

Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria

George G. Khachatourians, BA, MA, PhD

Abstract

MICROBIAL RESISTANCE TO ANTIBIOTICS IS ON THE RISE, in part because of inappropriate use of antibiotics in human medicine but also because of practices in the agricultural industry. Intensive animal production involves giving livestock animals large quantities of antibiotics to promote growth and prevent infection. These uses promote the selection of antibiotic resistance in bacterial populations. The resistant bacteria from agricultural environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional antibiotics. The author reviews trends in antibiotic use in animal husbandry and agriculture in general. The development of resistance is described, along with the genetic mechanisms that create resistance and facilitate its spread among bacterial species. Particular aspects of resistance in bacterial species common to both the human population and the agrifood industry are emphasized. Control measures that might reverse the current trends are highlighted.

Résumé

LA RÉSISTANCE MICROBIENNE AUX ANTIBIOTIQUES EST À LA HAUSSE, en partie parce qu'on utilise mal les antibiotiques en médecine humaine, mais aussi à cause des pratiques de l'industrie agricole. En élevage intensif d'animaux, on administre aux bestiaux d'importantes quantités d'antibiotiques afin de favoriser la croissance et de prévenir les infections. Ces utilisations sont propices à la résistance aux antibiotiques dans les populations bactériennes. Les bactéries résistantes de l'environnement agricole peuvent être transmises aux humains chez qui elles peuvent causer des maladies impossibles à traiter au moyen d'antibiotiques classiques. L'auteur passe en revue les tendances de l'utilisation des antibiotiques en élevage et dans l'agriculture en général. Il décrit l'apparition de la résistance, ainsi que les mécanismes génétiques qui créent la résistance et en facilitent la propagation dans les souches bactériennes. On insiste sur des aspects particuliers de la résistance de souches bactériennes communes à la population humaine et à l'industrie agroalimentaire. On met en évidence des mesures de contrôle qui pourraient casser les tendances actuelles.

Over the recent past the public has become increasingly alarmed by new scientific data that have made their way into the popular media about the connection between the overuse of antibiotics (or, more accurately, antimicrobial drugs) in both medicine and the agriculture–agrifood industry and the emergence and spread of antibiotic-resistant bacteria.¹⁻⁴ Microbial resistance to antibiotics is on the rise. More than 150 antimicrobial drugs are now available; these fall into some 20 classes and have 10 major targets of action, including the cell walls and membranes, nucleic acids, and protein and folate synthesis.⁵ With fewer new chemotherapeutic agents coming onto the market, the problem of resistance to drugs already in use has become a crisis in health care.^{6,7} One study estimated that the direct hospital costs of managing antibiotic resistance in the United States are US\$100 million to US\$10 billion per year, and the US Office of Technology Assessment has estimated that the minimal hospital costs of 5 types of nosocomial infection (e.g., surgical wound infection and pneumonia) due to antibiotic resistance were US\$4.5 billion per year (1992 dollars).^{8,9}



Education

Éducation

Dr. Khachatourians is with the Department of Applied Microbiology and Food Science, College of Agriculture, University of Saskatchewan, Saskatoon, Sask.

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The use of antibiotics to promote growth in livestock animals is one of the culprits. Antibiotic-resistant bacteria arising from agricultural practices enter human environments and move about with people and goods, thus creating transborder resistance. Not until recently did we suspect that the broad agricultural use of antibiotics could lead to widespread resistance in bacteria and the attendant effects on patients in health care settings and, after their discharge from institutions, the community at large. Fig. 1 shows the implications of the agricultural use of antibiotics in terms of the selection and transmission of antibiotic-resistant bacteria, as well as the consequences to the environment, the food chain and human health. Surveillance and prudent use of antibiotics by both medical and veterinary professionals are urgently needed to rectify the situation.

In this article I review trends in antibiotic use in animal husbandry and agriculture in general. The development of resistance is described, along with the genetic mechanisms that create resistance and facilitate its spread among bacterial species. Particular aspects of resistance in bacterial species common to both the human population and the agrifood industry are emphasized. Finally, some of the control measures that could be used to address the problem are highlighted.

