-exclusion programs ("biosecurity") help to limit the spread of some animal diseases. However, these programs are not usually designed to control commensal bacteria or even multiple zoonotic enteropathogens (e.g., *Salmonella* and *Campylobacter*); rather, they are designed to control a single or particular pathogen, such as *Salmonella* serotype Enteritidis. However, improved management and biosecurity likely will also reduce levels of other pathogens and improve overall herd or flock health.

ANTIMICROBIAL USE AFFECTS SHEDDING OF ENTEROPATHOGENS AND SUSCEPTIBILITY TO PATHOGENS

Treatment of animals with antimicrobials that are active against enteropathogens such as *Salmonella* (e.g., apramycin and oxytetracycline in pigs [72], oxytetracycline in calves [73], and oxytetracycline in poultry [73]) can reduce fecal shedding, providing a potential public health benefit by reducing pathogenic loads. In general, however, food animals are not treated with antimicrobials specifically to reduce fecal carriage and shedding of enteropathogens. Any public health benefits of this type would accrue indirectly.

Conversely, treatment may increase pathogen loads in the food chain by selecting for resistant nontarget pathogens with increased fitness, increasing the likelihood that animals will be infected with resistant pathogens and increasing the duration of infection. These effects may be specific to particular combinations of drug and bacterial species; for instance, when swine infected with *Salmonella* serotype Heidelberg were treated with ceftiofur or enrofloxacin, shedding was reduced compared with untreated controls infected with *Salmonella* [74].

Antimicrobials may increase the susceptibility of animals to infection by suppressing normal flora and increasing the probability that pathogens will colonize a site (the "competitive effect") or, if administered at the time of exposure to a resistant pathogen, by facilitating the infection because of a selective effect (the "selective effect") (see Barza and Travers, this supplement). Resistant nosocomial salmonellosis attributable to antimicrobial therapy occurs in horses [70], cats [75], and probably other species, although little is published on this subject. Between 3% and 26% of resistant *Salmonella* infections of humans are acquired through a selective mechanism associated with antimicrobial treatments, according to Barza and Travers (this supplement). Comparable estimates for animals remain to be determined.

Antimicrobials may prolong shedding or elevate levels of antimicrobial resistant pathogens in feces. In its Framework document, the FDA states a concern about antimicrobial use in food animals increasing the pathogen load in an animal's intestinal tract, which could increase infection risks for consumers. When challenged with *Salmonella* and exposed to antimicrobials in feed, poultry shedding increases and is prolonged compared with untreated controls, according to some studies [76, 77]. Other studies in swine do not indicate that the pathogen load increases; rather, it appears to decrease [74]. Further, a review of the published literature found that antimicrobial use in food animals is not always associated with increased pathogen loads [78]. Most of these studies, however, were conducted in the 1970s and 1980s, focused on *Salmonella,* and involved exposure challenges, which may not accurately reflect production environments.

POSTHARVEST FOOD SAFETY

Various government and industry programs are designed to reduce the flow of foodborne pathogens from animals to humans, including programs for meat and poultry inspection, standard operating procedures for sanitation, and the Hazard Analysis Critical Control Point (HACCP) system [79]. HACCP programs specifically focus on product safety and have been widely adopted, especially at slaughter and meat-processing plants. Some slaughter or processing HACCP programs include generic *E. coli* and pathogen testing as verification measures. These programs could also help to reduce the flow of antimicrobial-resistant pathogens associated with foods into humans.

ANTIMICROBIAL RESISTANCE AND ANIMAL HEALTH

Antimicrobial resistance is also a concern for animal health, but little is known about the magnitude of this problem. Surveillance of resistance in exclusive animal pathogens (e.g., *Moraxella bovis, Actinobacillus pleuropneumoniae,* and *Pasteurella multocida*) is poor compared with surveillance of zoonotic enteropathogens. Veterinary diagnostic laboratories typically test clinical outbreak specimens in limited fashion, often without identifying species. Because of costs, susceptibility testing of animal pathogens is performed only at the request of practicing veterinarians. Rarely do producers screen herds or flocks for bacteria that may be endemic, so few data are available on the prevalence of resistance in those bacteria. Lack of resources; cost of culture or sensitivity testing; perceived low priority; lack of coordination for collection; culture, and antimicrobial

testing methods; and concerns about sampling bias (because most bacterial infections of animals are not officially reportable except *Salmonella* in some countries) are some of the barriers to better surveillance.

Resistance among animal pathogens reduces the effectiveness of some drugs. This effect could potentially affect public health if drug use in food animals increases to compensate for this drop in effectiveness or if alternative drugs that are crucial to human health are used to treat animals. There is a belief among some veterinarians that new antimicrobials are needed to combat disease in animals [5]. Some of this perceived need appears to reflect experiences with reduced efficacy related to resistance. Antimicrobial resistance has been reported in a wide variety of animal pathogens—for example, *E. coli* of calves, pigs, and poultry; *Pasteurella multocida* and *Mannheimia (Pasteurella) haemolytica* from cattle; and *Actinobacillus pleuropneumonia* and *Streptococcus suis* from pigs [80–83]. However, other factors also play a role in perceived need (e.g., spectrum of activity, withdrawal time, nonresistance efficacy issues, pharmacodynamics).

Some animal pathogen surveillance has been organized in France [84], and in Denmark within the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) program, in which clinical isolates from diagnostic submissions are collected and tested for susceptibility to panels of drugs [36]. Other reports arise from diagnostic laboratories or researchers [85, 86]. In general, resistance is highly variable among animal pathogens in different geographic areas [62, 63]. Additionally, although some isolates of pathogens (e.g., *E. coli*) are resistant to multiple antimicrobials, others remain susceptible.

ANTIMICROBIAL RESISTANCE MONITORING–PROGRAMS IN BACTERIA OF ANIMAL ORIGIN

History of antimicrobial susceptibility monitoring in the United States. Susceptibility testing of bacterial isolates not only allows for discrimination between isolates, but for assessment of developing resistance. Susceptibility testing methods include disk diffusion [87], agar dilution [88], E-test (AB Biodisk), and broth microdilution [89, 90] assays. Determination of MICs by means of the broth microdilution assay is particularly useful in evaluating incremental changes in the development of resistance.

Because of public health concerns, the Food and Drug Administration Center for Veterinary Medicine proposed a postmarketing antimicrobial resistance–monitoring program for veterinary antimicrobials, especially fluoroquinolones. In 1996, the FDA, US Department of Agriculture, and the Centers for Disease Control and Prevention established the National Antimicrobial Resistance Monitoring System (NARMS; formerly the National Antimicrobial Susceptibility Monitoring Program but changed to NARMS–Enteric Bacteria) to monitor changes in antimicrobial susceptibilities of zoonotic pathogens from human and animal diagnostic specimens, from healthy farm animals, and from raw product of food-producing animals at slaughter and processing [91]. Nontyphoid *Salmonella* was selected as the sentinel organism, *Campylobacter* was added to the animal arm in 1998, and generic *E. coli* and *Enterococcus* species were added in 2000.

The goals and objectives of the monitoring program are as follows: (1) to provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms from the human and animal populations, (2) to facilitate the identification of resistance in humans and animals as it arises, (3) to provide timely information to veterinarians and physicians, (4) to prolong the life span of approved drugs by promoting the prudent use of antimicrobials, and (5) to identify areas for more detailed investigation. Data are published annually and may be accessed online (http://www.fda.gov/cvm/index/narms/narms_pg.htm). Additional data, including percent resistance by animal species for each year tested can be found at (http://www.ars-grin.gov/ars/SoAtlantic/Athens/arru).

This information may enhance prudent drug use to diminish the development and spread of resistance. For example, when analyses reveal major shifts or changes in resistance patterns in either animal or human isolates, outbreak investigations and field studies will follow. In the long term, these analyses can be incorporated into strategies to alter veterinary prescribing practices in collaboration with professional practitioner groups.

Other monitoring programs. Monitoring programs and methodologies differ from country to country; they are based on agricultural practices, monitoring needs, and antimicrobial uses and guidelines. In Europe, 13 countries (Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom) have established their own monitoring programs [92].

The Danish government established DANMAP to monitor trends in antimicrobial resistance among bacteria from animals, food, and humans and to monitor consumption of antimicrobial agents with the intent to model transmission of resistance from animals to humans [36]. Results from the DANMAP program are reported annually and may be accessed at the Zoonosis Centre home page (http://www.svs.dk).

The French Agency for Food Safety (Agence Francaise de Securite Sanitaire des Aliments, AFSSA) organized 2 types of surveillance programs [84]. One monitors resistance from nonhuman zoonotic *Salmonella* (AFSSA, Paris), and the other deals with bovine pathogenic strains by collecting resistance data from local public veterinary diagnostic laboratories (AFSSA, Lyon).

The Spanish government established a network, "Red de

Vigilancia de Resistencias Antimicrobialas en Bacterias de Origen Veterinario," which covers bacteria from sick animals, healthy animals, and food animals [93]. This network reports both qualitative (SIR [sensitive/intermediate/resistant]) and quantitative (MIC) data and provides information methods, analysis and reporting of data.

The Department for Environment, Food and Rural Affairs (DEFRA, formerly the Ministry of Agriculture, Fisheries and Food) from Great Britain compiles antimicrobial resistance and prevalence data in salmonellae. These data are reported by animal species and feed/feedstuffs. DEFRA can be accessed at http://www.maff.gov.uk. Prevalence data on *Salmonella* are also collected in Australia and published annually (http://www.imvs.sa.gov.au).

MONITORING SYSTEMS REQUIRE APPROPRIATE PLANNING

Operating properly designed monitoring programs increases the likelihood of obtaining relevant, high-quality data with which to assess antimicrobial resistance trends. Considerations include selection of sentinel and other relevant organisms, sampling and culture of the isolates, and test methodologies. Failure to standardize surveillance systems could lead to data that are subject to misinterpretation. Moreover, underreporting resistance could result in failure to implement mitigation strategies, with animal and public health consequences, such as lost drug efficacy and higher morbidity and mortality rates. Overreporting of data could lead to unnecessary actions being taken.

Some surveillance programs track *Salmonella* and *Campylobacter* in poultry operations. *Salmonella* in chickens appears to have a commensal relationship without affecting health and birds do little to exclude the organism once *Salmonella* is established [94]. Less is known about *Campylobacter,* which is difficult to recover early in production, often not appearing until 2–4 weeks after hatch [95]. Although environmental reservoirs of *Campylobacter* in poultry houses remain unknown [95–100], prevalence can approach 100% [95]. Nelson Cox (personal communication) implicated breeder stock as one source for its transmission.

Surveillance of resistance in commensals is important because they can be reservoirs of resistance determinants and because they are more ubiquitous than pathogens. Exchange of resistance genes occurs between pathogens and nonpathogens, even between gram-positive and gram-negative organisms [53]. Pathogenic bacteria such as *Salmonella* and *Campylobacter* are not typically present in the gut environment, although once acquired, particularly by animals, they can be carried in the host without sign of clinical disease [101]. The intestinal flora of animals that have been treated with antimicrobial agents can also serve as a reservoir of resistance factors [53]. Of particular interest are enterococci and *E. coli* that can play a role in transmission of mobile resistance genes [53].

Serotype. One of the most critical differences in analysis of resistance data between studies, especially in the case of *Salmonella,* includes accurate description of the serotype or serotypes involved. Generalizations of resistance in "*Salmonella*" will often be inaccurate because resistance between serotype can be significant. For *Campylobacter, C. coli* appears to acquire resistance more readily than *C. jejuni* [86]. Moreover, within serotypes, acquisition of resistance may act as a virulence attribute, altering colonization factors or pathogenesis, as occurs for *Salmonella* DT104. Exposing chicks to a resistant strain of DT104 increases colonization and shedding, whereas a similar exposure to a pan-sensitive strain of DT104 did not [102] (also see Swartz, this supplement).

Culture. Use of selective media may result in the selection of a subpopulation of bacteria with specific phenotypic and genotypic characteristics that do not represent the entire population (P. J. F.-C., unpublished observations), raising questions as to whether reports are truly representative of the general population of bacteria whenever antimicrobials are used as a selection factor. Additionally, multiple serotypes sometimes aggregate, suggesting that special care is needed when analyzing environmental specimens [103]. Moreover, "subpopulations" of bacteria within samples are poorly understood; some isolates are more virulent and better able to establish niches within hosts. Conversely, other populations may be extremely sensitive to antimicrobials and easily eliminated. Thus, isolation and characterization of dominant or phenotypically different (e.g., resistant) subpopulations may mask other important subpopulations.

ALTERNATIVES TO ANTIMICROBIALS IN FOOD ANIMALS

Alternatives to antimicrobials in food animal production include management practices that reduce the likelihood and effect of infectious diseases and also increase the production efficiency. Established veterinary steps to prevent or control infectious diseases include improved husbandry practices, quarantines and other biosecurity measures, and vaccinations. Other treatments include genetic selection to enhance disease resistance, uses of antiseptics such as teat dipping to prevent mastitis, vector control, and use of probiotics or other competitive microorganisms to exclude pathogens [104–106]. Moreover, control of viral and other infections can reduce secondary bacterial infections, thus reducing the need for antimicrobial therapy [107].

Herd health and good management. Although some important infectious diseases (e.g., tuberculosis and brucellosis in cattle, Marek's disease in poultry, and Aujeszky's disease in swine) have been controlled or eradicated, others remain en-

demic or epidemic in herds in the United States. One way to improve control of horizontally transmitted diseases depends on veterinarians and farmers implementing biosecurity practices that reduce or eliminate opportunities for exposure between farms or between groups of animals within a farm, such as all-in, all-out management [106]. Strict disease-control programs such as screening of breeding studs, hatcheries, and artificial insemination centers can reduce or prevent vertical transmission of pathogens. Good sanitation on farms further reduces the spread of certain diseases (e.g., mastitis in dairy cows). It also is important to maintain suitable ambient temperature and air and water quality for healthy animals. Poor air quality in confinement housing can predispose animals to respiratory disease and may decrease production in pigs and poultry; low temperatures can predispose piglets to diarrhea.

Host resistance and vaccines. Vaccines are available to prevent many important bacterial and viral infections of animals, including cattle (e.g., *E. coli, Salmonella* and viral diarrhea, viral and bacterial respiratory disease), pigs (e.g., leptospirosis, *E. coli* and viral diarrhea, bacterial pneumonia), and poultry (e.g., *Pasteurella* infection, Marek's disease) [3, 108, 109]. Efforts are under way to develop a vaccine to prevent coccidiosis in poultry, for which large quantities of prophylactic antimicrobials are used [3, 109]. After vaccines were introduced to control *Vibrio salmonicida* and *Aeromonas salmonicida* in salmon, Norwegian fish farmers dramatically reduced antimicrobial use [110].

Several efforts are under way to develop live-attenuated or killed vaccines for protecting chickens against *Salmonella.* A live-attenuated, orally administered vaccine is expected to provide better protection because it appears to stimulate cell-mediated immune responses [111]. One promising candidate vaccine contains several specific nonreverting and multiple attenuating mutations [112]. Other approaches target their mutations to genes affecting smooth lipopolysaccharide [113], auxotrophic mutants that require metabolites not available in animal tissues [114–116], and mutations in global regulatory pathways [117–120]. Still other candidate vaccines were developed by repeated passage through porcine neutrophils [121].

In these development efforts, investigators typically insert antimicrobial resistance genes, particularly tetracycline and nalidixic acid markers, into the chromosome of candidate vaccine strains to use them as markers. We are unaware of any cases in which this practice leads to any increase in environmental saturation of these resistance genes, and the likelihood that these genes will be transferred to other bacteria after testing or use of these vaccines is not known.

Biosecurity. *Salmonella* is readily introduced onto farms and, once present, disseminates widely. Measures to block its introduction and spread include limiting access to farm sites, requiring visitors to change clothing and boots, controlling birds and rodents, using *Salmonella*-free feed, and treating animals with disinfectant foot baths [106]. Large farms and high stocking densities also apparently facilitate the dissemination of *Salmonella.*

Effective cleaning of sites and disinfection procedures offer additional means to control infectious diseases. Many farms now follow an all-in, all-out policy with animals, permitting adequate cleaning and disinfection after pens and barns are emptied. This practice tends to reduce the spread of pathogens. For instance, pigs can be kept relatively free of *Salmonella* when raised in clean and disinfected environments [122–125]. However, because antimicrobial and quaternary ammonium compound resistance genes may be linked, high-level uses of disinfectants might lead to the development of resistance to antimicrobial agents. All-in, all-out systems also keep successive herds (and their resident microbiota) physically separated, thus reducing the degree to which resistant bacteria can disseminate.

Feeding systems. Probiotics consist of live beneficial bacteria (e.g., lactobacilli, bifidobacteria, propionibacteria), the benefits of which are similar to antimicrobial growth promoters [135]. However, their use in feed is limited, and results have been variable.

Other competitive-exclusion strategies entail displacing pathogens with organisms that are better suited to establish and maintain themselves in a particular biologic environment, possibly by producing chemicals that are toxic to competing pathogens [94]. Salmonellae can colonize broiler chicks at least in part because modern mass-production methods delay establishment of intestinal microflora [126]. However, feeding such chicks anaerobic cultures of normal intestinal adult fowl flora may prevent such infections [126, 127]. The results of experiments [128, 129] and commercial field trials [130, 131] support the workability of the competitive-exclusion concept. PREEMPT [132] was the first competitive-exclusion product approved by the FDA for use in the United States. Currently, competitive-exclusion products are under study in swine [105, 133] and cattle [134], with preliminary results indicating that they can be effective.

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Uses of Antimicrobials in Plant Agriculture

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Bacterial diseases of plants are less prevalent than diseases caused by fungi and viruses. Antimicrobials for prophylactic treatment of bacterial diseases of plants are limited in availability, use, and efficacy, and therapeutic use is largely ineffective. Most applications are by spray treatments in orchards. Monitoring and surveillance for drug resistance are not routinely done. In the United States, data on use of antimicrobials for treatment of bacterial diseases of plants are limited to streptomycin and oxytetracycline. Resistance to streptomycin has become widespread among bacterial phytopathogens; no resistance among these bacteria has yet been reported for oxytetracycline. No human health effects have been documented since inception of use of antimicrobials in plants in the 1950s. Transfer of antimicrobial resistance from marker genes in transgenic plants to bacteria has not been documented under natural conditions in field-grown plants. However, antimicrobial-resistance genes are being eliminated from use as marker genes because of concerns about possible transfer from plant genomes back to bacteria, with further horizontal transfer to the bacteria in the environment, or from plant genomes to animals by plant consumption. No new antimicrobials are expected to be used in plant agriculture because of high costs of development, regulatory constraints, and environmental and human health concerns. Alternatives to antimicrobials, such as biocontrol agents, transgenic plants, and novel chemicals, are being developed and marketed, although their efficacy remains to be determined.

CURRENT USE OF ANTIMICROBIALS IN AGRICULTURE

Antimicrobials originated from microorganisms isolated from the environment [1]. Although there are some studies of phenotypic antimicrobial resistance and a few studies of genetic determinants associated with resistance in natural isolates of commensal and phytopathogenic bacteria, as Salyers has pointed out, there are no systematic studies of microbes in an ecosystem (A. Salyers, personal communication). This lack of data is the case even for environments in which antimicrobials are used for managing bacterial plant diseases of fruit trees, for which antimicrobial use in the United States has proven to be economical [2]. The extent of naturally occurring antimicrobial resistance is not well known because, except for monitoring the target pathogen treated with antimicrobials, even fewer studies have monitored the resistance of nontreated, wild-type pathogens [3, 4] and commensal bacteria [5].

An estimated 40 million pounds of antimicrobials are used in the United States each year, of which ~0.1% is used in plant agriculture [6]. Antimicrobial use in US plant agriculture is limited in type and quantity used as a result of economics, lack of antimicrobial efficacy for a number of diseases, and environmental concerns. The US Environmental Protection Agency (EPA) has regulatory responsibility for antimicrobial use in plants, whereas the Food and Drug Administration regulates all other antimicrobial use. Eventually, the Food Quality Protection Act of 1996 may eliminate the use of antimicrobials in plant agriculture because the required reregistration and compliance with the higher standards involved may not be cost-effective.

Only 2 antimicrobials, streptomycin and oxytetracycline, are currently registered by the EPA for use in plant agriculture. Streptomycin and oxytetracycline are often grouped with fungicides in data reports, and both are used primarily as prophylactic treatments—that is,

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Table 1. Antibiotics registered for use in plant agriculture in the United States.

Crop use, crop	Disease	Disease agent	Registered treatment	
			Streptomycin[a,b]	Oxytetracycline[b,c]
Terrestrial food and/or feed crop use				
Apple	Fire blight	*Erwinia amylovora*	F	F
Bean	Halo blight	*Pseudomonas syringae* pv. *phaseolicola*	S	—
Celery	Bacterial blight	*Pseudomonas cichorii*	F*	—
Crabapple	Fire blight	*E. amylovora*	F	—
Nectarine	Bacterial leaf and fruit spot	*Xanthomonas campestris* pv. *pruni*	—	F
Peach	Bacterial leaf and fruit spot	*X. campestris* pv. *pruni*	—	F
Pear	Fire blight	*E. amylovora*	F	F
Pepper	Bacterial spot	*X. campestris* pv. *vesicatoria*	F*	—
Potato	Bacterial soft rot	*E. chrysanthemi, E. carotovora* subspecies *carotovora*	S	—
	Blackleg	*E. carotovora* subspecies *atroseptica*	S	—
Quince	Fire blight	*E. amylovora*	F	—
Tomato	Bacterial spot	*X. campestris* pv. *vesicatoria*	F* and S	—
Nonfood crops				
Sugar beets (grown for seed)	Bacterial rot/blight	*Erwinia* species	S	S
Tobacco	Wildfire	*Pseudomonas syringae* pv. *tabaci*	F* and S	—
Ornamental herbaceous plants, shrubs, and vines, and greenhouse ornamentals				
Anthurium	Bacterial blight	*X. campestris* pv. *dieffenbachiae*	F	—
Cotoneaster	Fire blight	*E. amylovora*	F	F
Chrysanthemum	Bacterial wilt	*E. chrysanthemi, E. carotovora* subspecies *carotovora*	F	C
Crabapple, flowering	Fire blight	*E. amylovora*	F	—
Elm	Lethal yellows	*Phytoplasma*	—	I
Dieffenbachia	Bacterial stem rot	*Erwinia* species	F	—
Hawthorn	Fire blight	*E. amylovora*	F	—
Palm	Lethal yellows	*Phytoplasma*	—	I
Philodendron	Bacterial leaf spot	*X. campestris* pv. *dieffenbachiae*	F	F
Pyracantha	Fire blight	*E. amylovora*	F	—
Quince, flowering	Fire blight	*E. amylovora*	F	—
Roses	Crown gall	*Agrobacterium tumefaciens*	F	—

NOTE. F, foliar; F*, foliar, seedling stage only; S, seed, seed piece, or bed treatment; C, cutting; I, internal injection.

[a] Adapted from 1992 US Environmental Protection Agency (EPA) Reregistration Eligibility document [7].

[b] Data from [8, 9].

[c] Adapted from 1993 US EPA Reregistration Eligibility document [10].

when disease is expected on the basis of previous experience, predictive systems, or recommendations of local agricultural advisors. Streptomycin is registered for use on 12 fruit, vegetable, and ornamental fruit crops, and oxytetracycline is registered for use on 4 fruit crops (table 1). Some data on minor uses for other crops and seed treatment are not available.

A major plant disease, fire blight, is caused by *Erwinia amylovora*, a relative of *Escherichia coli* and other enteric bacteria. Spray treatments may be used every 3–4 days (streptomycin) or 4–6 days (oxytetracycline) as prophylactic treatment to limit fire blight damage during blossom time, when fire blight damage is the most devastating [11]. Approximately 53,000 hectares (~131,000 acres) are sprayed annually with antimicrobials [12]. Blossom time may extend 6 weeks or more and differs among species and varieties. Residue studies described in the public literature are limited to streptomycin. These studies showed that fruit had no detectable streptomycin residue at the time of harvest, but streptomycin activity was still detectable in leaves [13]. The 1992 EPA fact sheet on streptomycin [7, p. 5] indicates that "all ecological effects data requirements are satisfied" and that streptomycin is nontoxic to birds, freshwater invertebrates, and honeybees and is slightly toxic to fish (both cold-water and warm-water species). Interestingly, streptomycin is reported to be "toxic to algae." The 1993 EPA fact sheet addressing oxytetracycline usage [10, p. 5] states, "oxytetracycline is practically non-toxic to birds, fish, aquatic inverte-

Table 2. Use of the antibiotic agent gentamicin in food crops by country.

Country, crop	Disease	Disease agent
Chile		
Tomato	Bacterial canker	*Clavibacter michiganensis* subspecies *michiganensis*
Pear	Fire blight	*Erwinia amylovora*
Central America (Costa Rica, Honduras, Guatemala, El Salvador)		
Potato	Blackleg	*Erwinia carotovora* subspecies *atroseptica*
	Bacterial wilt	*Ralstonia solanacearum*
Tomato	Bacterial speck	*Pseudomonas syringae* pv. *tomato*
Chili	Bacterial spot	*Xanthomonas campestris* pv. *vesicatoria*
Cauliflower and broccoli	Bacterial soft rot	*Erwinia* species
Cabbage	Bacterial black rot	*X. campestris* pv. *campestris*
Mexico		
Potato	Black leg	*E. carotovora* subspecies *atroseptica*
Apple, pear, and ornamentals	Fire blight	*E. amylovora*
Tomato and chili	Bacterial spot	*X. campestris* pv. *vesicatoria*
Agave	Bland rottenness of the heart of agave	*Erwinia* species
Watermelon	Bacterial spot	*Xanthomonas* species

NOTE. The gentamicin used is Agry-gent (Quimica Agronomica de Mexico, Rhode Island No. 4908, Residencial Campestre, C.P. 31238, Chihuahua, Chihuahua, Mexico).

brates and non-target insects such as honey bees." On the basis of limited public-domain data and on limited patterns of oxytetracycline use, the EPA waived all environmental data requirements. However, its open application in the environment remains a concern.

Recommended concentrations for streptomycin range from 50–200 ppm (50–200 μg/mL), depending on treatment objective and crop. For use in fire blight on apples and pears, an application rate of 24–48 ounces per acre (~2–4 L/hectare) is recommended. Oxytetracycline is used at concentrations of 150–200 ppm (150–200 μg/mL). For treatment of peaches and nectarines, the application rate at 150 ppm is 3 gallons per tree or 240 gallons per acre, which may be increased for large trees, not to exceed 500 gallons per acre per application. For treatment of pears, the application rate at 200 ppm is 50–100 gallons of solution per acre.

In 1999, the latest year for which data are available through the US Department of Agriculture [14], 30% of the pear acreage received a total of 6000 pounds of streptomycin and 40% of the acreage received a total of 12,000 pounds of oxytetracycline. Apples received >15,000 pounds of streptomycin on ~20% of the acreage, or 3000 pounds of oxytetracycline on 5% of the acreage. In 1997, 39,800 pounds of streptomycin and 26,800 pounds of oxytetracycline were used, mostly on pears and apples. Streptomycin use has decreased over the decade, but oxytetracycline use has increased, except in 1999. One reason for the increased use of oxytetracycline is the increasing prevalence of streptomycin resistance in the target bacterium, *E. amylovora* [6, 15, 16].

Most worrisome is the use of gentamicin for plant agriculture in Latin America (table 2). The extent and quantity of antimicrobial use in this region are not known, and the degree of human exposure is unclear. The American Society for Microbiology and others persuaded the EPA that fruits and vegetables treated with gentamicin should not be imported, and a tolerance level for gentamicin in food should not be considered because of the importance of gentamicin in human medicine. The concern was that any unnecessary residues on food could compromise use of this antimicrobial, which is the last economically feasible drug for some human bacterial infections. No data are available on gentamicin use in agriculture in Latin America or on the occurrence of antimicrobial resistance of bacteria on fruits and vegetables from Latin America.

ANTIMICROBIAL RESISTANCE IN PLANT PATHOGENS

Antimicrobial resistance in plant pathogenic target bacteria began to appear as early as the 1960s, a few years after introduction of use of streptomycin [15, 17]. Resistance has also been found to be linked with copper resistance [16, 18]. Genetically, resistance genes may be chromosomal or carried on plasmids or transposons; all genetic forms are found in environmental, human, and plant pathogenic strains [19, 20]. Tetracycline resistance has not been reported in target bacteria—that is, the pathogen—but it has been found in plant surface–associated (phylloplane) bacteria [5].

Although there is at present no evidence for a correlation

between the agricultural use of azoles as fungicides and fungal resistance in humans, such concerns have been expressed [21], and research on this issue is merited. In principle, the same concerns that apply to development of resistance with the use of bacterial antimicrobials are applicable to antifungals. The reverse concern may apply to antiviral agents, which have not yet been used in plants.

Antimicrobial-resistance genes have been used as selectable markers in producing transgenic plants. Under optimized laboratory conditions, the *nptII* gene (conferring resistance to kanamycin) could be transferred from transgenic sugar beets to the soil bacterium *Acinetobacter* sp. BD413 at a frequency of 10^{-9} to 10^{-10} [22]. This gene can also be transferred from transgenic potatoes to *Acinetobacter* BD413 and *Pseudomonas stutzeri* ATCC 17587, both of which harbor plasmids carrying the *nptII* gene with a small deletion [23]. In these experiments, detectable marker rescue was dependent on sequence homology in the recipient cells. Even if such transfer were to occur, Gebhard and Smalla [22] point out that the promoter sequences used in the transgenic constructions are not active in most bacteria, so that the recipients would not express a kanamycin resistance phenotype. Also, most of the antimicrobial-resistance genes used as marker genes are widely disseminated in environmental bacteria. Nevertheless, such use is being phased out because of concerns about potential transfer of these bacterial antimicrobial resistance genes from plant chromosomes back to bacteria, with subsequent horizontal transfer among bacteria in the environment [12].

At the genetic level, little information exists on the extent of antimicrobial susceptibility and resistance occurring naturally in environmental bacteria. Consequently, implications for human health from resistance arising from these sources remain problematic. Alternatives to antimicrobials under investigation include biocontrol agents [24, 25], transgenic plants, and novel chemicals. Some of these agents or compounds have been recently marketed, but efficacy and safety over time still remain to be determined.

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Human Diseases Caused by Foodborne Pathogens of Animal Origin

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Many lines of evidence link antimicrobial-resistant human infections to foodborne pathogens of animal origin. Types of evidence reviewed include: (1) direct epidemiologic studies; (2) temporal evidence; (3) additional circumstantial evidence; (4) trends in antimicrobial resistance among *Salmonella* isolates; and (5) trends in antimicrobial resistance among other pathogens, such as *Campylobacter jejuni*. Commensal microorganisms in animals and humans may contribute to antimicrobial resistance among pathogens that cause disease among humans. For instance, enterococci of food-animal origin, particularly strains that are vancomycin resistant, have been linked to strains found in the human gastrointestinal tract. The latent period between the introduction of a given antimicrobial and emergence of resistance varies considerably, but once the prevalence in a population reaches a certain level, control becomes extremely difficult.

INTRODUCTION

Approximately 500 species of commensal bacteria colonize the human gastrointestinal tract, producing disease only when normal anatomic or immunologic defenses are abrogated. The principal invasive intestinal bacterial pathogens of food-animal origin are *Campylobacter, Salmonella, Listeria, Escherichia coli* O157 (and other Shiga toxin– and enterotoxin-producing strains of *E. coli*), *Yersinia,* and *Vibrio* (table 1). Nearly all are common commensals in cattle, swine, and poultry that sometimes cause invasive infection in animals and humans (except for *E. coli* O157, a colonizer of cattle). *Vibrio,* an exception, is found in seawater and shellfish. Other microorganisms of food-animal origin, such as *Enterococcus* species and *E. coli* strains that produce neither Shiga toxin nor enterotoxin, also may enter and mix with commensal bacteria in the human gastrointestinal tract.

Since 1996, FoodNet has conducted surveillance for bacterial pathogens in foods, keeping track of infections associated with particular food sources. For example, chicken usually is associated with *Campylobacter* and *Salmonella;* consumption of uncooked eggs with *Salmonella enteritidis;* ground beef with *E. coli* O157; pork with *Yersinia;* and shellfish with *Vibrio.* Although annual rates of bacterial infections fluctuate moderately, some more substantial changes include a decline in 1996–1999 in the rate of *Campylobacter* infections by 26% and of *E. coli* O157 infections by 22% (table 2) [2]. In 1999, there were 4533 (17.7 per 100,000 population) culture-confirmed *Salmonella* infections and 3794 (14.8 per 100,000 population) *Campylobacter* infections in the 8 FoodNet surveillance states (26.9 million population) (table 3) [2]. The comparative seriousness of foodborne infections is reflected by hospitalization rates; for example, 88% of patients with *Listeria* infections required hospitalization, compared with 36% for *Yersinia,* 37% for *E. coli* O157, and 22% for *Salmonella* (table 4) [2].

The health impact of these foodborne infections becomes ever more serious because of the growing rate of antimicrobial resistance among these foodborne pathogens, a problem that has been recognized for >3 decades. For example, in its 1969 report, the Swann Committee in England recommended that antimicro-

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Table 1. Reported and estimated illnesses, hospitalization rates, and case fatality rates for known foodborne bacterial pathogens in the United States.

Bacteria	Estimated total cases	Reported cases by surveillance type			Hospitalization rate	Case fatality rate
		Active	Passive	Outbreak		
Campylobacter species	2,453,926	64,577	37,496	146	0.102	0.0010
Salmonella, nontyphoidal	1,412,498	37,171	37,842	3640	0.221	0.0078
Escherichia coli O157:H7	73,480	3674	2725	500	0.295	0.0083
E. coli, non-O157 STEC	36,740	1837	—	—	0.295	0.0083
E. coli enterotoxigenic	79,420	—	2090	209	0.005	0.0001
E. coli, other diarrheogenic	79,420	—	2090	—	0.005	0.0001
Listeria monocytogenes	2518	1259	373	—	0.922	0.2000
Vibrio vulnificus	94	—	47	—	0.910	0.3900
Vibrio, other	7880	393	112	—	0.126	0.0250
Yersinia enterocolitica	96,368	2536	—	—	0.242	0.0005
Clostridium perfringens	248,520	—	6540	654	0.003	0.0005
Brucella species	1554	—	111	—	0.550	0.0500
Total	4,492,418					

NOTE. Data from [1].

bials be used to treat animals only when prescribed by a veterinarian, and that penicillin and tetracycline no longer be used in "subtherapeutic" doses to promote growth of food animals. Since 1969, other advisory committees have endorsed the Swann Committee report and similarly recommended that antimicrobial drugs used to treat human disease not be used as growth promoters in food animals [3–5]. In the early 1970s, most countries in Western Europe banned the use of penicillin and tetracycline as growth promoters, whereas the United States did not.

Since then, the National Research Council and the Institute of Medicine of the US National Academy of Sciences issued several reports regarding human health risks associated with uses of antimicrobial drugs in food-animal production [6, 7]. In 1989, an Institute of Medicine Committee conducted a quantitative risk assessment, concluding that existing data were not adequate to demonstrate directly that the subtherapeutic use of antimicrobials in animal feeds was a definite hazard to human health [6].

In a 1999 report, the National Research Council Committee on Drug Use in Food Animals concluded that use of antimicrobial agents in food-animal production "is not without some problems and concerns" [p. 9, 7]. As a principal concern, it identified uses of antimicrobials in food animals that could enhance the development of antimicrobial resistance and its transfer to pathogens that cause disease in humans. The 1999 report also acknowledged a link between antimicrobial-resistant infections in humans and antimicrobial use in food animals, although the incidence of infections may be low. It recommended establishing integrated national databases to support a science-driven policy for approving antimicrobials for use in food animals.

TYPES OF EVIDENCE THAT LINK HUMAN HEALTH RISKS TO ANTIMICROBIAL USE IN FOOD ANIMALS

Several types of evidence might link the risks of humans becoming infected with antimicrobial-resistant pathogens to use of such drugs in food animals, including (1) direct epidemiologic studies, (2) emergence of resistance among bacteria associated with animals before the emergence of resistance among closely related pathogens associated with humans, (3) addi-

Table 2. Rate and percentage change of bacterial pathogens detected by FoodNet at 5 original sites.