Use of antibiotics in agriculture

Antimicrobial agents are usually used appropriately as therapeutic agents against bacterial infections, but they may also be used inappropriately in both human medicine (e.g., in response to demands from patients rather than according to medical indications) and agriculture (e.g., as growth-

promoting and prophylactic agents in animals). In addition to medical misuse, inappropriate use of antibiotics in the agricultural setting is a major contributor to the emergence of antibiotic-resistant bacteria. This situation was first documented in 1963, when increased levels of resistance in a particular strain of *Salmonella typhimurium* were observed at several British feedlots; several resistant isolates were subsequently identified over a period of 3 years.¹⁰

About 90% of the antibiotics used in agriculture are given as growth-promoting and prophylactic agents, rather than to treat infection.^{7,8} The recommended levels of antibiotics for feeds were just 5–10 ppm in the 1950s but have been increased by 10- to 20-fold since then.^{9,11} When high-energy feed for meat and dairy cattle, sheep and goats is supplemented with low levels of antibiotics (e.g., 35–100 mg of bacitracin, chlortetracycline or erythromycin per head per day or 7–140 g of tylosin or neomycin per ton of feed), there is a 3% to 5% increase in the rate of weight gain and feed efficiency (conversion of daily feed intake into meat).¹² To improve the growth of swine, 2–500 g of bacitracin, chlortetracycline, erythromycin, lincomycin, neomycin, oxytetracycline, penicillin, streptomycin, tylosin or virginiamycin is added to each ton of feed,¹² and for poultry, the same agents are used, but at 1–400 g per ton of feed. Even these low quantities of antibiotics encourage the selection of antibiotic-resistant bacteria, but sometimes feeds contain more than the recommended concentrations. In an examination of 3328 feeds in the US National Swine Survey, up to 25% of the feeds contained antibiotics at concentrations higher than the recommended levels.¹⁰ Other practices may also affect the use of antibiotics and the development of resis-

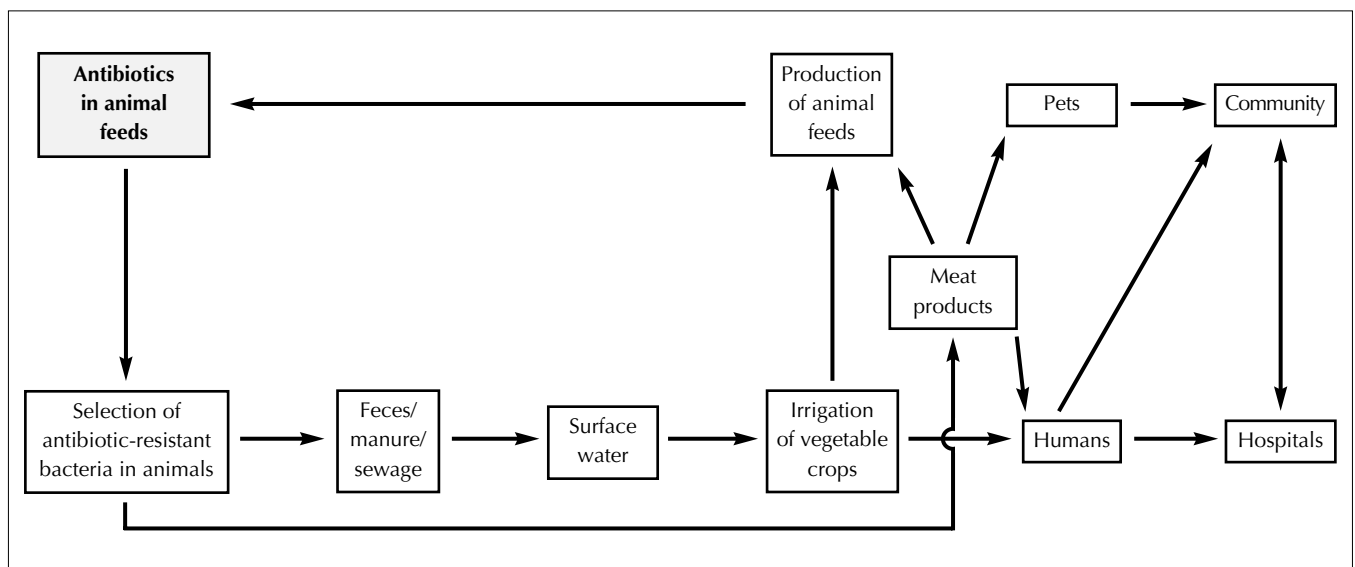


Fig. 1: The agricultural use of antibiotics in animal feed can result in the selection and transmission of antibiotic-resistant bacteria. These bacteria move through the environment by a variety of routes, and their presence ultimately has consequences for human health.



tance. For example, farmers with large operations were more likely than those with small farms to use antibiotics in feeds, and those working with a veterinary consultant were twice as likely to use such additives.¹⁰

The scale of agricultural use of antibiotics is enormous: in terms of annual quantities, their use in animals is 100 to 1000 times that in the human population.¹⁻³ Spraying of crops, particularly fruit trees, to eliminate surface bacteria is also implicated.^{8,9} Overall, annual estimates for applications of antibiotics in the US agrifood industry are over 8 million and 22 000 kg for animals and fruit trees respectively.^{8,9} Although sales of penicillin and tetracycline for use in animal feed have been declining, sales of other agents are on the rise.

Genetic basis for antibiotic resistance

Two conditions are needed for antibiotic resistance to develop in bacteria. First, the organism must come into contact with the antibiotic. Then, resistance against the agent must develop, along with a mechanism to transfer the resistance to daughter organisms or directly to other members of the same species.

Each antibiotic operates at a specific site within the bacterial cell. For example, some target the cell walls (e.g., bacitracin, cephalosporins and penicillins), whereas others target cell membranes (e.g., ionophores and polymyxins), cell components responsible for the synthesis of proteins (e.g., aminoglycosides, chloramphenicol and tetracycline), RNA (e.g., rifamycins), DNA (e.g., nalidixic acid and quinolones) or particular biochemical pathways such as folate synthesis (e.g., methotrexate and sulfonamides).^{3,5,9} Thus, when resistant organisms arise, their resistance is specific to particular antibiotics.

Bacteria have evolved diverse mechanisms to transmit resistance traits to other members of their own species and to other species. Genetic traits for antibiotic resistance are coded for in 2 places in bacteria: the chromosomes and the extrachromosomal elements (Figs. 2 and 3). Mutations can cause chromosomal genes that usually code for antibiotic sensitivity to start coding for resistance; such mutations occur at the rate of one per million to one per billion cells. The extrachromosomal elements (plasmids and transposons) are smaller pieces of circular DNA, each equivalent in size to about 1% of a chromosome. Plasmids can be either nonconjugative or conjugative; the latter can move from one bacterium to another. Genetic exchange is another mechanism by which antibiotic-resistant plasmids can move between bacteria (Fig. 2). Some bacteria are considered "promiscuous," because once they have acquired antibiotic-resistant plasmids, intra- and inter-species transfer of resistance occurs irrespective of the environment (i.e., whether or not antibi-

otics are present).^{5-7,9,13} Unusual transfer of antibiotic-resistant DNA sequences between bacterial species and between different ecological niches (e.g., between humans and ruminants) have been documented. For example, in *Staphylococcus aureus*, a gram-positive bacterium, the chromosomal gene for resistance to methicillin originated as a staphylococcal β -lactamase gene and a segment of a penicillin-binding gene originating from an unknown donor bacterium, perhaps *Escherichia coli*, a gram-negative bacterium.¹⁴

As far as mechanisms of resistance are concerned, some bacterial species are normally and inherently insensitive to certain antibiotics, whereas others are sensitive. Sensitivity has 3 requirements: a target for reaction, a mechanism for transport into the cell before the antibiotic action takes place and absence of enzymes that could inactivate or modify the antibiotic. A change in any of these prerequisites could render an antibiotic-sensitive bacterium resistant to the drug. For example, one or more mutations might be acquired that change the target of action, the uptake, efflux or extrusion of antibiotic, or the ability of the bacterium to inactivate or modify the antibiotic.^{7,9}