Pathogen	Rate per 100,000 population in				% Change, 1996–1999
	1996	1997	1998	1999	
Campylobacter	23.5	25.2	21.4	17.3	−26
Salmonella	14.5	13.6	12.3	14.8	−2
Typhimurium	3.9	3.9	3.7	3.6	−8
Enteritidis	2.5	2.3	1.4	1.3	−48
Escherichia coli O157	2.7	2.3	2.8	2.1	−22
Yersinia	1.0	0.9	1.0	0.8	−20
Listeria	0.5	0.5	0.6	0.5	0
Vibrio	0.1	0.3	0.3	0.2	+100
Total	51.2	50.3	46.9	40.7	−21

NOTE. Data from [2].

Table 3. Cases and incidence rates of foodborne diseases in the United States.

Pathogen	Cases	Incidence rate/100,000
Bacterium		
Salmonella	4533	17.7
Campylobacter	3794	14.8
Shigella	1031	4.0
Escherichia coli O157	530	2.0
Yersinia	163	0.6
Listeria	113	0.5
Vibrio	45	0.2
Total	10,209	
Parasite		
Cryptosporidium	474	1.5
Cyclospora	14	0.04
Total	488	

NOTE. Data from [2]. Surveillance occurred in 8 states (Connecticut, Georgia, Minnesota, and Oregon, and selected counties in the states of California, Maryland, New York, and Tennessee) through >300 clinical laboratories. The total population assessed was 25.6 million.

tional circumstantial evidence linking antimicrobial use in food animals to resistance among foodborne pathogens that do not tend to be transmitted between individuals, (4) trends in antimicrobial resistance among *Salmonella* isolates, (5) trends in antimicrobial resistance among other pathogens such as *Campylobacter jejuni* and *E. coli* O157:H7 isolates, and (6) studies suggesting that farmers and family members may be more likely than the general population to acquire antimicrobial-resistant bacteria of food-animal origin.

Direct epidemiological evidence. In a prospective study, Levy et al. [8] determined that tetracycline resistance among *E. coli* in fecal samples from farm chickens increased within a week after the introduction of tetracycline-supplemented feed to the flock. Tetracycline-resistant intestinal coliforms also increased among members of the immediate farm family. After chickens received medicated feed for 3–5 months, fecal samples from farm family members contained bacterial populations in which 80% of coliforms were tetracycline resistant, compared with 6.8% in coliforms in fecal samples from neighbors. Approximately 6 months after tetracycline was removed from the feed, percentages of tetracycline resistance in coliforms in fecal samples from farm family members approximated those found before tetracyclines were introduced.

Evidence indicates that antimicrobial-resistant *E. coli* and *Salmonella* species are transmitted from farm animals to humans. For instance, in a 1985 outbreak of multidrug-resistant *Salmonella* serotype Newport in California, transmission of the pathogen was traced by genetic means from human infections to hamburger consumption at fast-food restaurants, then to meat-processing plants, and finally back to the dairy farms where the cattle were raised [9]. The outbreak strain contained a single large plasmid that conferred resistance to several antimicrobials including chloramphenicol, which was apparently used by the dairy without approval by the US Food and Drug Administration.

Such "trace-back" studies are difficult because cattle, hogs, and poultry increasingly are mass produced, transported over great distances, and mass processed. Another potential problem is that considerable time lags typically exist between antimicrobial use in food animals and the identification of antimicrobial-resistant infections in humans. Such studies require isolation and identification of the same resistant strain from humans and animals, and success is therefore more likely in outbreaks than in sporadic cases. However, finding such a genetically defined resistance strain that caused human disease at each step back in the food-production chain (food item, food market, slaughterhouse, feedlot, and farm) would provide "smoking gun" evidence.

Temporal evidence: emergence of resistant strains in animals before those strains appear in humans. Multidrug-resistant *Salmonella* Typhimurium DT104 emerged in 1988 among cattle in England and Wales before it became common in humans [10]. It was subsequently isolated among poultry, sheep, and pigs. Given that DT104 infections occur relatively frequently among humans living on or visiting farms, it is not unreasonable to speculate that extensive use of antimicrobial drugs in food animals may have helped to select for such resistant strains, which subsequently infected humans. Other data from England and Wales indicate that resistance to ampicillin in *Salmonella* Typhimurium was more frequent among isolates from bovines in 1981 (13%) and 1990 (66%) than among humans (5% and 17%, respectively) [11].

Many *Salmonella* serotypes, including *Salmonella* Typhimurium, commonly include relatively high percentages of resistant strains, and resistance levels appear to be similar in strains associated with animals and humans. However, certain less com-

Table 4. Percentage of people hospitalized in the United States because of infections with foodborne pathogens.

Pathogen	% Hospitalized
Listeria	88
Escherichia coli O157	37
Yersinia	36
Vibrio	25
Salmonella	22
Shigella	14
Campylobacter	11

NOTE. Data from [2].

mon *Salmonella* strains isolated from humans, such as *Salmonella* serotypes Braenderup, Javiana, and Enteritidis (accounting for ~2%, 2%, and 15%, respectively, of human *Salmonella* isolates), remain susceptible to commonly used antimicrobials such as sulfamethoxazole, tetracycline, streptomycin, chloramphenicol, and ampicillin [12]. It would be informative to monitor veterinary and food isolates of these *Salmonella* strains to determine whether antimicrobial resistance emerges earlier or later than in comparable isolates from humans.

Temporal differences in emergence of resistant strains in animals and humans might also be evident among *C. jejuni* with use of newer antimicrobial drugs on the farm. For example, in the United States, fluoroquinolone use in poultry began in 1995. Two years later, 14% of chicken samples obtained in Minnesota markets (from 15 poultry-processing plants in 9 states) contained ciprofloxacin-resistant *C. jejuni* [13]. According to statewide surveillance, the proportion of human infections due to quinolone-resistant *C. jejuni* increased during the same period, from 1.3% of all *C. jejuni* infections in 1992 to 10.2% in 1998 [13]. This evidence suggests that chickens may serve as a reservoir of quinolone-resistant *C. jejuni.*

Before the emergence of quinolone-resistant *Campylobacter* in the United States, the prevalence of quinolone-resistant bacterial isolates from poultry and humans increased in Europe, coinciding with greater use of these drugs in both veterinary and human medicine. In the Netherlands, for example, the prevalence of quinolone resistance among *Campylobacter* strains isolated between 1982 and 1989 from poultry products increased from 0% to 14%, whereas the prevalence in humans increased from 0% to 11% [14]. Perhaps accounting for these trends, the fluoroquinolone enrofloxacin was introduced in the Netherlands in 1987 to treat and prevent *E. coli* diarrheal disease and *Mycoplasma* infections in poultry (and less commonly in pigs) [14]. Less-likely causes include the quinolone flumequine, which was used in veterinary medicine in the Netherlands since about 1981; norfloxacin, which was introduced in 1985 to treat human urinary tract infections; and other quinolones, such as ciprofloxacin, pefloxacin, and ofloxacin, which were not introduced for human use until late 1988, early 1989, and late 1989, respectively [14].

In the United States, enrofloxacin and sarafloxacin were licensed for use in poultry and were widely used in the mid- to late 1990s to reduce mortality from *E. coli* and *Pasteurella multocida* infections in chickens and turkeys. Evidence of fluoroquinolone resistance in *Campylobacter* isolates obtained from infected humans suggested that use of these antimicrobial agents in poultry had contributed to fluoroquinolone resistance in *Campylobacter,* and in October 2000, the US Food and Drug Administration announced its intention to withdraw approval for their use in poultry.

The prevalence of fluoroquinolone resistance among common veterinary *Salmonella* isolates appears to increase before human isolates, suggesting that resistant strains move from food-animal sources to humans. Overall, however, there has been little such resistance in human *Salmonella* isolates in the United States, but in recent years, there has been a trend toward decreased susceptibility to fluoroquinolones. The only reported cases of human infection due to fluoroquinolone-resistant *Salmonella* in the United States have been an individual in New York who had an *Salmonella* Schwarzengrund infection (acquired in the Philippines) and an outbreak of 11 cases of a similar *Salmonella* Schwarzengrund infection in an Oregon nursing home [15].

Enterococcus faecium infections are major problems in hospitalized patients, particularly those in intensive care units. Plasmid-mediated, high-level (*van*A) vancomycin resistance in *E. faecium* emerged in humans in France in 1986 [16], causing concern because such infections were essentially untreatable. In the 1990s, vancomycin-resistant enterococci (VRE) infections emerged in the United States, and by 1998, >21% of nosocomial enterococcal infections in the United States were due to VRE. In Europe, VRE infections have not increased at the same rate and to the same degree as in the United States, suggesting the possibility of a different epidemiology.

The glycopeptide antimicrobial avoparcin was approved for growth promotion in farm animals in Europe in 1974. In 1994, VRE were isolated from pig herds and on farms in the United Kingdom [17]. In 1995, VRE from pigs, poultry, and humans were isolated in Germany, and this emergence appeared to be associated with the high-volume use of this and other glycopeptides as growth promoters in food animals [18]. This has not been the case in the United States, where vancomycin use in hospitalized patients has been extensive, but avoparcin has not been approved for use in food animals. Europeans are frequently fecal carriers of VRE types also found in animals and presumably ingested from food [19–21]. Furthermore, *Tn*1546-like elements of VRE carry single nucleotide (T or G) variants, with G variants found only in poultry isolates, whereas swine isolates carry the T variant [22]. However, among human VRE isolates, these G and T mutations are evenly distributed, suggesting that food animals are the source of vancomycin resistance genes in humans rather than the reverse. Furthermore, human isolates from a Muslim country, where swine are not raised or consumed, carry only the G mutation [22].

Because of concerns about increasing resistance to glycopeptide antibiotics, avoparcin use was banned in Denmark in 1995, in Germany in 1996, and in all European Union states in 1997. Although the prevalence of glycopeptide resistance among *E. faecium* of porcine origin in Denmark remained at ~20% from 1995 to 1997 [23, 24], its prevalence in *E. faecium* of swine decreased to 6% by 2000. Genetic evidence suggested a link between glycopeptide and macrolide resistance, so this trend may reflect the decreased veterinary use of tylosin (which

may have been selecting for glycopeptide resistance) since 1998 in addition to the ban on avoparcin [24].

Between 1996 and 2000, the prevalence of vancomycin resistance in *E. faecium* from poultry dropped from 42.5% to 5.8% [24]. Meanwhile, in Germany, the proportion of VRE-positive poultry meat samples decreased from 100% in 1994 to 25% in 1997, and the carrier rate in fecal specimens from humans in the community dropped from 12% in 1994 to 3% in 1997 [25]. Because VRE were not monitored when avoparcin use began, it is impossible to ascertain whether there were temporal differences in the emergence of resistance in poultry and humans. However, the prevalence of VRE among poultry and human isolates declined at similar rates after the discontinuation of avoparcin use in agriculture.

Monitoring resistance to quinupristin-dalfopristin (QD), a combination of 2 streptogramins, may provide a better opportunity to evaluate temporal emergence of resistant strains in animals and humans. QD was introduced clinically for treatment of VRE after 1996; another streptogramin, virginiamycin, has been used as an agricultural growth promoter in Europe and the United States for decades, mainly for poultry. QD-resistant *E. faecium* are now found in the United States, the Netherlands, and Denmark [26–28]. The gene *sat*A, which confers resistance to both virginiamycin and QD, has been found in *E. faecium* strains of both animal and human origin. It has also been demonstrated that this gene can be transferred among strains of *E. faecium* within the mammalian intestinal tract [29].

In the United States between July 1998 and June 1999, in chickens purchased in grocery stores, *E. faecium* were found in 5% of chickens cultured in nonselective broth and in 62% cultured in selective broth [30]. Of the 20 *E. faecium* strains isolated in nonselective broth, 55% were QD resistant; with selective broth, 58% of 407 chickens sampled contained QD-resistant *E. faecium* strains. Meanwhile, 3 of ~300 human stool samples collected from outpatients contained QD-resistant *E. faecium* [30]. This low but significant proportion of QD-resistant *E. faecium,* despite the low rate of human carriage, suggests that humans are acquiring resistant organisms through the consumption of poultry treated with virginiamycin. Continued monitoring of the proportions of QD-resistant *E. faecium* in humans and poultry might provide further evidence to determine whether resistance in human isolates can be attributed to the use of virginiamycin in food-animal production.

Circumstantial evidence linking antimicrobial resistance to drug use in food production. Antimicrobial use in food animals is implicated in certain human infections involving drug-resistant pathogens such as *Salmonella* species and *C. jejuni,* which are rarely transmitted from person to person. Although the evidence is circumstantial, several types of observations link steps in meat and poultry production to consumption of such food products and subsequent development of disease involving antimicrobial-resistant pathogens.

Such observations include the following: (1) according to a 2001 report [31], 70% of all antimicrobial agents used in the United States (24.5 million pounds per year) are administered to livestock for nontherapeutic purposes; (2) antimicrobial-resistant non-Typhi *Salmonella* are found in high proportions among isolates from swine, including sulfamethoxazole (23%), tetracycline (50%), ampicillin (12%), and streptomycin (23%) [32]; (3) similarly, high proportions of poultry and ground meats are contaminated with antimicrobial-resistant pathogens or potential pathogens [13, 30, 33]; (4) specific drug-resistant strains persisted for up to 14 days in stool samples obtained from volunteers who ingested either 10^7 cfu of vancomycin-resistant *E. faecium* of chicken origin or 10^7 cfu of virginiamycin-resistant *E. faecium* of swine origin [34]; and (5) among *Bacteroides* species in the human intestinal tract, horizontal transposon transfer of resistance to tetracycline (*tet*Q) and erythromycin (*erm*F and *erm*G) has been observed [35]. Between 1970 and the 1990s, carriage of *tet*Q in individuals from the community increased from 23% to >80% of isolates, whereas *erm*F and *erm*G increased from <2% to 23%. Transfer of these transposons occurs when donor bacteria are first stimulated with low levels of tetracycline; once acquired, however, these resistance genes are stably maintained in the absence of antimicrobial selection.

***Resistance to antimicrobials among human isolates of* Salmonella.** The human disease burden with salmonellosis is considerable, estimated at 1,400,000 *Salmonella* infections annually in the United States and causing 16,000 hospitalizations and nearly 600 deaths [1]. Most human salmonellosis results from contaminated food of animal origin, although water and reptiles are other possible sources of infection. Antimicrobial resistance patterns among *Salmonella* isolates from humans are of interest in determining whether resistant pathogens are commonly transferred between food animals and humans, because *Salmonella* infections are infrequently transferred from person to person.

In 1996, the National Antimicrobial Resistance Monitoring System (NARMS) began to compile data describing antimicrobial susceptibilities of every 10th *Salmonella* isolate and every fifth *E. coli* O157:H7 isolate. Surveillance is carried out through 16 health departments in states and in 2 high-density population centers (New York City and Los Angeles) that represent 37% of the US population. According to NARMS data, susceptibility to individual antimicrobials of non-Typhi *Salmonella* from humans did not change significantly from 1996 to 1999 [12]. This may indicate that overall resistance patterns are in equilibrium or clonal balance.

Analyzing trends in resistance among *Salmonella* serotypes can also be informative. The >2200 serotypes of *Salmonella*

enterica vary widely in niche and intrinsic pathogenicity, with *Salmonella* serotypes Typhimurium, Enteridis, Newport, and Heidelberg among the strains most commonly isolated from human infections (table 5). Susceptibility patterns among *Salmonella* serotypes varied widely in 1999. Resistance to most antimicrobial agents has been essentially absent among isolates of *Salmonella* serotypes Braenderup and Javiana and of low prevalence in *Salmonella* Enteritidis (9% of isolates resistant to tetracycline, 10% to ampicillin), whereas *Salmonella* Heidelberg isolates showed somewhat higher frequencies of resistance (24% to streptomycin, 19% to tetracycline, 19% to sulfamethoxazole). The most prevalent serotype, *Salmonella* Typhimurium, showed even higher frequencies of resistance to ampicillin (41% of isolates in 1999), chloramphenicol (29%), streptomycin (43%), sulfamethoxazole (45%), and tetracycline (42%), whereas *Salmonella* serotype Hadar was also highly resistant to ampicillin (23%–42% of isolates from 1996–1999), streptomycin (31%–93%), and tetracycline (91%–100%) [12].

Because the majority of human *Salmonella* infections are foodborne, the emergence of new resistance patterns among human isolates is likely to result from agricultural practices. For example, a strain of *Salmonella* Typhimurium, phage type DT104, has emerged in Europe and the United States [37] with resistance to 5 antimicrobial drugs: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT). In the 1999 NARMS compilation, 28% of the 362 isolates of *Salmonella* Typhimurium had the ACSSuT resistance pattern [12], and 12% of these isolates were also resistant to kanamycin, 3% to ceftiofur, and 1% to ceftriaxone. Ciprofloxacin resistance has not been observed among *Salmonella* Typhimurium human isolates in the United States, but 1996 data from the United Kingdom indicate that 14% of 5-drug–resistant isolates of DT104 had a decreased susceptibility (MIC, $\geqslant$0.25 μg/mL) to ciprofloxacin [38].

In addition to DT104, another multidrug-resistant *Salmonella* Typhimurium strain, bearing the resistance pattern AKSSuT, has emerged in the United States. Of the 700 *Salmonella* Typhimurium strains referred to the US Centers for Disease Control and Prevention and tested in 1997–1998, 11% had this pattern (compared with 38% that were DT104) [39]. The frequency of *Salmonella* Typhimurium with this resistance pattern has decreased from 34% in 1996 to 28% in 1999 among human isolates, whereas the frequency among animal isolates did not change. Human *Salmonella* strains resistant to 8 or more antimicrobials increased in frequency from 0.3% of all non-Typhi isolates in 1996 to 2% in 1999 [40].

Salmonella Newport made up 4.9% of 3751 human *Salmonella* isolates between 1997 and 1999. Among these, a particular strain had a unique pulsed-field gel electrophoresis (PFGE) pattern and resistance to 7 antimicrobials (ampicillin, chloramphenicol, cephalothin, clavulanic acid, streptomycin, sulfamethoxazole, and tetracycline [ACCephClavSSuT]) plus intermediate resistance to ceftriaxone. This pattern increased among all US *Salmonella* Newport isolates, from 1.3% in 1998 to 17.2% in 1999 [41]. Over the same interval, 56 *Salmonella* Newport isolates with a similar resistance pattern (1% of all animal *Salmonella* isolates) were noted among strains isolated from a particular processing facility, and a similar strain of animal origin increased from 8.3% in 1998 to 27.3% in 1999 [41].

Table 5. The 20 most frequently reported *Salmonella* serotypes from human sources reported to the Centers for Disease Control and Prevention in 1999.

Salmonella serotype	No. of isolates	%
Typhimurium	7631	23.5
Enteritidis	5102	15.7
Newport	2508	7.7
Heidelberg	1687	5.2
Muenchen	1328	4.1
Javiana	1111	3.4
Montevideo	814	2.5
Thompson	613	1.9
Oranienburg	606	1.9
Infantis	540	1.7
Braenderup	499	1.2
Hadar	498	1.2
Agona	481	1.2
Saint Paul	446	1.1
Typhi	359	1.1
Poona	230	0.7
Mississippi	226	0.7
Paratyphi B	200	0.6
Mbandaka	160	0.5
Java	143	0.4
Other	5710	17.6
Unknown	1571	4.8
Total	32,463	98.7

NOTE. Data from [36].

Contemporaneous parallel data from the United States on antimicrobial drug resistances in human and animal isolates of *Salmonella* are limited. The earlier appearance of a higher prevalence of a specific drug resistance or resistance pattern in animal sources might suggest the direction of flow, but development of a rough equilibrium might eventually be anticipated. Limited insight into this phenomenon may be provided by data from England and Wales, where the prevalence of resistance to ampicillin in *Salmonella* Typhimurium isolates was higher among bovines in 1981 (13%) and 1990 (66%) than among humans (5% and 17%, respectively) [11].

Comparisons of serotype distributions in *Salmonella* between humans and animals at the time of slaughter provide additional evidence. Using a mathematical model developed to predict

serotype distribution of *Salmonella* isolates among humans on the basis of data from farm animals, Sarwari et al. [42] observed a significant mismatch between predicted and actual human serotype distributions. For example, although the model predicted that *Salmonella* serotype Kentucky should comprise 14% of all isolates from humans, in reality <1% of human cases are due to this serotype. For *Salmonella* Typhimurium the mismatch is in the opposite direction, with the model predicting this serotype to comprise 12% of all human isolates when in fact it was observed in 29% [42].

At least in part, however, these discrepancies may be explained by the fact that the model assumed an equal probability of causing illness for all *Salmonella* serotypes and food categories. Early volunteer studies with a number of *Salmonella* serotypes suggested that all serotypes were equally capable of causing human disease [43], but these studies were limited and did not include many of the serotypes now commonly isolated from humans and animals. Animal studies have indicated that certain *Salmonella* serotypes are more likely to cause invasive disease and bacteremia. Thus, it is reasonable to expect that *Salmonella* serotypes differ in their ability to infect the human intestinal tract and to cause disease, likely accounting for the mismatch between the predicted and observed results with the above-noted mathematical model.

Antimicrobial-resistant* C. jejuni *and* E. coli *O157:H7. *Campylobacter* causes an estimated 2.4 million cases of illness annually in the United States, with an estimated hospitalization rate of 10.2% and a case fatality rate of 0.1% [1]. Foodborne transmission accounts for ~80% of cases, with chickens the most common source of such infection. According to a 1998 FoodNet study, 11% of 858 human isolates tested nationwide were ciprofloxacin resistant [44]. Among 67 individuals not treated with antimicrobials, diarrhea lasted longer (12 days) when the isolates were ciprofloxacin resistant than when they were ciprofloxacin susceptible (6 days) ($P = .02$). Resistance to ciprofloxacin among human isolates of *Campylobacter jejuni* increased from 13% in 1997 to 18% in 1999, according to NARMS surveillance data [12]. In a detailed study of *C. jejuni* infections (6674 in 1992–1998, amounting to 20.7 cases per 100,000 population), quinolone-resistant *C. jejuni* isolates increased from 1.3% in 1992 to 10.2% in 1998.

A 1999 survey indicated that 44% of 180 chickens tested in 3 states were contaminated with *Campylobacter,* and antimicrobial resistance occurred frequently (65% of isolates were resistant to tetracycline, 32% to nalidixic acid, 24% to ciprofloxacin, and 5% to erythromycin); 24% of isolates were resistant to 3 or more drugs, but resistance to both a macrolide and ciprofloxacin occurred in only 2.5% of isolates [45]. In a similar survey [12], ciprofloxacin-resistant *C. jejuni* were isolated from 14% of 91 domestic chicken products from retail markets in the Minneapolis–St. Paul, Minnesota, area. Six of 7 molecular subtypes of quinolone-resistant *C. jejuni* identified among isolates from poultry products were also present among human isolates of the same species, implicating the poultry as a source of drug-resistant *C. jejuni* infections [13]. Case-control studies suggest additional risk factors, including pets and raw milk [46–48].

Meanwhile, *E. coli* O157:H7 accounts for an estimated 73,480 illnesses annually in the United States, leading to a hospitalization rate of ~29.5% and a case fatality rate of 0.8% [1]. Among 802 isolates collected between 1996 and 1999, 6.9% were resistant to a single drug, and 5.9% were multidrug resistant [49]. The most prevalent resistances were to sulfamethoxazole (10%), tetracycline (4%), and streptomycin (2%). Less than 2% of isolates were resistant to ampicillin, ceftiofur, cephalothin, chloramphenicol, trimethoprim-sulfamethoxazole, or kanamycin. From 1996 to 1999, there were only very minor changes in resistance to individual antimicrobials [12]. Antimicrobial treatment remains inadvisable for *E. coli* O157:H7 gastroenteritis because it may predispose patients to develop hemolytic-uremic syndrome.

ARE FARMERS MORE LIKELY THAN OTHERS TO ACQUIRE ANTIMICROBIAL-RESISTANT BACTERIA OF FOOD-ANIMAL ORIGIN?

Even in the absence of antimicrobial selection, *E. coli* of animal origin can colonize the human intestinal tract and that of other animals. In a study by Marshall et al. [50], *E. coli* of porcine and bovine origin were engineered to contain a transferable multiple resistance plasmid and bearing a selectable chromosomal marker. The bacteria were then fed back to host animals, which were housed adjacent to, but separate from, potential secondary hosts. These mutant microbial strains of bovine and porcine origin persisted in their original hosts for most of a 4-month test period [50]. The inoculated strain was also isolated from multiple secondary hosts, including humans, with direct or indirect contact with the inoculated donors. The bovine mutant was excreted by 2 caretakers for more than a month.

Hummel et al. [51] studied a pig-farming community in which the streptothricin antimicrobial nourseothricin was added to pig feed as a growth promoter. After 2 years of nourseothricin use, coliform organisms containing plasmids conferring nourseothricin resistance were found in 33% of fecal isolates of pigs with diarrhea, in 17%–18% of those from employees of the pig farms and their families, and in 16% of outpatients in adjacent communities. Nourseothricin had not been used in humans in the region [51].

In the Netherlands, pig farmers showed a higher prevalence of antimicrobial resistance among fecal *E. coli* than did abattoir workers and urban and suburban residents [52]. *E. coli* from fecal samples of pig farmers were 53%–84% resistant to com-

monly used antimicrobials (amoxicillin, tetracycline, trimethoprim, sulfonamides), whereas samples from their pigs were 92%–100% resistant [53]. Only 4% of *E. coli* isolates from farmers were resistant to the same antimicrobials as those of pigs from their farms, and there were only very limited similarities in biotype, plasmid content, and DNA restriction patterns of *E. coli* isolated from farmers and their pigs [54].

VRE were found in 50% of the turkey fecal samples, 39% of fecal samples from turkey farmers in Europe, 20% of fecal specimens from turkey slaughterers, and 14% of specimens from area residents [20]. Turkey flocks receiving avoparcin in feed had a higher prevalence of VRE (60%) than flocks not receiving the glycopeptide (8%). The percentage of VRE relative to the total enterococcal populations in each of the 4 groups was low (2%–4%) [55]. Although the PFGE patterns of VRE isolated from the different groups were heterogeneous, the same PFGE pattern was found among human and animal isolates, and similar *van*A containing transposons were found in VRE isolates from both groups. These results suggest that animals serve as a reservoir for *van*A resistance in Europe, where avoparcin use was permitted until recently.

In the early 1990s in the US Pacific Northwest, cattle isolates of *Salmonella* Typhimurium DT104 increased in frequency, reaching 73% of *Salmonella* Typhimurium isolates in 1995, and thereafter decreasing to 30%. Human patients infected in the Northwest with R-type ACSSuT resided in postal zip code areas of above-average cattle farm density ($P < .05$), whereas patients infected with other R types did not. Although the prevalence of salmonellosis in humans did not change, the strain involved (DT104) did. In addition, people with *Salmonella* Typhimurium (R-type ACSSuT) infection in the Northwest were more likely to have had direct contact with livestock compared with humans infected with other strains of *Salmonella* Typhimurium.

Since 1991, *Salmonella* species resistant to expanded spectrum cephalosporins have been noted in South America, Europe, North Africa, and the Middle East, and this resistance may be spreading to the United States. For example, according to a review of domestically acquired ceftriaxone-resistant *Salmonella* infections in the United States associated with an AmpC β-lactamase detected in 1996–1998, 3 of 13 patients had visited a farm within the 5 days before the illness began [56]. Other reports indicate a strong association between humans becoming infected with multidrug or cephalosporin-resistant *Salmonella* and farm exposure to such pathogens. Evidence includes the following:

- Consuming beef, pork, and chicken products and having contact with farm animals were risk factors for developing *Salmonella* Typhimurium DT104 infections in the United Kingdom, according to a 1993 case-control study [10, 57]. Other case reports describe antimicrobial-resistant *Salmonella* infections among members of farm families with direct or indirect contact with infected farm animals [58, 59].
- In 1976, several calves newly introduced on a Connecticut farm developed infection due to *Salmonella* Heidelberg resistant to chloramphenicol, tetracycline, and sulfamethoxazole [60]. The farmer and his pregnant daughter became infected with the same antimicrobial-resistant strains. The daughter delivered a son 9 days after the calves came to the farm, and 3 days after delivery, the newborn infant developed gastroenteritis and bacteremia from *Salmonella* Heidelberg with the same antimicrobial resistance profile [60].
- In the late 1970s, outbreaks of *Salmonella* Typhimurium (multiantimicrobial resistant) of phage types 193 and 204 occurred among calves on >300 farms in the United Kingdom and caused 211 human infections after entering the food supply, 30 of which developed in people on farms where outbreaks involved multidrug-resistant (chloramphenicol, streptomycin, sulfonamide, and tetracycline [CSmSuT]) strains [61].
- In 1977, an outbreak of multidrug-resistant *Salmonella* infections among 3 of 4 members of a family who worked on a dairy farm in Kentucky was transmitted, apparently through unpasteurized milk [62].
- A human *Salmonella* infection in the United States due to ceftriaxone-resistant *Salmonella* Typhimurium was reported in 2000 in a 12-year-old boy with gastroenteritis; this strain and 1 of 4 isolated from nearby cattle with salmonellosis were indistinguishable and resistant to 13 antimicrobials [63]. Four days before the onset of fever and abdominal pain, the boy had finished a 10-day course of treatment with amoxicillin-clavulanate for a sinus infection.

ECOLOGICAL AND ENVIRONMENTAL EFFECTS OF ANTIMICROBIAL RESISTANCE AMONG COMMENSAL MICROORGANISMS COMMON TO FARM ANIMALS AND HUMANS

Salmonella, Campylobacter, and heat-stable enterotoxin-producing *E. coli* (STEC) species are, except for STEC species, endemic in food animals and capable of producing invasive disease. By contrast, *Enterococcus* species are commensals in the human and animal gastrointestinal tracts and invade adjacent tissues or bloodstream when mucosal barriers are breached after surgery or for conditions such as diverticulitis, bowel neoplasms, or vascular compromise, or when introduced into otherwise sterile body areas. Moreover, *Enterococcus* species may contaminate the skin of hospitalized patients and may colonize those receiving antimicrobials to which enterococci are not susceptible. Such patients are also susceptible to pericatheter or bacteremic infections.

Vancomycin, a glycopeptide antimicrobial, became available in the late 1950s for treatment of serious penicillin-resistant *Staphylococcus aureus* infections, and by the 1960s and 1970s was used increasingly to treat methicillin-resistant *S. aureus,* or other *S. aureus* and enterococcal infections in individuals allergic to penicillin. Since the 1980s, vancomycin use has accelerated considerably in the United States, where it is used to treat penicillin-resistant or penicillin- and aminoglycoside-resistant (streptomycin, gentamicin) enterococcal infections and also *Clostridium difficile* enterocolitis.

Resistance to vancomycin (or the related glycopeptide teicoplanin) in clinical isolates was first reported in Europe in 1988 and in the United States in 1989. Since then, according to the National Nosocomial Infections Surveillance System, VRE increased among hospitalized patients 20-fold through 1993 [64]. Although *Enterococcus faecalis* is the most frequent pathogen among enterococci causing human disease, vancomycin resistance is far more prevalent among *E. faecium.*

Treating patients with antianaerobic antimicrobials such as clindamycin and metronidazole for multiple conditions appears to promote high-density (>6 log per gram) colonization with VRE, primarily by inhibiting intestinal anaerobes [65]. In contrast, the use of antimicrobials with minimal activity against anaerobes but with activity against susceptible Enterobacteriaceae, such as cephalexin, trimethoprim-sulfamethoxazole, or ciprofloxacin, does not produce such high-density colonization. Environmental contamination may further contribute to nosocomial spread of infection with VRE. Eighty percent of environmental specimens from incontinent patients with >4 log per gram VRE in stool showed VRE, whereas ~10% of environmental samples from patients with lower concentrations of VRE in stool showed VRE.

Several lines of evidence suggest that antimicrobial-resistant enterococci of food-animal origin can colonize the human gastrointestinal tract:

- Ingesting vancomycin-resistant *E. faecium* associated with chickens or virginiamycin-resistant *E. faecium* associated with pigs led to resistant strains appearing in stools of volunteers for up to 14 days, suggesting multiplication during intestinal transit [34].
- In Europe, where the glycopeptide antimicrobial avoparcin was used for years as a feed additive, carriage of VRE is as high as 28%, considerably higher than in the United States, where VRE are relatively absent outside the nosocomial environment [66]. Although VRE among strains causing nosocomial infections was low in Europe, *van*A-positive enterococci were readily detected outside hospitals in several European countries [55].
- After avoparcin use on Danish farms was suspended in 1996, prevalence of resistance to this antimicrobial among *E. faecium* isolates declined from 82% to 9% in 1998 [67]. After a similar ban in Germany, VRE declined in poultry [18], and VRE prevalence in the intestinal flora of healthy individuals in the same area fell from 12% in 1994 to 3% in 1997.

TIME LAGS FOR RESISTANCE TO AN ANTIMICROBIAL AFTER ITS INTRODUCTION

Resistant microbial strains may "emerge" under the continuing selective pressure of a given drug, one of its congeners, or a linked antimicrobial. This process may involve 2 latent periods: first, one occurring after a drug is introduced into human or veterinary medicine and before resistant strains are identified; and second, another occurring after resistance is recognized and before it becomes so prevalent that it leads to significant therapeutic failures. During the past 50 years, such latent periods have varied from a few years to several decades, depending on the specific antimicrobial agent, the mechanism by which resistance was spread (vertically or horizontally), and the amount of antimicrobial agent in use. Limiting or even banning use of specific drugs during the second latent period might avoid such therapeutic failures. However, once resistance reaches a certain threshold level, avoiding such failures may become impossible.

Resistance has typically been initially identified in nosocomial infections. Pertinent instances of the emergence of resistance to specific antimicrobial agents include the following:

- Penicillin G was introduced into clinical medicine in the mid-1940s. Even though ~90% of *S. aureus* isolates before 1946 were susceptible to penicillin, by 1952, ~75% of *S. aureus* isolates at the Boston City Hospital where this antimicrobial was widely used had become penicillin-resistant. By the mid-1960s, the majority of *S. aureus* isolates in hospitals were penicillin resistant, and for the past 20–30 years, 90% of all human *S. aureus* strains have been penicillin resistant [68].
- Methicillin-resistant *S. aureus* (MRSA) was described initially in England in 1961 and soon became an important cause of nosocomial outbreaks of infections around the world. The prevalence of MRSA among *S. aureus* isolates differed markedly among countries in the 1980s: 0.1% in Denmark, 4% in Germany, 15% in the United States, and 29% in France. The prevalence of MRSA in the United States rose from 2.4% in 1975 to 29% in 1991 [68]. Once such high prevalence has been reached, it has proved extremely difficult to reduce resistance levels, despite the introduction of infection-control practices.
- In the mid-1980s, the fluoroquinolones were introduced into clinical medicine and, among other things, were used to treat infections due to MRSA and to eradicate the carrier state. Unfortunately, in the late 1980s and early

1990s, resistance to fluoroquinolones rapidly developed. Over that short interval, >80% of MRSA at a large tertiary-care hospital in New York City had become resistant to fluoroquinolones [69].

- Vancomycin resistance among clinical enterococcal isolates, particularly *E. faecium* isolates highly resistant to penicillin, was initially recognized in the late 1980s in France and England. In the Massachusetts General Hospital, 99% of enterococcal isolates were susceptible to vancomycin in 1993; by 1995, the percentage of resistant strains had increased to 9%; and most recently (1997–2000), 13%–16% of strains have been vancomycin resistant. This increase has persisted despite real-time reporting of vancomycin-resistant isolates, greater attention to infection-control measures, and exhortations to restrict vancomycin use.
- In a burn treatment unit where topical use of gentamicin had begun in 1964, 90% of *Pseudomonas aeruginosa* isolates were gentamicin susceptible in 1965. By 1967, with continued intensive use of topical gentamicin, only 9% of *P. aeruginosa* isolates remained susceptible. In 1969, topical use of gentamicin was discontinued, and a year later, 95% of *P. aeruginosa* isolates from burns were again gentamicin susceptible [70].
- In Finland, the frequency of erythromycin resistance among group A streptococci increased from 5% in 1988–1989 to 13% in 1990, leading to recommendations to reduce the use of macrolide antimicrobials [71]. Macrolide consumption dropped by 50% in 1992. Erythromycin resistance among group A streptococci reached 19% in 1993 and then steadily declined to a level of 8.6% in 1996.
- *Streptococcus pneumoniae* began to show intermediate penicillin resistance in the 1970s in South Africa; in the 1980s, highly penicillin-resistant strains began to appear in Spain. By the mid-1990s, the prevalence of penicillin resistance among pneumococcal isolates in the United States had reached 20%–25%, with even higher levels in isolates from children in day care centers. In the 1997 study of lower respiratory tract isolates of *S. pneumoniae* at tertiary-care hospitals in the United States, the prevalence of resistance ranged from 30% to 60% [72].