Antimicrobial drugs used for human therapy often have significant structural similarities to those used for animals, which leads to the potential for additional problems. For example, resistance to ormetoprim, a veterinary medicine, might foster resistance to trimethoprim, a structurally similar compound that is specified for use in humans.^{1,9} Bacteria resistant to streptogramin, quinupristin and dalfopristin were found in turkeys that had been given virginiamycin, but not any of the other antibiotics; however, all of these compounds have a similar structure.^{1,7} The use of animal feed supplemented with tylosin has resulted in the development of erythromycin-resistant streptococci and staphylococci not only in the animals but also in their caretakers.^{1,7}

Occurrence of antibiotic resistance in selected organisms

Salmonella

Multidrug-resistant *Salmonella typhimurium* definitive type 104 (DT 104) initially emerged in cattle in 1988 in England and Wales and was subsequently found in meat and meat products from other domestic animals, as well as unpasteurized milk from other locations.¹⁵ Human illness occurred through contact with farm animals and consumption of beef, pork sausages and chickens. The number of DT 104 isolates from humans in Britain increased from 259 to 3837 between 1990 and 1995.¹⁶ The proportion of antibiotic-resistant *Salmonella* associated with human infections rose from 17% to 31% of isolates between



1979/80 and 1989/90,¹⁶ and the proportion of *Salmonella* isolates exhibiting antibiotic and multidrug resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline increased from 39% to 97% in the same period.^{16,17} In the 1990s, 90% of all DT 104 isolates obtained from humans have been multidrug resistant, and more recent isolates are resistant to fluoroquinolone as well.¹⁵ In 1997 an interagency workshop with representation from Canada, the US, the United Kingdom and the Netherlands reported a rise in the number of multidrug-resistant DT 104 isolates, and resistance to trimethoprim and fluoroquinolone was also reported.¹⁷

A study of aquacultural products including fish and shellfish exported to the US from Canada between 1986 and 1989 reported high levels of antibiotic resistance.¹⁸ The study questioned the alleged benefits of administering subtherapeutic levels of medication in feeds. DT 104

isolates have also been found in poultry, sheep and pigs.¹⁹ In the Pacific Northwest, 4% of the human isolates of *S. typhimurium* strains were DT 104 in 1989, but this proportion had risen to 43% by 1994.²⁰ In the latter study 25 isolates from human and cattle sources were phage typed and shown to be identical.

Poultry that are given antibiotics often carry antibiotic-resistant strains of *Salmonella* or antibiotic-resistant transposons, which eventually reach humans through poultry meats, eggs and other foods. A 1985 outbreak of *Salmonella newport*, which was traced from dairy cows and calves, through the carcasses and the meat-packing plant to the restaurants and grocery stores to which the meat had been shipped. Similarly, drug-resistant, animal-associated *Salmo-*

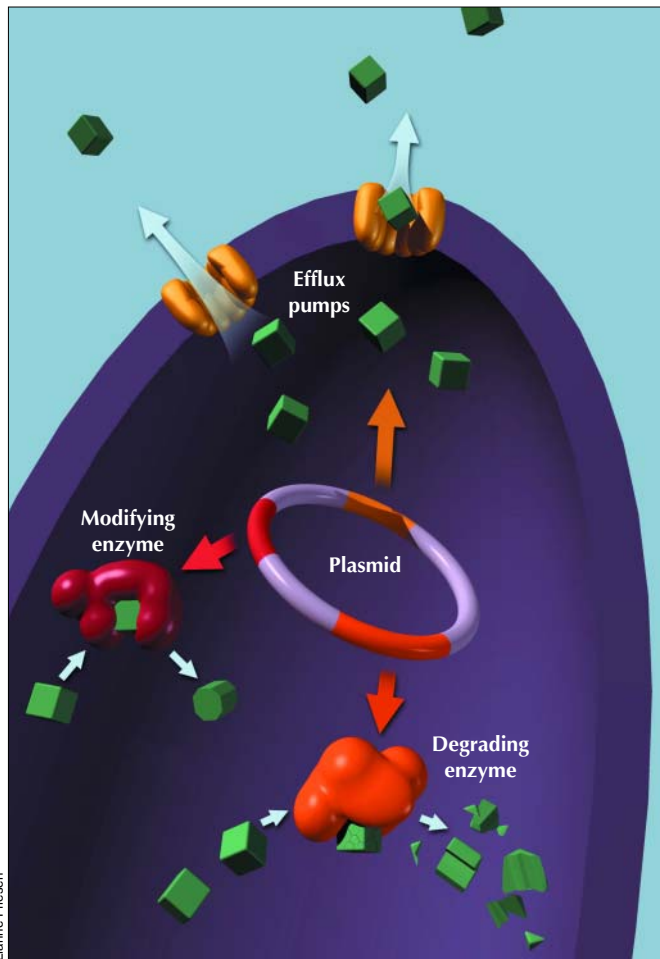


Fig. 2: Transfer of antibiotic resistance from one bacterium to another can occur by means of bacterial plasmids. Plasmid-mediated resistance mechanisms include efflux pumps, which remove antibiotic (represented as cubes) from the cell; modifying enzymes, which render the antibiotic ineffective by changing its conformation; and degrading enzymes, which degrade the antibiotic altogether.

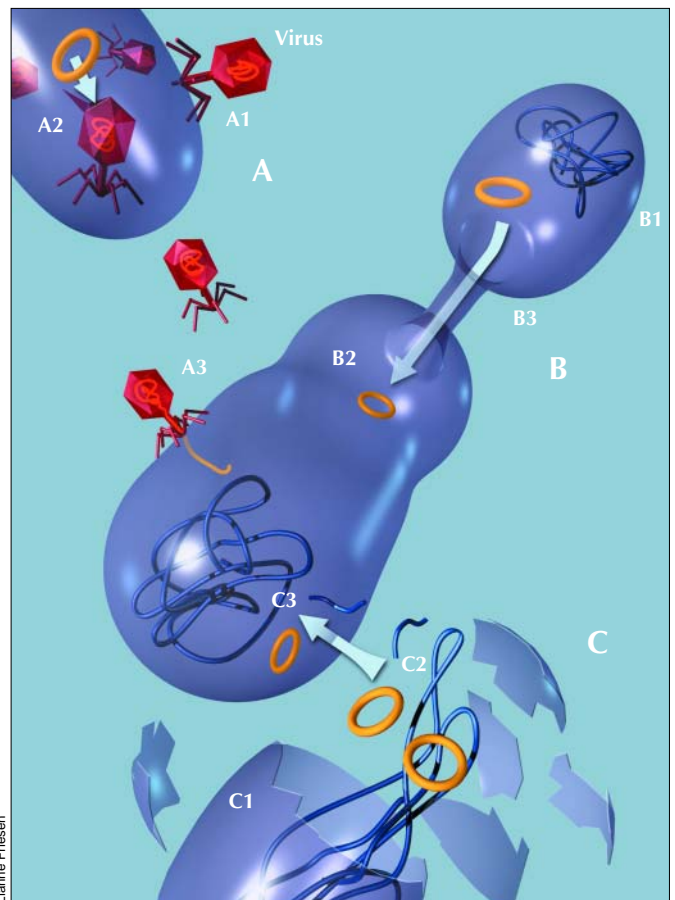


Fig. 3: Resistance genes are transferred to other bacteria in the following ways. A: A virus infects a plasmid-bearing cell (A1), where it replicates, picking up a plasmid in the process (A2). The new viruses, which now carry the plasmid and the associated resistance mechanisms, go on to infect other cells (A3). B: A donor bacterium (B1) may transfer plasmid DNA (B2) to another cell through a pilus (B3). C: When bacteria are lysed (C1), they release their plasmids and chromosomal DNA (C2), which can then enter other bacteria (C3).



nella strains can spread to humans through pets that eat pet foods contaminated by infected poultry products. In fact, the spectrum of modes of transmission of antibiotic-resistant *Salmonella* to humans is broad and includes domestic and wild animals, fish and crustaceans, and insects and rodents.^{1,9}