A major decrease in prescription of a particular antimicrobial does not necessarily reduce resistance to that drug. Between 1991 and 1999, the annual number of sulfonamide prescriptions in the London Hospital dropped from 320,000 to 77,000. Despite this major decline in sulfonamide use, prevalence of sulfonamide resistance among *E. coli* clinical isolates in this hospital remained high: 39% in 1991 and 46% in 1999. Genes for sulfonamide resistance on integrons or plasmids were frequently found in these strains. Among explanations to account for the lack of decline in sulfonamide resistance are the following: (1) it is a slow process, (2) additional compensatory mutations allow resistant strains to persist in the absence of selection, (3) continued use of sulfonamides in agriculture (80 tons trimethoprim-sulfamethoxazole sold in food animals in 1998) may permit recurrent transfers of resistant organisms to humans via the food chain, and (4) close linkage of sulfonamide-resistance genes to other resistance determinants selects for the latter and maintains sulfonamide resistance [73].

Farm animals exposed to an antimicrobial over prolonged periods develop a microbial flora resistant to the antimicrobial, much as occurs in a hospital intensive care unit. In a herd of pigs maintained on subtherapeutic concentrations of tetracycline for 9 years, the prevalence of tetracycline resistance, predominantly plasmid mediated, in the fecal coliform population averaged >90% [6]. This herd of pigs, established in 1963, received antimicrobials routinely, but it did not receive any single antimicrobial continuously. After 1972, the herd was no longer exposed to any antimicrobial agents, at which time tetracycline resistance among fecal coliforms was >90%. In subsequent years, resistance declined, but slowly, and remained at 57% 8 years after exposure to tetracyclines and other antimicrobials ceased. Similarly, although glycopeptide resistance among *E. faecium* from broilers and pigs in Denmark declined markedly after avoparcin use was banned, resistant *E. faecium* could still be found almost 6 years later [24].

Such development of antimicrobial resistance in both pathogenic bacteria and commensals of humans and food animals is informative. The latent period between introduction of a new class of antimicrobials and the emergence of the initial resistant strains varies considerably from drug to drug. This interval may be only several years, as in the selection of penicillin- or ciprofloxacin-resistant *S. aureus,* or several decades, as in the selection of penicillin-resistant *S. pneumoniae* and vancomycin-resistant *E. faecium.*

In human medicine as well as on the farm, the apparent absence of antimicrobial resistance cannot provide assurance that it will not become a problem. It appears to be clear that once the prevalence of resistance rises, the time in which to act (reduction of specific antimicrobial use; institution of infection control measures) is limited. Once antimicrobial resistance reaches high prevalence levels in hospitals (e.g., ~25% for MRSA, ~15%–20% for VRE) or on farms, the resistant strains become endemic and extremely difficult, if not impossible, to reduce in prevalence, except perhaps over prolonged periods of time. Monitoring programs can be helpful in recognizing the spread of resistance while there is still time to control it.

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Potential Mechanisms of Increased Disease in Humans from Antimicrobial Resistance in Food Animals

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There are at least 5 potential mechanisms by which antimicrobial resistance can have adverse effects on human health. The first, called the "attributable fraction," relates to individuals who become infected only because they are taking an antimicrobial agent (for unrelated reasons) to which the pathogen is resistant: the antimicrobial agent, by suppressing their normal microbiota, renders them more vulnerable to infection. A second mechanism involves the linkage of virulence traits to resistance traits so that resistant organisms may be more virulent than susceptible organisms. A third mechanism is that treatment may be rendered ineffective by the choice of a drug to which the pathogens are resistant or may be complicated by the need to use an agent with less desirable attributes than would otherwise be the case. A fourth mechanism is the animal equivalent of the attributable fraction: resistant pathogens acquired by this mechanism in food animals may then be transmitted through the food chain to humans. Last, resistance traits can be acquired by the commensal flora of animals; from this reservoir, resistance traits could find their way through the food chain to commensals and pathogens of humans.

Simply showing that a growing proportion of pathogenic and commensal organisms isolated from food animals are resistant to antimicrobial agents is not enough to prove a human health threat. Rather, it must be demonstrated that, as a result of such antimicrobial resistance, infections are more numerous, or are more severe, or are less easily treated (i.e., outcomes are worse or treatments more costly) than would be the case otherwise. This article reviews the mechanisms by which antimicrobial resistance per se could cause adverse effects on human health (table 1).

The relationship between antimicrobial drugs, antimicrobial resistance, and pathogenicity of microorganisms is exceedingly complicated, as is illustrated by the first mechanism. The commensal microbiota of the intestine and other epithelial surfaces normally exerts a protective effect against colonization and infection by exogenous organisms. Treatment with antimicrobial agents often results in a reduction in various components of this microbiota. Therefore, subjects taking antimicrobial agents are, paradoxically, at increased risk of certain infections, both while taking the antimicrobial and for some days or weeks thereafter, until the microbiota is reconstituted. By means of statistical and epidemiologic analyses, a subset of patients can be identified who become infected only because their resistance to infection has been diminished by consumption of an antimicrobial. This subset of subjects has been called the "attributable fraction" (i.e., the fraction of infected subjects in whom this was the presumed mechanism), "etiologic fraction," or "excess cases." Although this phenomenon could theoretically apply also to subjects infected by antimicrobial-susceptible pathogens (provided they stopped taking the antimicrobial agent before exposure to the pathogen), virtually all evidence of the effect has emerged from studies of infection by

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Table 1. Potential mechanisms by which antimicrobial resistance could cause an increased burden of infection in humans.

Mechanism	Description	Consequence
(1) "Attributable fraction"	Subjects who are taking an antimicrobial agent for unrelated reasons have diminished colonization resistance and become infected by a pathogenic organism resistant to the antimicrobial agent	Increased number of infections (infection of people who would not otherwise have been infected)
(2) Genetic linkage of resistance traits and virulence factors	Increased virulence of the pathogen acquiring the resistance trait	More infections, more severe infections, prolonged duration of infections
(3) Resistance to commonly used antimicrobial drugs	Initial therapy may be ineffective, or a less desirable agent than the usual one must be chosen	Worse outcomes through choice of an ineffective drug, or necessity of choosing a more toxic or more expensive drug
(4) "Attributable fraction" (equivalent of mechanism 1) in food animals	Increased amount of drug-resistant pathogens in food animals and in the food chain, creating a reservoir of drug-resistant pathogens that could be transferred to humans	More infections and more antimicrobial-resistant infections in humans
(5) Acquisition of resistance traits by nonpathogenic, commensal organisms in food animals	In food animals, creation of a reservoir of drug resistance traits that could be transferred via commensals to human pathogens	More antimicrobial-resistant infections in humans

antimicrobial-resistant strains (see Barza and Travers, in this supplement). This mechanism should not be confused with the phenomenon, demonstrated in humans and laboratory animals, by which the treatment of salmonella infections with certain antimicrobial agents appears to prolong shedding of the organism in the feces. That effect, the mechanism of which is uncertain, is seen primarily with drugs to which salmonella are susceptible and, therefore, is not relevant to the issue of antimicrobial resistance.

A second potential mechanism by which antimicrobial resistance might increase the disease burden in humans is by genetic linkage of resistance traits and virulence factors, resulting in increased virulence of resistant strains. An increase in virulence would arise not directly from the antimicrobial-resistance mechanism itself but from linkage of the resistance genes to other virulence genes. Cotransfer of the resistance trait and virulence genes could make drug-resistant pathogenic strains intrinsically more virulent than drug-susceptible strains. As a result, the infective dose could be reduced compared with that usually required, or, for a given infective dose, more people would become infected. Theoretically, it is also possible that resistant strains could be less virulent, but there is little direct evidence of this.

Infection by antimicrobial-resistant microorganisms might have worse outcomes due to ineffective initial treatment, the need to use less desirable treatment options, or both. The initial empiric treatment choice might be an antimicrobial agent to which the pathogen is resistant, leading to a delay in effective therapy. Effective agents might be more toxic, more expensive, or more difficult to administer than the traditional choice, which could have health or economic consequences. These phenomena are considered in Travers and Barza (in this supplement).

A fourth mechanism by which antimicrobial resistance can increase the human disease burden arises from an increase in the amount of pathogens (number of organisms, variety of species, or both) in animals. Exposure of food animals to antimicrobial agents might lead to increased colonization of the animals by drug-resistant pathogens. This is the same mechanism as the attributable fraction in humans (mechanism 1) but applying in this case to animals. This increase in the number of pathogens in food animals could lead to an increase in the burden of pathogens in the environment and in the food chain down to the human consumer. Furthermore, as in humans, most of these pathogens would presumably be resistant to antimicrobial agents. This phenomenon is considered by McEwen and Fedorka-Cray (in this supplement).

Finally, antimicrobial resistance arising in food animals could involve not just obvious pathogens (such as salmonella and campylobacter strains) but relatively nonpathogenic microbes such as *Escherichia coli,* enterococci, or *Bacteroides* species. These organisms could become reservoirs of antimicrobial resistance elements that could colonize humans via the food chain or environment (see Summers, in this supplement). The resistance elements could be transferred to ordinary pathogens or to other commensal bacteria that sometimes cause human disease, such as *Klebsiella* and *Enterobacter* species. Because these commensal species are not associated with food animals, the sources of these resistances could be difficult to discover. Vancomycin-resistant enterococci are an example of the transfer of resistant commensals from animals to humans (see Swartz, in this supplement). These are organisms of low pathogenicity except in patients with compromised defenses.

Only mechanisms 4 and 5 (described above) depend directly on the presence of the resistance traits in food animals; mechanisms 1 through 3 operate whatever the source is of the re-

sistance traits. However, pathogens such as *Salmonella* spp. and *Campylobacter jejuni,* which are the subject of this report, originate largely from the food chain. Therefore, in this context, even mechanisms 1 through 3 relate to the presence of resistance traits in bacteria from food animals. It is possible that subjects could ingest susceptible strains of these pathogens, with acquisition of drug resistance occurring in the human intestine, but there is little evidence that such events occur frequently. By contrast, there is much evidence to indicate that the food chain already contains an abundance of antimicrobial-resistant pathogens. Therefore, for the purposes of this supplement, all 5 mechanisms can be considered as applying primarily to antimicrobial-resistant organisms arising from the farm. Taken together, these mechanisms provide an abundance of ways in which antimicrobial resistance originating in food animals can increase the impact of infectious diseases on humans.

SUPPLEMENT ARTICLE

Excess Infections Due to Antimicrobial Resistance: The "Attributable Fraction"

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Antimicrobial use causes a transient decrease in an individual's resistance to colonization by noncommensal bacteria ("competitive effect") and increases the likelihood of infection upon exposure to a foodborne pathogen. The additional "selective effect" of antimicrobial resistance results in a >3-fold increase in vulnerability to infection by an antimicrobial-resistant pathogen among individuals receiving antimicrobial therapy for unrelated reasons. Combining the increase in vulnerability to infection with the prevalence of taking an antimicrobial agent, it is possible to estimate the attributable fraction, or the number of excess infections that occurred as a result of the unrelated use of an antimicrobial agent to which the pathogen was resistant. Calculations based on estimates of the annual infection rates and attributable fractions of infections with nontyphoidal *Salmonella* and *Campylobacter jejuni* suggest that resistance to antimicrobial agents results annually in an additional 29,379 nontyphoidal *Salmonella* infections, leading to 342 hospitalizations and 12 deaths, and an additional 17,668 *C. jejuni* infections, leading to 95 hospitalizations.

THE NORMAL MICROBIOTA AND "COLONIZATION RESISTANCE"

All epithelial surfaces of the body have a microbiota (flora or microflora) composed of commensal organisms. The components of this microbiota differ by body site (skin, oral cavity, intestinal tract, and vagina) and even differ between contiguous sites (e.g., stomach vs. small intestine vs. colon). The microbiota is highly complex, consisting of dozens of species that tend to keep each other in balance by mechanisms that are incompletely understood. These mechanisms include competition for consumption of limited supplies of nutrients, suppressive effects by products of bacterial metabolism on bacterial growth, and secretion by some bacterial species of products inhibitory to other species (e.g., bacteriocins). One of the most important functions of the normal microbiota is to occupy the epithelial surface and thereby impede colonization by new microorganisms. This effect is called "colonization resistance."

Which components of the normal microbiota are responsible for resistance to colonization by different pathogens has been a subject of interest. In most body sites, anaerobic organisms outnumber facultative bacteria by at least 10 to 1. Although aerobic or facultative species may also play a role in colonization resistance, anaerobic species have drawn much attention, in part because they are so numerous that their suppression would create a significant ecological vacuum [1]. A study of patients colonized by vancomycin-resistant enterococci showed that proliferation of the resistant organisms in the intestine was fostered by administration of antimicrobial agents with potent activity against anaerobic bacteria [2]. In some circumstances, individual species appear to be crucial to the preservation of colonization resistance. For example, acid production by lactobacilli in the vagina creates a local environment inhospitable to colonization by *Candida* species. Sup-

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pression of lactobacilli by antimicrobial agents results in vaginal overgrowth by *Candida.*

INCREASED VULNERABILITY TO INFECTION RESULTING FROM TREATMENT WITH ANTIMICROBIAL AGENTS

Antimicrobial agents suppress colonization resistance both during the treatment period and for days or even weeks afterward until the normal microbiota is restored. During this period, subjects have enhanced vulnerability to infection by intestinal pathogens; that is, a lower dose of pathogens than usual will cause infection or a higher proportion of subjects than usual will become infected from exposure to a given dose of pathogens.

Table 1 summarizes 2 distinct effects of taking an antimicrobial agent around the time of exposure to a pathogen: the competitive effect and the selective effect. The competitive effect results from a decrease in colonization resistance and applies to both antimicrobial-susceptible and -resistant organisms when the drug is taken before but not during exposure to the pathogen. When the drug is taken during exposure to the pathogen, infection by a drug-susceptible pathogen will tend to be prevented, whereas infection by a resistant pathogen will proceed because the drug inhibits the normal microbiota but not the pathogen. This particular instance has been called the "selective effect" (inhibition of the normal microbiota but not the pathogen). In some instances, a low inoculum may cause colonization but not symptomatic infection by pathogens, such as *Salmonella.* Subsequent ingestion of an antimicrobial drug to which the pathogen is resistant may then lead to proliferation of the organism and symptomatic infection.

Many studies have examined the facilitating effect of exposure to an antimicrobial agent given for unrelated reasons, such as for the treatment of upper respiratory tract infection, on the risk of infection by foodborne pathogens (table 2). It is generally assumed in these studies that the antimicrobial agents are being taken for relatively mild illness and that the patients do not have a serious underlying disease that could, in itself, predispose the patient to intestinal infection. Virtually all of these studies have found an appreciable enhancing effect of taking an antimicrobial on the risk of infection by foodborne pathogens.

We conducted a random-effects meta-analysis of the studies listed in table 2 that deal with antimicrobial-resistant strains and afford a definite odds ratio (OR) [3, 4, , 6–8, 10]. We omitted the study by Pavia et al. [9] because it deals with drug-susceptible strains, and the studies by Holmberg et al. [5] because an OR cannot be calculated. On the basis of the remaining 6 studies, the cumulative OR value for infection risk among subjects taking an antimicrobial to which the pathogen was resistant was 3.7 (95% CI, 2.7–5.0).

In the studies dealing with drug-resistant pathogens, the observed enhancement of infection risk resulting from exposure to an antimicrobial agent could reflect either the competitive effect or the selective effect (table 1). A random-effects meta-analysis of studies by Adler et al. [3] and Spika et al. [7] yields an OR of 5.33 (95% CI, 1.35–21.0) relating the effect of antimicrobial intake on infection with resistant *Salmonella,* compared with uninfected controls. This effect can be considered to be the products of the competitive effect of antimicrobial intake and of the selective effect of antimicrobial intake. Because the only outcome considered is antimicrobial-susceptible infection, the study by Pavia et al. [9] describes only the competitive effect; the OR presented is 4.3 (95% CI, 1.3–13.5). We can make a rough estimate of the selective effect alone by dividing the cumulative OR derived from the Adler et al. [3] and Spika et al. [7] studies by the estimate provided by Pavia et al. [9], thus canceling out the competitive effect and yielding an OR of 1.24 for the selective effect alone.

A more direct estimate of the selective effect is provided by analysis of 3 studies that compare the antimicrobial exposure histories of subjects infected by drug-resistant as opposed to drug-susceptible strains [4, 6, 10]. Because all patients were infected, the 3 studies are free of any differential bias that favors recollection of drug exposure by patients who are symptomatic as opposed to asymptomatic. Because the competitive effect should apply equally to drug-susceptible and drug-resistant infections, it should cancel out between the groups, allowing measurement of the selective effect due to antimicrobial resistance per se. A cumulative random-effects meta-analysis of

Table 1. Competitive and selective effects of antimicrobial exposure on infection risk.

	Infection risk when antimicrobial agent is taken	
Antimicrobial susceptibility of pathogen	Before exposure to pathogen	During exposure to pathogen
Pathogen susceptible to antimicrobial agent	Increased vulnerability to infection by pathogen (competitive effect)	Infection prevented
Pathogen resistant to antimicrobial agent	Increased vulnerability to infection by pathogen (competitive effect)	Infection facilitated (selective effect)

Table 2. Frequency of antimicrobial therapy in people infected with antimicrobial-resistant and -susceptible *Salmonella* strains.