Enterobacter* and *Campylobacter

Quinolone antibiotics have as their main target the A subunit of bacterial DNA gyrase, the enzyme that “unwinds” the double strands of DNA during replication. One mechanism for the development of resistance to these agents is mutations in the *gyrA* subunit of this enzyme (Fig. 4). The approval of fluoroquinolones for veterinary use in Europe led to the emergence of antibiotic-resistant *Campylobacter jejuni* in human and chicken populations. The use of these compounds to treat infections caused by *Enterobacter* and *Campylobacter* in the Netherlands led to increases in the number of fluoroquinolone-resistant strains, which appeared in humans who consumed poultry.²¹ The prevalence of enrofloxacin-resistant strains of *Campylobacter* in poultry and humans increased from 0% to 14% and from 0% to 11% respectively.²¹ Koenaard and associates²² reported a higher prevalence of quinolone-resistant *Campylobacter* isolates from sewage plants receiving effluents from poultry abattoirs. However, the increase in frequency of development of resistance to fluoroquinolones could have an alternative explanation. A recent study in Vancouver by Waters and Davies²³ revealed a natural resistance to fluoroquinolone ciprofloxacin in bacterial populations isolated within city-area soil. DNA sequencing revealed a high degree of variation in DNA gyrase, the target of fluoroquinolones; thus, even without any selective pressure, these bacteria showed the same alterations in the target sequence as those isolated from clinical and laboratory environments.

Escherichia coli

A recent longitudinal study of *E. coli* O157:H7 dissemination related the therapeutic and subtherapeutic (in feed) use of antibiotics and the occurrence of antibiotic-resistant isolates on Wisconsin farms.²⁴ Over a 14-month period, subtherapeutic use of antibiotics (penicillin, sulfamethazine, chlorotetracycline, oxytetracycline and neomycin, 0.25 to 1 kg per ton of feed or added to drinking water) and therapeutic use of sulfamethazine to treat diarrhea correlated well with the emergence of antibiotic-resistant *E. coli* O157:H7 on the farms. Restriction endonuclease digest profiles of cellular DNA showed changes in the resistant bacterial isolates. The possibility of transmission of *E. coli* O157:H7 through birds that ate animal feed or drank

animal water was implied.

Vegetables and fruits can also be a source of some antibiotic-resistant bacteria. Consumers may be infected with *E. coli* O157:H7 if crop farmers use antibiotics for phytosanitation or if crops are fertilized with animal manure. Outbreaks of *E. coli* O157:H7 associated with apple cider²⁵ and potatoes²⁶ were traced to contact between fresh produce and manure. Antibiotic-resistant *E. coli* and *Campylobacter* strains can emerge from irrigation water, run-off from animal-processing plants or manure from intensive livestock operations, where *E. coli* O157:H7 may survive for up to 2 months in fresh or inadequately composted manure.

In Great Britain pigs and calves are treated with both apramycin (an antibiotic used specifically for animals) and gentamicin (which is used for both animals and humans). Porcine strains of antibiotic-resistant *E. coli* were found in