Study	Subjects	Infections caused by resistant pathogens	No. with recent antimicrobial use	No. of drug-resistant infections associated with recent antimicrobial use	OR (95% CI)
Adler et al. [3]	76 patients on a pediatric ward	36 patients with multidrug-resistant strain (single strain)	49 patients received semisynthetic penicillin or ampicillin; time frame not stated	28	3.2 (1.1–9.8)
Riley et al.[a] [4] (CDC survey)	(a) 485 geographically dispersed patients, all nontyphoidal *Salmonella*	105 patients with strain resistant to ⩾2 drugs	13 patients received ⩾1 antimicrobials within preceding 4 weeks	13	3.3 (1.5–7.2)[a]
	(b) 43 patients receiving antimicrobial drugs	13 patients with strain resistant to ⩾2 drugs	25 patients received ampicillin or penicillin in preceding week	12	15.7 (1.8–709.3)
Holmberg et al. [5]	(a) 21 patients with *Salmonella* serotype Newport infection	10 patients with resistant strains	7 patients received amoxicillin or penicillin in preceding week	7	Undefined[b]
	(b) 39 patients	10 patients with resistant *Salmonella* Newport, 29 household controls	7 patients	7	Undefined[b]
	(c) 37 patients	10 patients with resistant *Salmonella* Newport, 27 patients with non–*Salmonella* Newport	7 patients	7	Undefined[b]
MacDonald et al.[a] [6] (CDC survey)	485 geographically dispersed, all nontyphoidal *Salmonella*	117 patients with strain resistant to ⩾1 drugs	63 patients with antimicrobial use in preceding 4 weeks	23	2.0 (1.1–3.64)[a]
Spika et al. [7]	133 patients total: 45 patients with multidrug-resistant *Salmonella* Newport, 88 controls	Epidemic of 45 patients with multidrug-resistant *Salmonella* Newport infection	13 patients with penicillin or tetracycline use in preceding month	11	13.9 (2.8–132.8)
Ryan et al. [8]	100 patients total: 50 patients with *Salmonella* serotype Typhimurium infection, 50 matched controls	Epidemic of 50 patients with multidrug-resistant *Salmonella* Typhimurium infection	Not stated	Not stated	5.5
Pavia et al. [9]	72 patients with *Salmonella* serotype Havana infection	0 resistant strains (all 72 were susceptible)	19 patients with antimicrobial use in preceding 45 days	—	4.3 (1.3–13.5)
Lee et al.[a] [10] (CDC survey)	758 geographically dispersed patients, all with nontyphoidal *Salmonella*	232 (31%) strains resistant to ⩾1 drugs; 189 (25%) strains resistant to ⩾2 drugs	126 patients with antimicrobial use in preceding 4 weeks	49	5.1 (3.25–8.01)[a]

[a] The effect measures in these studies compare groups infected with resistant versus susceptible strains on the basis of antimicrobial intake. All others compare antimicrobial intake among groups infected with either resistant or, in the case of the study by Pavia et al. [9], antimicrobial-susceptible strains compared with uninfected controls.

[b] Undefined, $P < .001$.

these 3 studies yielded an OR for infection risk of 3.3 (95% CI, 1.8–6.0).

In summary, among subjects taking an antimicrobial agent for unrelated reasons, there is a severalfold greater risk of infection directly attributable to antimicrobial resistance of the infecting pathogen.

ATTRIBUTABLE FRACTION

An attributable fraction can be estimated by combining the calculated OR with the proportion of the population recently treated with an antimicrobial agent. The attributable fraction reflects the proportion of all cases that would not have occurred in the absence of recent or concurrent treatment with an antimicrobial agent to which the bacterium was resistant. These cases are sometimes called "excess cases." The calculation uses the equation $[(OR - 1) \times P]/\{1 + [(OR - 1) \times P]\}$, where P is the proportion of the general population with exposure to antimicrobial agents.

In a review of outbreaks of drug-resistant infection caused by nontyphoidal *Salmonella,* Cohen and Tauxe [11] considered 16%–64% of cases to be in the attributable fraction. Although that analysis did not separate the competitive effect from the selective effect of taking an antimicrobial agent, the magnitude of the OR that we have calculated for the selective effect alone (related directly to resistance of the pathogen) is similar to the one used in those calculations, which supports their estimates.

To estimate the role of antimicrobial resistance in increasing the infection burden from nontyphoidal salmonellae, we calculated an attributable fraction by using the OR for infection risk calculated from the cumulative meta-analysis described above, together with recent estimates of the proportion of subjects taking an antimicrobial agent. For this latter figure, recent estimates are 6.6% [12], 13% [9], and 15% [10]. Differences in these estimates likely reflect selection bias in the studies and differing selection criteria; for example, the estimate of 13% refers to people taking any antimicrobials within the past 45 days. On the basis of this range of estimates, and using 3.3 as the OR for infection risk, we estimate that an attributable fraction of between 13% and 26% of drug-resistant *Salmonella* infections are acquired through a selective mechanism due to exposure to antimicrobial agents.

RISK ASSESSMENT

Excess cases of nontyphoidal* Salmonella *infections in the United States annually. Each year, an estimated 1,412,498 infections in the United States are caused by nontyphoidal salmonella, leading to 16,430 hospitalizations and 582 deaths [13]. Of these, 26%, or 367,249, infections are caused by strains resistant to 2 or more antimicrobial agents [14]. If we assume that rates of hospitalization and death resulting from infections with drug-resistant strains are similar to those resulting from infections with drug-susceptible strains (an assumption that may underestimate the rates for drug-resistant strains if they are more virulent), then strains resistant to 2 or more antimicrobial agents cause 4272 hospitalizations and 151 deaths. The attributable fraction due to antimicrobial resistance could be 13%–26% on the basis of our own estimate, or as high as 16%–64% on the basis of the data of Cohen and Tauxe [11]. Using a low estimate of 10% for the attributable fraction, calculations show that 36,724 infections, 427 hospitalizations, and 15 deaths occur in the United States each year as a direct result of antimicrobial resistance among nontyphoidal *Salmonella.* Using a conservative estimate of 80% for the percentage of infections originating from food animals (see Swartz, this supplement), 29,379 infections, 342 hospitalizations, and 12 deaths in the United States each year can be attributed to antimicrobial-resistant *Salmonella* from food animals.

Attributable fraction for* Campylobacter jejuni *infections. One report provides useful data for estimating an attributable fraction for infection by antimicrobial-resistant *Campylobacter.* A study of subjects with quinolone-resistant *Campylobacter* infections concluded that quinolone use contributed to a maximum of 15% of cases [15]. For purposes of this assessment, we will assume that the attributable fraction was 5%.

There are 2,453,926 infections, 13,174 hospitalizations, and 124 deaths caused by *C. jejuni* each year in the United States [13]. Eighteen percent of these, or 441,707 infections, are caused by strains resistant to at least one antimicrobial agent. Again assuming that rates of hospitalizations and death are similar for infections with drug-resistant strains and with drug-susceptible strains, then 2371 hospitalizations and 22 deaths result each year from infection with *Campylobacter* strains resistant to at least one antimicrobial agent. If the attributable fraction is 5%, this translates to 22,085 infections, 119 hospitalizations, and 1 death in the United States each year as a result of infection by quinolone-resistant *C. jejuni.* If 80% of *C. jejuni* infections arise from food animals (Swartz, this supplement), then antimicrobial resistance in these animals contributes to 17,668 infections and 95 hospitalizations per year.

Weaknesses in estimates. Some of the estimates above are derived from only one or two studies; thus, the estimates could be inappropriately high or low. In addition, the estimates for both *Salmonella* and *Campylobacter* may be underestimates because the rates of hospitalization and death are based on the rates for all strains, whereas the hospitalization and death rates for antimicrobial-resistant strains may be higher than for susceptible strains (see Travers and Barza, this supplement). Where ranges of data were available, we used conservative estimates that would be more likely to underestimate than to overestimate the effect of antimicrobial resistance on human health.

CONCLUSIONS

Compelling data show that antimicrobial resistance of pathogens is associated with an increased risk of infection among subjects taking an antimicrobial drug for unrelated reasons. This risk can be expressed in the form of an attributable fraction—that is, a proportion of infections that would not have occurred had the pathogen not been resistant to antimicrobial agents. Because the taking of antimicrobial agents for a variety of reasons is common in the United States, antimicrobial resistance contributes to an appreciable number of cases of illness, hospitalization, and death that would not have occurred in the absence of this resistance—so-called excess cases. Because the incidence of antimicrobial resistance has been rising steadily, it is likely that, all else being equal, the number of excess cases of infection will increase.

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Morbidity of Infections Caused by Antimicrobial-Resistant Bacteria

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Antimicrobial resistance can have 2 effects on the outcome of infection: there can be an accompanying change in the virulence of the organism, and there can be a poorer response to treatment because of the empiric choice of an antimicrobial to which the organism is resistant. We have reviewed published studies relating antimicrobial resistance to the outcomes of infection caused by enteric pathogens. The data for *Salmonella* and *Campylobacter* infections suggest that antimicrobial-resistant strains are somewhat more virulent than susceptible strains—that is, they cause more prolonged or more severe illness than do antimicrobial-susceptible strains. However, not all studies corrected for possible differences in age and underlying diseases between patients infected by antimicrobial-resistant and -susceptible strains of *Salmonella*. Two studies of *Campylobacter* infection suggest that poorer outcomes with antimicrobial-resistant pathogens could be related to the initial choice of an ineffective antimicrobial for treatment. Estimates from various sources indicate that fluoroquinolone resistance, likely acquired from the administration of antimicrobials to food animals, leads to >400,000 excess days of diarrhea in the United States per year compared with the duration that would occur if all of the isolates were susceptible. Antimicrobial resistance also could account for an extra 8677 days of hospitalization for nontyphoidal salmonellosis, mainly arising from food animals.

ANTIMICROBIAL RESISTANCE AND INFECTIONS

Antimicrobial resistance can have 2 principal effects on the outcome of infections. First, antimicrobial resistance may be associated with a change in the virulence of the strain, as measured by the incidence of disease complications, hospitalizations, and deaths. An increase in virulence could result from linkage of resistance factors to other virulence genes, such as those for adherence, invasion, and toxin production; in that case, acquisition of the resistance trait by the pathogen would be accompanied by acquisition of additional virulence genes. Similarly, a decrease in virulence could occur if acquisition of the resistance trait by the pathogen were accompanied by loss of certain virulence factors.

A second effect of antimicrobial resistance is the possible complication of the choice of treatment agents. Because treatment is usually begun before the antimicrobial susceptibilities of the pathogen are known, the initial choice of antimicrobial agent must be made empirically. Antimicrobial resistance may lead to an inappropriate choice of antimicrobial for initial therapy, leading to a poorer response, or suspected resistance may force a less desirable choice of antimicrobial drug (e.g., more toxic or more expensive). These issues are becoming more important as the prevalence of resistance to commonly used agents increases.

In the following sections, we discuss studies relating to these 2 effects. In some instances, it is difficult to know whether a worse outcome is due to greater virulence or a poorer response to treatment of the resistant pathogen. In those cases, we have accepted the authors' preferred explanation, if given.

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ANTIMICROBIAL RESISTANCE AND VIRULENCE

Several studies have addressed the relation between antimicrobial resistance and the apparent virulence of intestinal pathogens. Holmberg et al. [1] reviewed data from the CDC for community-based and nosocomial outbreaks of nontyphoidal salmonellosis occurring in the United States between 1971 and 1980. In community-based outbreaks caused by drug-susceptible strains, the death rate was 3 (0.2%) of 1321, whereas for multidrug-resistant strains, it was 7 (3.4%) of 205. For nosocomial outbreaks, the comparable figures were 2 (1.0%) of 202 for susceptible strains and 30 (11.7%) of 256 for multidrug-resistant strains.

In a more recent study from the CDC [2], among subjects with culture-confirmed nontyphoidal salmonellosis diagnosed in 1989–1990, 31% were infected by organisms resistant to at least one antimicrobial and 25% by organisms resistant to 2 or more antimicrobials (i.e., multidrug resistant). Subjects with infection caused by antimicrobial-resistant organisms were significantly more likely to be hospitalized than those with antimicrobial-susceptible infections (35% vs. 27%, $P = .006$), and this difference persisted even after correction for underlying illness. Subjects infected by resistant organisms also tended to be ill longer (median, 10 vs. 8 days) and hospitalized longer (median, 5 vs. 4 days). Most subjects were treated with an agent to which the organism was susceptible: therefore, the difference in hospitalization rates probably reflects a somewhat higher virulence of the infecting organism rather than an inappropriate choice of antimicrobial for treatment.

The studies cited above have 2 potentially confounding factors, one related to host susceptibility in terms of age and the other to potential differences in virulence between serotypes of *Salmonella.* It is known that the ability of *Salmonella* strains to produce disease is greatest in the very young and very old and is greater in subjects with serious underlying diseases. The study of Lee et al. [2] matched subjects for underlying disease, but that of Holmberg et al. [1] did not. Neither study controlled for age.

Second, the studies did not take account possible differences in the distribution of *Salmonella* serotypes between those infected by susceptible and resistant strains. Certain serotypes are more virulent than others in animals [3] and in humans [3–5]. Recent studies showing differences in the serotype distribution of *Salmonella* between food animals and humans [6] could be explained by a difference in the ability of these serotypes to cause infection. Nevertheless, if the widespread use of antimicrobial agents in animal husbandry is selecting for antimicrobial-resistant serotypes that happen to be more virulent, the effect on human health will be the same as if antimicrobial resistance were an independent virulence factor. From this point of view, a failure to correct for serotype distribution is unimportant.

Data supporting an increase in virulence for infections by antimicrobial-resistant strains of *Campylobacter jejuni* are beginning to emerge. A multistate surveillance study of FoodNet sites by Marano et al. [7] found that among subjects not treated with an antimicrobial agent or an antimotility agent (Imodium [McNeil Consumer] or Lomotil [Searle]), diarrhea lasted significantly longer when caused by a fluoroquinolone-resistant strain rather than when caused by a susceptible strain (mean duration, 12 vs. 6 days, $P = .02$).

EFFECT OF ANTIMICROBIAL RESISTANCE ON RESPONSE TO TREATMENT

There is increasing resistance of *C. jejuni* to fluoroquinolones. In a study in Minnesota spanning 1992–1998, ~10% of isolates were resistant to these drugs [8]. Many of the resistant strains were acquired abroad, but ~3% of infections acquired domestically were fluoroquinolone resistant [8]. Approximately 85% of patients were treated with an antimicrobial agent; of these, 65% were treated with a quinolone and 25% with a macrolide. Among subjects treated with a fluoroquinolone, the mean duration of diarrhea was 7 days for those infected by a quinolone-susceptible strain but 10 days for those infected by a quinolone-resistant strain ($P = .03$), a difference of 3 days [8]. The multistate surveillance study of FoodNet sites by Marano et al. [7] found that among patients treated with a fluoroquinolone, diarrhea lasted significantly longer among those infected by a fluoroquinolone-resistant strain than by a fluoroquinolone-sensitive strain (mean duration of diarrhea, 8 vs. 6 days, $P = .02$), a difference of 2 days.

Because fluoroquinolones are active against most bacterial diarrheal pathogens, they have become a favored choice for diarrheal illness. Macrolides are active against *Campylobacter* but not many other diarrheal pathogens. The increasing resistance of *Campylobacter* species to fluoroquinolones complicates the initial choice of treatment.

In summary, the data for nontyphoidal *Salmonella* and *Campylobacter* infections suggest that antimicrobial-resistant strains are somewhat more virulent than susceptible strains. The studies of *Salmonella* infections did not completely control for host factors (age and underlying diseases). In 2 studies of *Campylobacter* infections [7, 8], worse outcomes for resistant strains could be related to the choice of an ineffective antimicrobial for treatment.

RISK ASSESSMENT

***Excess days of illness related to fluoroquinolone resistance of* C. jejuni.** There are estimated to be 2,453,926 *Campylobacter* infections per year in the United States [9]. Recent data from the US National Antimicrobial Resistance Monitoring System indicate that 18% of strains are now fluoroquinolone resistant (see table 2 in Swartz, this supplement), which would amount to 441,707 infections by resistant strains per year. Data from a

Table 1. Studies comparing outcomes of infection according to antimicrobial susceptibility of pathogens.

Study	Pathogen	Study group	Outcome of infection		Adequacy of matching of cases and controls
			Virulence of organism	Effect of choice of antimicrobial	
Holmberg et al. [1]	Nontyphoidal *Salmonella*	Community-acquired outbreaks, United States, 1971–1980	Death rate, 0.2% for drug-susceptible vs. 3.4% for multidrug-resistant organisms	Not assessed	Not matched for age or serotype
Holmberg et al. [1]	Nontyphoidal *Salmonella*	Nosocomial outbreaks, United States, 1971–1980	Death rate, 1% for drug-susceptible vs. 11.7% for multidrug-resistant organisms	Not assessed	Not matched for age or serotype
Lee et al. [2]	Nontyphoidal *Salmonella*	Community outbreaks, United States, 1989–1990	Hospitalization rate, 35% for resistant vs. 27% for susceptible ($P = .006$); longer illness (median, 10 vs. 8 days) and hospitalization (median, 5 vs. 4 days) for resistant	Not assessed	Difference in hospitalization rates persisted even after matching for underlying disease; could not match for age or serotype
Smith et al. [8]	*Campylobacter jejuni*	Community acquired	—	Among subjects treated with quinolone, median for diarrhea was 7 days if susceptible strain vs. 10 days if resistant strain ($P = .03$)	—
Marano et al. [7]	*C. jejuni*	Community acquired, multistate surveillance (FoodNet)	Among untreated patients, diarrhea lasted longer for infection by Cipro-resistant than Cipro-sensitive strains (mean, 12 vs. 6 days, $P = .02$)	Among subjects treated with fluoroquinolone, diarrhea lasted longer with infection by resistant than susceptible strain (mean, 8 vs. 6 days, $P = .02$)	Similar results when corrected for underlying illness

FoodNet multistate survey show that 58% of these resistant strains are domestically acquired [10]. Accordingly, 256,190 *Campylobacter* infections per year are domestically acquired and resistant to fluoroquinolones. In the community-based study by Smith et al. [8], 15% of patients received no antimicrobial therapy, and 65% of treated patients received a fluoroquinolone. Applying these values to the 256,190 fluoroquinolone-resistant infections, 38,428 would be treated by no antimicrobial. Of the 217,762 treated infections, 141,545 would be treated with a fluoroquinolone.