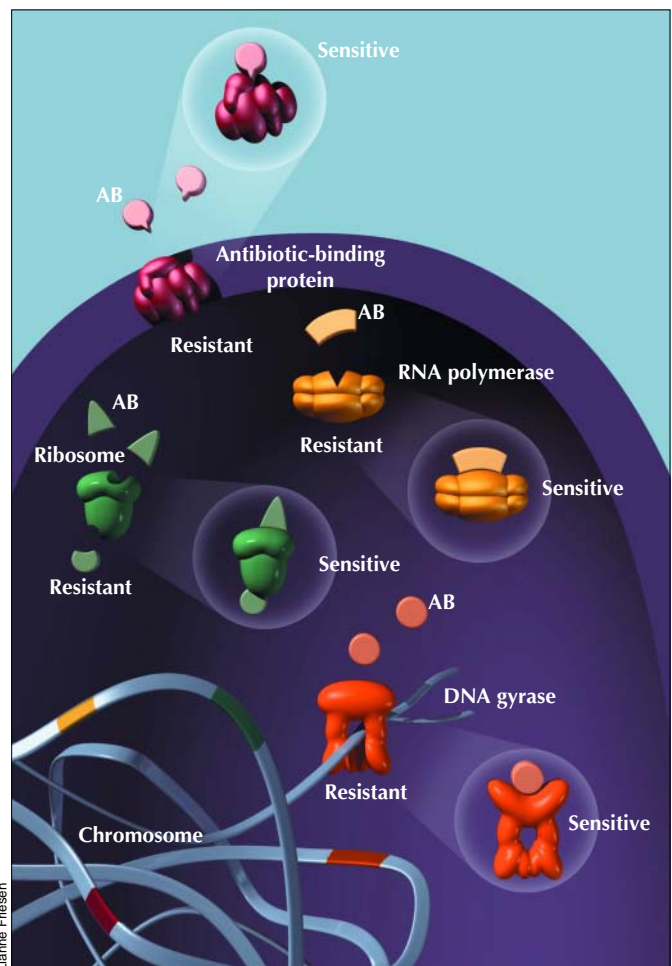


Fig. 4: Chromosomal mutations in bacteria may mediate changes to the targets of antibiotic (AB) action, rendering them incapable of binding with (and therefore resistant to) the antibiotic. Targets include antibiotic-binding proteins, ribosomes, RNA polymerase and DNA gyrase. The interactions between antibiotics and sensitive cell components are shown within the 4 large circles.



a pig farmer. Gentamicin-resistant strains isolated from human clinical samples showed resistance to apramycin after the introduction of this drug into animal husbandry practice. Similarly, nourseothricin-resistant strains were isolated from both animals and humans soon after its introduction as a growth-promoting agent for pigs.¹

The use of nourseothricin as a porcine growth-promoting agent in the former East Germany between 1983 and 1990 resulted in the development of an antibiotic-resistant transposon.^{2,7,27} Two years after its introduction, resistant isolates of *E. coli* were found in porcine guts and meat products and subsequently in the intestinal microflora of the pig farmers and their families, as well as in patients with urinary tract infection and the general public of the municipality. By 1990 the same transposon had been found in *Shigella* and other human enteric bacteria.

Enterococci

Vancomycin has been used since the 1950s in human medicine and since the 1980s in farm animals, where it has been identified as a source for reservoirs of resistant enterococci. Vancomycin-resistant enterococci were first isolated from sewage treatment plants in Britain and small towns in Germany and later from manure samples from pig and poultry farms.²⁸ These bacteria have been transmitted to humans through the food chain in Germany, Norway and the Netherlands.²⁹ Molecular genetic analysis of vancomycin-resistant isolates from the feces of pigs and poultry in Denmark and of the transposon Tn1546, isolated from humans, suggests that there has been transmission among humans, farm animals and household pets.²⁸

Avoparcin is an antibiotic that acts in the same way as vancomycin, except that it is used solely for veterinary practice and in animal feeds. Vancomycin-resistant enterococci isolates from Denmark and Germany are cross-resistant to avoparcin and to teicoplanin. There may be a link among the use of avoparcin, the selection for vancomycin-resistant enterococci and the colonization of humans by these bacteria through the food chain. One possibility is that vancomycin resistance is associated with animals being fed avoparcin and analogous antibiotics. Avoparcin is not used in North America as a feed additive. However, the annual use of this drug in feeds in Denmark and Australia has been reported as 24 000 and 62 642 kg respectively,^{28,29} whereas the use of vancomycin for humans is 24 and 582 kg respectively.² In spite of the problem, the feed additive manufacturing industry in Europe has protested the withdrawal of avoparcin from use in farm animals.³⁰ Because of its carcinogenic potential, avoparcin has not been licensed in Canada or the US. However, it has been used illegally for veal feed in the US.³¹ Vancomycin-resistant enterococci are not present in

the normal fecal flora of the US population, nor are they found in vegetarians or on farms not using avoparcin.³² There are now restrictions on the use of this drug in Denmark and Britain, and in the US there is a prohibition on the extralabel use of fluoroquinolones and glycopeptides for animal feeds.³²