The data from Marano et al. [7] (table 1) for untreated subjects indicate an excess of 6 days of diarrhea for each of 38,428 subjects, or 230,568 excess days of diarrhea, presumably related to the virulence of the infecting strain. Again, from the data of Marano et al. [7] (table 1), for the 141,545 subjects treated with a fluoroquinolone, there would be a mean excess of 2 days of diarrhea, or a total of 283,090 excess days, presumably related to the poorer response of the disease to treatment. (The data of Smith et al. [8], shown in table 1, would suggest an excess of 3 days, rather than 2 days, of diarrhea among subjects with fluoroquinolone-resistant infections treated with a fluoroquinolone, but we have used the more conservative value of Marano et al. [7]). In total, one can infer 513,658 excess days of diarrhea per year in the United States due to the fluoroquinolone resistance of domestically acquired *C. jejuni.* If 80% of *Campylobacter* infections arise from food animals (see Swartz, this supplement), one can attribute 410,926 excess days of diarrhea to fluoroquinolone resistance in the *Campylobacter* strains of domestic farm animals. This figure would be expected to increase as the rate of fluoroquinolone resistance increases.

***Excess morbidity attributable to antimicrobial resistance of nontyphoidal* Salmonella.** Hospitalization and the personal distress associated with *Salmonella* should be considered as significant consequences of such infections. In the case of hospitalization, data exist to support significant differences in days of hospitalization associated with resistant and susceptible *Salmonella* infection, thus allowing calculation of the excess days of hospitalization associated with resistant *Salmonella* infection arising from food animals. A study by Lee et al. [2] has shown the median duration of hospitalization to be 4 days for infections with susceptible strains and 5 days for infections with resistant strains. We used the above data to construct a model assessing the number of hospitalizations and the excess duration of hospitalization (in days) due to foodborne *Salmonella* infection. The model has the following form: annual number of *Salmonella* infections (49,812 on the basis of the rate reported by Marano et al. [7] per 100,000 population multiplied by population size of 281,421,906 reported in the 2000 US Census [11]), multiplied by the fraction infected with resistant strains (0.26 [12]), multiplied by excess proportion of hospitalizations among resistant *Salmonella*-infected individuals ($0.35 - 0.27 = 0.08$ [2]), multiplied by the median days of hospitalization among those infected with resistant strains (5 days) [2]. The model estimates $49{,}812 \times 0.26 \times 0.08 \times 5$ days $= 5180$ excess days of hospitalizations per year, among patients who would not otherwise have been hospitalized, that can be attributed to resistant strains of *Salmonella.* In addition, we can calculate an extra day of admission on account of antimicrobial resistance among patients who would have been hospitalized anyway (i.e., $49{,}812 \times 0.26 \times 0.27 \times 1 = 3497$ extra days of hospitalization). The total extra days of hospitalization due to drug resistance is $5180 + 3497 = 8677$. Previous reports have estimated that as much as 90% of resistant *Salmonella* present in food is a result of subtherapeutic administration of antimicrobials to food animals [13].

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Review of Assessments of the Human Health Risk Associated with the Use of Antimicrobial Agents in Agriculture

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To our knowledge, no comprehensive risk assessment of agricultural uses of antimicrobial agents has been published. The published risk assessments of antimicrobial use in farm settings are all subject to multiple, serious limitations in scope, including (1) limitation to one species of microorganism; (2) limitation to one or a very few related antimicrobial agents; (3) limitation to a single outcome (death, hospital days, number of illnesses, etc.); (4) limitation to one species of farm animal (e.g., chicken or swine); and (5) limitation to therapeutic use, despite reason for concern about misstated, off-label, or illegal use. In addition, all of the risk assessments reviewed overlooked important issues by accepting 2 further limitations: (6) limiting the scope of the analysis to what has already happened and ignoring the effects of continuing the practices of recent years; and (7) examining only the effects on the species of microorganism that was initially affected and ignoring the cross-species spread of resistance by plasmid transfer. After our review of the risk assessments now available, we propose a comprehensive scheme for organizing existing knowledge and dealing with critical gaps.

NEED FOR A MODEL TO EVALUATE RISK

Antimicrobials are used extensively in both veterinary and human medicine, and the problem of emerging resistance to these important drugs thus substantially affects both fields. The emergence of antimicrobial resistance in human populations is a public health problem of continually growing importance, and the emergence of antimicrobial resistance in animal populations has important economic implications. The association between animals and humans suggests that resistance does not arise separately in these 2 groups. The prudent use of antimicrobial agents in both groups requires an ability to relate the risk of emerging antimicrobial resistant pathogens to antimicrobial use.

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The nature of the risk to human health due to antimicrobial use in animal husbandry is inherently indirect. Without measurements of direct exposure, it is not possible to estimate directly the associated risk of possible outcomes. A quantitative and technically thorough assessment of risk is therefore difficult. However, an evaluation of the risk, although not scientifically elegant, can indeed be carried out. Such evaluations of risk rely on a number of best estimates and subjective judgments, and as such, they may vary significantly. It is therefore important to consider the current body of such risk evaluations as a whole. Their inherent uncertainty is a necessary product of the uncertainty associated with the choice of a model and the estimation of parameters included in the model. An objective review of the risk models presented is necessary for continued refinement of methodology.

Here, we review some methodologies that have been used to estimate the human health risk associated with the administration of antimicrobial agents in agriculture. We are not interested in reproducing the results

of previous studies or evaluating their specific estimates; rather, we are interested in the methods. This critique of the methods, rather than the results, will serve as a basis for future efforts of our own and of other groups. At present, quantitative knowledge about risks is very limited and affected by much uncertainty, but broader and more reliable assessments are possible.

The investigators who have prepared the risk assessments now available have had good reasons for these limitations. Despite their narrow focus, these risk assessments have been expensive, time-consuming, and difficult. In addition, some authors have had specific problems in mind and, understandably, focused on only those problems. Overall, however, enormous gaps remain, and it is critical that decision makers understand that the problems brought to light to date are likely to be a tiny part of the whole problem. There are 2 particular issues of grave concern: first, the tendency to limit the scope of the analysis to what has already happened and to ignore the effects of continuing the practices of recent years; and second, the tendency to examine the effects of antimicrobial resistance on only the species of microorganism that was initially affected and to ignore the cross-species spread of resistance by plasmid transfer.

There is no documentation of the spread of antimicrobial-resistant organisms to humans or animals from plants; indeed, to our knowledge, no studies have been performed to determine such an association. Given what we know today, such a study could be done, at least to determine whether humans or animals became colonized with resistance determinants in bacteria that could be attributed to an origin in plant-associated bacteria.

RISK MODELS UNDER REVIEW

We first review 5 reports that address the microbiological risks associated with antimicrobial use in animals and attempt to estimate them. The 5 models are described in the following reports: (1) the 1989 Institute of Medicine (IOM) Committee report [1], (2) the 2001 US Food and Drug Administration (FDA)–Center for Veterinary Medicine (CVM) report [2], (3) 2000 Animal Health Institute report by Cox and Popken [3], (4) the 2000 Heidelberg Appeal Nederland (HAN) Foundation report [4], and (5) the 2000 Bywater and Casewell letter [5]. This is not to be considered a global evaluation of all such models; rather, we have focused on those models evaluating human health risk associated with antimicrobial use in animals. By focusing the review in such a way, we hope to look at a group of comparable evaluations that may highlight the problems inherent in such modeling (and perhaps point to solutions).

Most assessments of cancer risks now rely on a sequence of 4 steps proposed by a committee of the National Academy of Sciences [6]. That paradigm might be adaptable to the emergence of antimicrobial resistance, but it seems more natural to adapt a multiplicative model with probabilities (or number of people or infections) of moving from step to step in a well-defined chain of events. Indeed, all but one of the risk assessments reviewed here have used such a multiplicative model, although the specific steps have varied.

END POINTS EVALUATED

The end points considered in these models often included hospitalization (or number of days of hospitalization, as in the Cox and Popken model). Other possible end points that might be considered in a risk assessment are serious distress and death, as well as meningitis and other specific medical conditions. This is by no means an exhaustive list of potential end points of concern.

The risk assessments under review consider specific clinical outcomes. A more general outcome of significant concern is simply the risk of a shift toward resistance in bacterial populations, regardless of immediately discernible clinical significance.

1989 IOM MODEL OF RISK ASSESSMENT

Nature of the model. The 1989 IOM Committee developed and used a statistical model of the risk of death from antimicrobial-resistant *Salmonella* (all strains) in the United States, where the resistance was caused by the subtherapeutic use of penicillin-ampicillin or the tetracyclines in animal feed [1]. The model is based on 5 successive steps, all assumed to be necessary and to take place in sequence. These steps, with that Committee's best guesses at the time, are as follows: (1) annual number of cases of culture-confirmed cases of *Salmonella* reported in the United States (50,000); (2) fraction of these human cases due to bacterial strains resistant to penicillin-ampicillin or the tetracyclines (15%); (3) death rate among cases with drug-resistant salmonellosis (1.0%); (4) fraction of these deaths associated with infections by bacterial strains of farm origin (70%); and (5) proportion of this fraction resulting from subtherapeutic use of penicillin-ampicillin or the tetracyclines in animal feed (90%). The number of deaths is then estimated by multiplying these numbers together. For example, the best estimates above produce an estimated $50{,}000 \times 15\% \times 1\% \times 70\% \times 90\% = 47.25$ deaths per year.

In practice, the committee used 3 estimates for each of these parameters—its own best estimate, and high and low estimates intended to include ~95% of what the committee thought might be provided by experts in the field. The Committee took each combination of 1 of the 3 numbers from each of the 5 sets, a total of 243 combinations, and with those obtained 243 different estimates of the number of deaths. Approximately

90% of these estimates lay in the range of 1 to 400 deaths, which the committee took to be a fair indication of the degree of uncertainty in the model. The model was then extended to other antimicrobials and to specific uses (prophylaxis; growth promotion) of the antimicrobials.

Additional assumptions. The model further assumes that all deaths of interest in this context are expressed in a logical chain—that is, that no relevant deaths are missed by the reporting network.

Data required by the model. The only data required for this model are the 5 parameters described above: (1) annual number of reported cases of salmonellosis; (2) fraction of these human cases due to resistant strains; (3) death rate among cases with drug-resistant salmonellosis; (4) fraction of deaths associated with infections by bacterial strains of farm origin; and (5) proportion of this fraction resulting from subtherapeutic use of antimicrobial agents in animal feed.

Strengths of the model. The model is simple and easy to understand, straightforward in concept, and requires nothing in the way of computation beyond simple multiplication. Interested people can readily see what happens if the parameters are changed. It is flexible and could be extended easily to other microorganisms, other antimicrobials, and other yes-no end points. Estimates of each of the 5 required parameters can be readily produced, although they may be highly uncertain in some analyses.

One of the required 5 parameters is a statistic (number of cases reported). The others are subjective probability estimates but bounded to the interval 0 to 1 (all are percentages) and estimable. Except for the case fatality rate (best estimate, 1%), reasonable changes in these proportions would have little effect of the output of the model.

Limitations of the model. The model is limited to yes-no responses for which the data are available or estimable with some reliability. In practice, this means that the report is limited to deaths. Even for deaths, the uncertainties are rather large, as reflected in the range that includes 90% of the estimates. The Committee discussed 6 specific limitations:

1. At the time the report was written, only *Salmonella* could be assessed with reasonable confidence.
2. The model itself may be incorrect. Other chains of critical events might be developed, and with present knowledge, they might give different estimates.
3. The model requires that estimates of the 5 parameters be conditionally independent. The structure of the model assures that this is at least partly true, but it could not be verified for the full set of parameters.
4. It is limited to the case mix reported to the US Centers for Disease Control and Prevention. Although the most severe cases of salmonellosis are likely to be reported, including nearly all recognized deaths, there is a likelihood that some recognized deaths and a possibly large number of unrecognized cases of salmonellosis may be unreported.
5. The model deals only with lethal infections. Morbidity, which is surely great, and the role of salmonellosis in contributing to deaths from other causes are not included.
6. The model is limited by the range and quality of the data available.

Each of these limitations is discussed at length in the Committee's report. No other serious problems are evident.

Overall uncertainty of estimates. The 1989 IOM report considered uncertainty at length and dealt with it in part by supplementing its own best estimates with what it considered to be a probable range of estimates that might be offered by other qualified people. Overall, this report dealt with uncertainty at considerably greater length than any of the other reports we discuss here.

Utility for the present purpose. The model is simple and straightforward and deals directly with the problem addressed in the present report. No modifications, other than updating the estimates of the parameters, are needed.

2001 FDA-CVM REPORT

Nature of the model. The 2001 FDA-CVM report [2] was prepared as part of the process surrounding the Guidance for Industry, No. 78 [7], on the use of antimicrobials in food animals.

The specific risk assessed is the risk to human health from the ingestion of chickenborne *Campylobacter* that are resistant to fluoroquinolones (FQs), where the resistance is acquired as a result of use of the drug on the farm. Such matters as cross contamination of other foods and interhuman spread of farm-related resistance are not included. The model assumes that resistant bacteria pass through the food supply, infect humans, and are treated in the same manner as susceptible bacteria. Furthermore, the model assumes that drug resistance in *Campylobacter* on the carcass is due to antimicrobial drug use. The end points examined are counts and probabilities—of *Campylobacter* infection, of infection with resistant strains, of infections by resistant strains and treated with FQ, and so on, all generally subdivided into 3 classes of illness (nonbloody stools, bloody stools, and invasive infection). The authors performed several kinds of sensitivity analyses to determine the sensitivity of the final estimates to various levels of error in the input parameters.

The model itself depends largely on the multiplication of probabilities of various steps in a causal chain. For example, the authors assume that one would find 21,912 cases of confirmed campylobacteriosis defined by nonbloody stools if the active surveillance program of FoodNet had covered the entire US population. From this, they proceed stepwise to estimate

29,215 cases before accounting for failures to identify the organisms in specimens examined, to 30,916 to account for specimens not examined for *Campylobacter,* to 204,744 to account for patients who sought medical care but without specimens submitted, to 998,753 cases in the US population during a 1-year period. The number of these associated with the consumption of contaminated chicken is then estimated as 566,297, of which 116,657 seek care, of which 52,846 are treated, of which 29,118 are treated with FQ, of which 5,707 are FQ-resistant strains. Thus, the model uses a multiplicative approach to expand from confirmed uses in a special study to all cases in the United States, then to reduce that number to severe infections by resistant strains.

The general multiplicative approach is reminiscent of the IOM approach, but more elaborate and (12 years later) with reasonably reliable data to support many of the inferences rather than committee judgments. On the other hand, the FDA-CVM model does not go as far as the IOM report in discerning possible errors in the multiplicative parameters, despite the attention to sensitivity analysis (see below). The multiplicative approach to estimate total error is a valuable innovation that was not possible before the availability of FoodNet data.

The model and data allow for one interesting internal check. The authors note that their assumptions imply that the ratio of chicken-attributed cases to amount of chicken consumed should be the same for resistant and sensitive strains, and they show that the ratio is about twice as high for resistant strains as for all strains combined. The report discusses possible reasons for this discrepancy, but a 2-fold difference seems rather small to the present committee, given the roughness of the data and the number of assumptions required.

Additional assumptions. Appendix B in the FDA-CVM report [2] lists and discusses 13 important assumptions in the model. However, all of these have to do with the values of various input parameters, and not with whether the model itself is correct or whether certain probabilities follow the beta distribution as hypothesized. Two critical assumptions are as follows: first, the level of risk as calculated does not account for cases originating from chicken and contaminating other foods or the spread from chicken to other animal hosts and resulting in human exposure; and second, the current level of risk of contracting campylobacteriosis from consumption of chicken is contained within the range of risk ascertained from studies conducted in the 1980s.

Data required by the model. The model requires the following data:

- The US population.
- The FoodNet catchment area population.
- FoodNet enteric/invasive numbers of *Campylobacter* infections by geographic location.
- Proportion of culture-confirmed enteric infections with bloody diarrhea.
- Probability that an infected person seeks medical care.
- Probability that one who seeks care provides a stool specimen.
- Probability that the specimen is tested for *Campylobacter.*
- Probability that a tested specimen with *Campylobacter* is reported as positive.
- Probability that a *Campylobacter* case is chicken related.
- Probability that a chicken-related case is FQ resistant.
- Probability that a case is treated with an antimicrobial agent.
- Probability that the antimicrobial agent is FQ.
- Total prevalence of *Campylobacter* on broiler carcasses.
- Prevalence of FQ resistance among the contaminated carcasses.
- Per capita consumption of boneless, domestically reared chicken in the United States.

Strengths of the model. The model is mathematically simple, easy to use, and readily updated as new data become available to refine parametric estimates or to replace assumptions with observations.

Limitations of the model. As noted, the FDA-CVM model estimates only numbers and probabilities of various kinds of infection. However, small modifications could extend the model to other end points. The FDA-CVM report cites specific reasons why it does not estimate hospital days.

Overall uncertainty of estimates. The sensitivity analyses in the FDA report are helpful. However, we are troubled by the large number of parameters required, each of which may have some degree of error, and the large number of assumptions, some of which may be affected by substantial error. Overall, we believe that the FDA model is subject to considerable uncertainty as a result of its replacement of a few big assumptions with several smaller assumptions. Despite these matters, we regard the CVM model favorably as a serious attempt to estimate risks in the face of limited knowledge.

Utility for the present purpose. The FDA-CVM model deals only with counts and probabilities, not with the consequences of infection such as morbidity, hospital days, cost, or death. A modification of the FDA-CVM model could be devised to estimate population effects. However, it would still be focused on the effects of FQ resistance in chickens, and it could not capture the risks of future FQ use in feed, including the risks of antimicrobial-resistance gene transfer to other kinds of microorganisms.

2000 REPORT BY COX AND POPKEN

Nature of the model. Cox and Popken present (in draft form) a model for the relation between *Campylobacter jejuni* infection of humans, FQ use in chicken flocks, and extra days of hos-

pitalization [3]. Their model includes the effects of other factors that modify the risk, including the use of non-FQ antimicrobials (especially erythromycin). The model considers FQ use to combat illness (not for growth promotion), considers spread by ingestion only, and uses colony-forming units as the measure of microbial load.

Cox and Popken properly stress the amplification or reduction of microbial load at the time of consumption for various groups of actors: farmer, transporter, processor, and consumer. This valuable feature of the model appears to be unique.

The model of Cox and Popkin, like the IOM and FDA models, is multiplicative and depends on estimates of the probabilities of going from one to the next step in a hypothesized causal chain. It differs from them in paying more attention to events on the farm and during transportation to a processing plant, and in its focus on the single end point of changes in the duration of hospitalization. Special features of the model include explicit modeling of microbial load, dose-response modeling, and explicit linkage between *Campylobacter* loads in chickens and human illness.

The model deals only with risks from surface contamination of chickens and only with duration of illness. The model begins with the assumption that 90% of chickens have cecal colonization with *Campylobacter,* a figure that seems adequately established. Cecal colonization is modeled as a binomial probability event. The model then assumes that the proportion of chickens with surface contamination is uniformly distributed between 0.20 and 0.56, the figures reported in 2 studies of surface flora. The authors note that tests for FQ resistance do not always yield unambiguous results, and they cite evidence that the degree of resistance is bimodal rather than yes-no, but they do not state the proportion. They briefly refer to several recent studies that found resistance rates of ~10%. Probabilities of infection and illness are then estimated as a function of the amount (cfu) of *Campylobacter* in the chickens eaten. Sensitivity analyses are performed for some of the critical parameters.

Additional assumptions. Cross contamination from uncooked chicken is not regarded as a significant problem, despite considerable evidence to the contrary. Also, the model assumes that antimicrobial-susceptible and antimicrobial-resistant organisms respond to their environments in the same way. Moreover, the model assumes that all people aged >1 year are equally susceptible and does not consider special populations, such as immune-deficient people.

Their computations express the estimated contamination loads as geometric means—an approach that produces means lower, sometimes much lower, than arithmetic means. This approach is not established as correct and may be seriously misleading for risk assessments when the distribution of loads is highly skewed. They do not say how they handled zero loads (which would produce geometric mean loads of zero when converted back to an arithmetic scale; zero values did occur in the data they use). For 3 of the 4 steps in estimating final loads, they assume triangular distributions; the fourth is assumed to be quadratic (all on $\log_{10}$ scales). No reason for the change in distribution form is given.

The annual chicken-attributable rate of infection among the chicken-eating population is estimated as the product of 3 terms: the percentage of chickens contaminated, the annual infection rate in the general population, and the estimated proportion of infections in people aged >1 year.

Once the average number of colony-forming units consumed is estimated, the probability of infection is estimated by an approximation to the beta Poisson model, a 2-parameter model. The parameters for *C. jejuni* were based on *C. pylori* loads ranging from 8×10^2 to 2×10^9. In this model, the probability of infection at a given dose increases with dose, but the probability of illness given infection decreases with dose. The latter result may be because it is conditional on infection; the form of the unconditional relationship is not stated. The probability of infection with doses <500 cfu is assumed to be zero.

Data required. The Cox and Popken model uses numerous kinds of data, including the following:

- Average surface microbial load on chickens at the farm.
- Multiplier for changes during transportation.
- Multiplier for changes during processing (rinsing, scalding, etc.).
- Multiplier for changes during further processing (heating, freezing).
- Percentage of *Campylobacter* spp. associated with poultry consumption.
- Estimated annual cases and rates in various age categories.
- Estimated annual rate in chicken-eating population.
- Average annual consumption rate in pounds, converted to whole chickens.
- Average number of servings per chicken, in 3 broad population groups (white, black, other).
- Population proportions in those 3 groups.
- Probability that treatment will be prescribed for a *Campylobacter* illness.
- Probability that the treatment will be an antimicrobial (itself estimated as a function of various measures including invasive illness and enteric illness with and without bloody diarrhea).
- Probability that the antimicrobial will be FQ.
- Estimated proportion of human *Campylobacter* illness from resistant isolates that result from consumption of chicken.
- Proportion of chicken carcasses with resistant strains of *Campylobacter.*
- Proportion of colonies on those carcasses that are resistant.

- Difference in duration of illness between infections with sensitive and resistant *Campylobacter.*

Limitations of the model. This model, like the others here, deals with the present frequency and distribution of resistance and not with what may be found at a future time if present practices do or do not continue. Similarly, it does not deal with the transfer of resistance among distinct species of bacteria. The narrow focus on number of days of hospitalization is a serious limitation, and it is not clear that the model can be adapted to other end points.

Overall uncertainty of estimates. The overall uncertainty of estimates is not addressed in depth. The estimates of the proportion of human infections that are related to chickens are remarkably lower than figures in the literature; no explanation is offered.

Utility for the present purpose. Some strong features are present in this model, but it is likely to require too many parameters and assumptions for broad use. We note that the model development was industry sponsored and that no final version has been published in a peer-reviewed journal.

REPORT OF THE HAN

The HAN Foundation was established in the Netherlands in 1993 and named after the Heidelberg Appeal, a declaration signed in 1992 by >3500 scientists [4]. HAN is an independent nonprofit alliance of scientists and science supporters whose aim is to ensure that scientific debates are properly aired, and that decisions that are taken and actions proposed are founded on sound scientific principles. HAN promotes a greater role for science and what it regards as realistic risk analyses in influencing public opinion and policy decisions.

Nature of the model. The HAN report does not present a formal risk assessment, either qualitative or quantitative, and no risk assessment model is proposed. It is simply an overview of how the risk might (or might not) manifest itself using reviewed literature. The analysis involves determining the spread of resistance to growth-promoting antimicrobial agents beyond the sphere of livestock production, documenting the spread to humans, and determining the risk to humans of the use of antimicrobial agents as growth promoters in animals, particularly in relation to other potential risk factors. Finally, the HAN report reviews the generalizability of specific risks, with the example being avoparcin use and incidence of vancomycin-resistant enterococci.

Additional assumptions. Critical assumptions include the following: the absence of data to support the existence of specific risk is assumed to suggest that no currently estimable risk exists. Any gap in the chain of risk leads to uncertainty surrounding the ultimate risk of concern: death. The precautionary principle should not be applied. Overall, the absence of analyzed data precludes a formal risk assessment.

Data required. The data required for the analysis described in the HAN report include the host range of bacteria in question, documented cases of bacterial transfer, the frequency of human gut colonization with animal bacteria, the frequency of plasmid-transposon transfer in the human gut, and the frequency of transfer of antimicrobial resistance from animal to human bacteria in human gut.

HAN report conclusions. The HAN report concludes that the human health risk associated with the use of antimicrobial agents as growth promoters cannot be properly assessed for lack of data. The report further concludes that the contribution of antimicrobial resistance in bacteria infecting animals to the incidence of antimicrobial resistance in bacteria infecting humans cannot be fully assessed for lack of data. These conclusions are based on the assumption that data are necessary at every step of the risk chain.

The HAN report concludes that the use of antimicrobial agents as growth promoters in animals has not compromised the human therapeutic use of related antimicrobials. However, this conclusion assumes that an inability to reject a null hypothesis is the same as full acceptance of the null hypothesis—that is, that the absence of a statistically demonstrated effect indicates that there is no effect in reality. This acceptance is in contrast to the previous assertions in the HAN report that the data are simply not available. Citing data from Kirst et al. [8] that show a lack of association between avoparcin use and the incidence of vancomycin-resistant enterococci in the United States, United Kingdom, and Denmark, the HAN report concludes that epidemiological data do not show an increase of infectious diseases as a result of the use of antimicrobial agents as growth promoters. Again, the report ignores statistical issues such as the power of such studies to document an association.

The HAN report concludes that there are essentially no thorough, documented, in vivo cases showing the spread of antimicrobial resistant gram-positive bacteria from livestock to humans. A criticism of this conclusion is that epidemiological associations depend on plausible mechanisms of association, not necessarily on documentation of those mechanisms. The report also concludes that transfer of antimicrobial resistance from animals to humans is only part of the entire risk chain; the major parts of the risk chain include a microbiological-genetic part, an animal-human transfer part, and an epidemiological part. Assessing the human health risk in relation to the use of antimicrobial agents as growth promoters involves making a full scientific inventory. Beneficial aspects such as animal welfare in relation to the use of antimicrobial agents as growth promoters and the influence of this use of antimicrobial agents on the spread of pathogenic zoonotic organisms also need to be taken into consideration.

BYWATER AND CASEWELL

Nature of the model. In a Letter to the Editor, Bywater and Casewell [5] assess the effect of antimicrobial resistance in different bacterial species and the contribution of animal sources to resistance in human infections. These authors selected 31 people considered to have experience and wide knowledge of microbiology and asked them to complete and return a questionnaire. Of these, 22 responded, and 20 of the responses were usable. The questions were based in part on those used by the Public Health Laboratory Service in setting its priorities, and the list of 20 organisms covered came from a report of the Scientific Steering Committee of the European Union Directorate General XXIV. The authors assigned scores for increasing degrees of importance for each of 3 features for each species or subgroup. The 3 features were as follows: (1) the burden of ill health, assessed according to the prevalence and severity of infection, such as mortality, postinfection sequelae, and treatment cost, is scored 1 (negligible) to 5 (major burden); (2) the extent to which, for each organism, antimicrobial resistance restricts treatment choice, scored 1 to 5 (resistance to usual treatments rare or antimicrobial treatment seldom required, to resistance common and can leave few or no treatment options); and (3) the extent to which, for each species, an animal source may contribute to resistance in human infection, scored 0 to 5 (not a source to the main source of resistance in human infection); these were then arbitrarily assigned scores of 0%, 1%, 5%, 20%, 50%, or 80%.

The authors then multiplied the mean scores for the first 2 of these to get a "resistance impact" for that species and normalized these effects to sum to 100%. The normalized scores were then multiplied by the mean factor assessing the extent to which an animal source may contribute to resistance in human infection, in order to estimate the perceived contribution of individual species and of possible animal sources of those species to antimicrobial resistance in humans.

The perceived contribution of animal sources (third question) was <1% for 12 of the 20 species and ranged up to 3.44% for nontyphoid salmonellae and 3.38% for *Campylobacter*. Overall, the mean scores indicated that animal sources might account for 3.88% of the human antimicrobial resistance problem.

Additional assumptions. Important assumptions in this analysis include the following:

- That the sample respondents were in fact knowledgeable about the issue at hand, and that the 20 (of 32) respondents adequately represented the nonresponders.
- That the possible responses (1 to 5) for the first 2 questions are equally spaced on some linear scale (necessary for the mean to have the assigned meaning) and that the arbitrary scores for the third are correct.
- That the same subjective criteria are used by each respondent for each of the 20 organisms.
- That the normalization process correctly divides the total burden of illness among the 20 organisms surveyed.

Data required. The data required for this approach to risk analysis are simple: 3 judgmental scores for each strain or group to be analyzed from each expert surveyed.

Limitations of the model. The authors do not discuss the weaknesses in their model or the uncertainties in its output. However, these appear to be serious. Two are of special concern to us. First is the selection of experts and the reliability of their responses. It is not clear from the published article that all the respondents were sufficiently expert in the quantitative analysis of risk, or that they brought to bear the extensive knowledge and thought needed for a reliable response to each of the 3 questions per organism.

Second is the compression of all aspects of ill health into a single scale without examination of the implications of doing so or the effects of different kinds of ill health on the final estimate. What is, in fact, the implied trade-off between moderate illness and death? Between death and expense? If such disparate end points are to be weighed on the same scale, the inherent conversion factors should be teased out and examined.

A further difficulty, although not a weakness in the model as used by the investigators, is that it produces relative estimates (percentages of the overall problem of infection) rather than absolute estimates (numbers of illness or deaths).

Overall uncertainty of estimates. In the absence of more information about how well the experts were qualified in the quantitative analysis of risk, and of how they made their judgments, we cannot judge the uncertainty of the estimates.

Utility for the present purpose. The approach of a mail survey of selected experts in clinical microbiology does not seem appropriate for the present purpose. We find the low proportion of resistance related to animal sources lacking in credibility.

RISKS ASSOCIATED WITH ANTIMICROBIAL USE IN FOOD ANIMAL PRODUCTION

Critique. In this section, we comment on a critique that was directed toward risk assessments of antimicrobial resistance. We try to identify the points that have some validity, although in general, the matters raised are trivial, unsupported, or of doubtful validity.

Response of Bayer Corporation to the FDA-CVM proposal. In a document released in February 2001 [9], the Bayer Corporation argues against the FDA-CVM proposal of 31 October 2000 to withdraw the approval of enrofloxacin (a FQ) for poultry. This document is not directly relevant to this review because it is not a risk assessment itself, but rather a rebuttal

of the CVM analysis. However, it is helpful in summarizing the counterarguments, and so we include it here.

Bayer concludes the following: the use of enrofloxacin in poultry does not pose a threat to public health; withdrawal of enrofloxacin would severely limit therapeutic options available to the veterinarian and would significantly increase suffering and mortality in poultry; and withdrawal would not result in a meaningful reduction of FQ-resistant *Campylobacter* infections in humans. In the Executive Summary of the report, Bayer cites 15 points in support of its conclusions. Most of these have to do with the value or the interpretation of some of the parameters used in the CVM model, or with appropriate measures and interpretations of disease in humans. These points should be accepted or rebutted in specific applications of the model. The Bayer report also stresses 3 points that deal with other matters:

1. The drug is expensive and used sparingly for therapeutic purposes only, and off-label use for poultry is prohibited by law.
2. The evidence that poultry are a major source of campylobacteriosis is largely circumstantial, as is the assertion that many *C. jejuni* strains that colonize chickens are different from those that colonize humans.
3. FQs are frequently effective in treating infections classified as FQ "resistant" based on in vitro testing.

The effect of each of these factors should be considered, although we believe that they are overstated.

CONCLUSIONS

Of concern is the fact that the risk assessments reviewed in this report consider only a few narrow and specific clinical outcomes, without consideration of the more general (and possibly more significant) outcome of a shift toward more resistant bacterial populations (regardless of present clinical significance). Given currently known mechanisms of resistance transfer across bacterial species, the risk of such a shift has serious potential consequences, including risks to human health.

Some fundamental questions remain unaddressed by the current literature on risk assessments. How much does antimicrobial use in animal populations (particularly subtherapeutic use) simply accelerate shift toward resistance resulting from human use of the same or similar drugs? What is the relative risk to humans of such use in comparison with human antimicrobial use?

The assessment of human health risk associated with antimicrobial use in animals is fraught with pitfalls, most notably the lack of specific data required by the models, the inherent complexity of the issues, and the choice of appropriate outcomes to consider. Compounding the problem is the fact that historical data used may not accurately predict future risk. The models do not consider the slowdown in recent years in the development of new antimicrobial agents for use in animal and human populations, nor do they consider the cumulative effect of antimicrobial use on the proportion of resistance in bacterial pools.

We conclude that there is a need for a new approach to the assessment of risks associated with antimicrobials in use on farms. What has been done to date is useful, but it is circumscribed by the vast scope of the problem and the cost and difficulty of assessing each piece of it. A new approach should have several characteristics:

- Reduced demand for time, money, and other resources.
- Common format, so that assessments of different aspects of risk can be readily combined.
- Reduced demands for data.
- Easy comprehension by people not expert in the details.
- Ready adaptation to meet special needs for specific purposes without losing the common core needed for a comprehensive view of the problem.

We conclude that a multiplicative model could meet these criteria, and indeed, this is the approach adopted by most of the risk assessments we have reviewed. We conclude further that these risk assessments have generally used much the right scheme for breaking the problem into successive steps that can be separately modeled, with estimates that can be multiplied together. However, there have been some variations in the details, and some of the models have gone well beyond what is needed for a general understanding of the problem as a whole.

The following set of 4 estimates, each dependent on those that come before, seems to meet these criteria.

Annual number of symptomatic infections by the organism of interest in a specific risk assessment. This step excludes asymptomatic infections, even though they may be of importance for diseases that can spread directly from person to person. We do not suggest that the counting of symptomatic events is easy, and much effort and judgment may be required at this stage because many cases of infection are not reported, and even the patient may not recognize an illness if the symptoms are mild and transitory. Whether an infection is symptomatic may not be defined precisely; it is in fact somewhat vague and subject to interpretation. An example of how such an estimate may be derived is in the FDA-CVM risk assessment, which included estimation of the number of symptomatic *Campylobacter* infections in the United States in 1998 and 1999.

Fraction of those occurrences in which the bacterial strain was clinically resistant to the antimicrobial or class of antimicrobials under study. This again requires a sharp line, now dividing resistant from nonresistant strains, and it requires some judgment about whether resistance as determined in the laboratory translates directly into resistance at the bedside. It also assumes that each infection is clonal and that mixed strains

are not frequent enough to merit separate study. Again, a precise dichotomy may be defined, but in practice, things are not so neat because resistance itself is on a graded scale. A risk assessment might include a sensitivity analysis to explore the effects of reasonable variations in the assumptions regarding these points.

Annual number of occurrences in which infection by a resistant strain led to the specific outcome under study. This limitation to counting outcomes precludes study of the severity of the outcomes except as defined by being above or below some limit (such as >3 days of hospitalization). However, several levels of severity could be examined in parallel, or the basic model could be extended to include severity as a special supplementary study. Some judgment about whether resistance led to the outcome may be needed if the resistant strain retains sensitivity to other antimicrobials, but unrecognized resistance leads to a delay or other problem in implementing more effective treatment.

Fraction of the above outcomes in which the antimicrobial resistance was a result of the farm use or category of uses under study. This again will require informed judgment, and the best judgment may be quite uncertain. Further modeling may help. For example, the IOM risk assessment divided this step into 2: the proportion of farm origin, and the proportion arising on the farm that were a result of subtherapeutic use.

This general approach is illustrated with 2 specific examples in Travers and Barza (this issue), each with a different bacterial strain, a different antimicrobial agent, and a different set of problems and uncertainties in the estimate. The 2 examples are *Salmonella* and penicillin-tetracycline resistance (an update of the 1989 IOM report); and *Campylobacter* infections and FQs (a modest revision of the FDA-CVM report).

The estimates outlined above should provide the basic data necessary for a simple approach to the assessment of human health risk posed by antimicrobial use in animals. Regulatory procedures should invoke the precautionary principle when even these data are scarce, until such high-quality data can be collected for inclusion in risk assessments and risk management.

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