The conjugative transposons carrying resistance to avoparcin and vancomycin have a resistance gene cluster.³³ *Enterococcus faecalis* has been reported to transfer plasmids harbouring antibiotic-resistance traits to other enterococci and to *Listeria monocytogenes* in water treatment plants in Germany.³⁴ Multidrug-resistant and vancomycin-resistant enterococci are commonly isolated from humans, sewage, aquatic habitats, agricultural runoff and animal sources, which indicates their ability to enter the human food chain.³⁵ *Enterococcus faecium* conjugative transposons can be transferred from animals to humans. Such conjugative transposons can also transfer vancomycin resistance to *Staphylococcus aureus*, streptococci and lactobacilli. The vancomycin-resistant enterococci are of special concern, because they cause illness and death in patients in hospital settings, especially those who are immunocompromised. Infection with vancomycin-resistant enterococci in some groups of US hospital patients is on the rise; these resistant organisms now represent 20% to 40% of all enterococci causing hospital-acquired infections,³⁶ and the spread of vancomycin-resistant staphylococci has already been reported from Japan.³⁶

Measures to reduce the impact of antibiotic resistance

Whether antibiotics are given as treatments to humans or animals or as additives in animal feeds, their misuse is at the heart of the antibiotic-resistance problem. The problem of medical misuse of antibiotics is significant but beyond the scope of this review. The extent of the problem in the agricultural setting is indicated by the fact that about half of all antibiotics used in the US are for animal husbandry, the primary compounds being penicillin and tetracycline. Only 10% of these drugs are given to treat infectious disease; the rest are given to promote growth or prevent disease.

The concept of emerging infectious diseases relates to diseases arising both from previously unknown pathogens and from known pathogens that have undergone some change that renders them more virulent. Many of the latter represent antibiotic-resistant forms. The emergence of such novel infectious diseases and the development of antibiotic resistance have a context unlike any other in medicine. In a perceptive article on emerging infectious diseases and the law, Fidler³⁷ made the point that microbes do not



respect international borders. Therefore, to curb emerging infectious diseases and antibiotic resistance, new international laws and cooperative efforts are needed. Almost 30 years ago the Swann Committee in the United Kingdom recommended strict adherence to certain regulations in the use of antibiotics for animal feed and prevention of infection.^{2,38} Reports to the US Office of Technology Assessment⁸ and the Swedish government in 1997³⁹ and recent reports from Denmark and the World Health Organization^{4,6,40} have put the problem and its solutions into both health^{40,41} and legal^{37,42} contexts, and have proposed a broad strategy for containment of antibiotic resistance. The elements of the strategy are to improve the rational use of antibiotics in human medicine, to reduce and eventually eliminate the use of antibiotics for purposes other than human medicine and the treatment of infection in animals, and to reduce the spread of antibiotic-resistant organisms by improving hygienic practices and relevant infrastructure in both hospital and public health settings.

To implement such a wide-ranging strategy will require new public health views and practices. The elements

of the strategy should reside in education, technical development, statutory regulation and surveillance of antibiotic use internationally. Denmark's comprehensive surveillance of the consumption of antibiotics and the occurrence of antibiotic-resistant strains in animals, foods and agricultural practice has enabled that country to maintain a low level of antibiotic resistance.^{1,40} A body of literature is emerging about new approaches to both intensive and small livestock operations, suggesting that attention to good animal husbandry practices, including management of crowding and general hygiene measures, are worthwhile options to reduce the need for antibiotics.^{43,44}

Poorly regulated use of antibiotics in medicine and agriculture has contributed to a build-up of reservoirs of antibiotic-resistant bacteria. The absence of newer antibiotics to treat the emerging infectious diseases caused by antibiotic-resistant organisms has heightened public apprehension. Levy^{3,9} has presented arguments supporting the view that the problem of resistance is ecological and represents an imbalance between sensitivity and resistance in bacterial populations. Some data suggest that if anti-

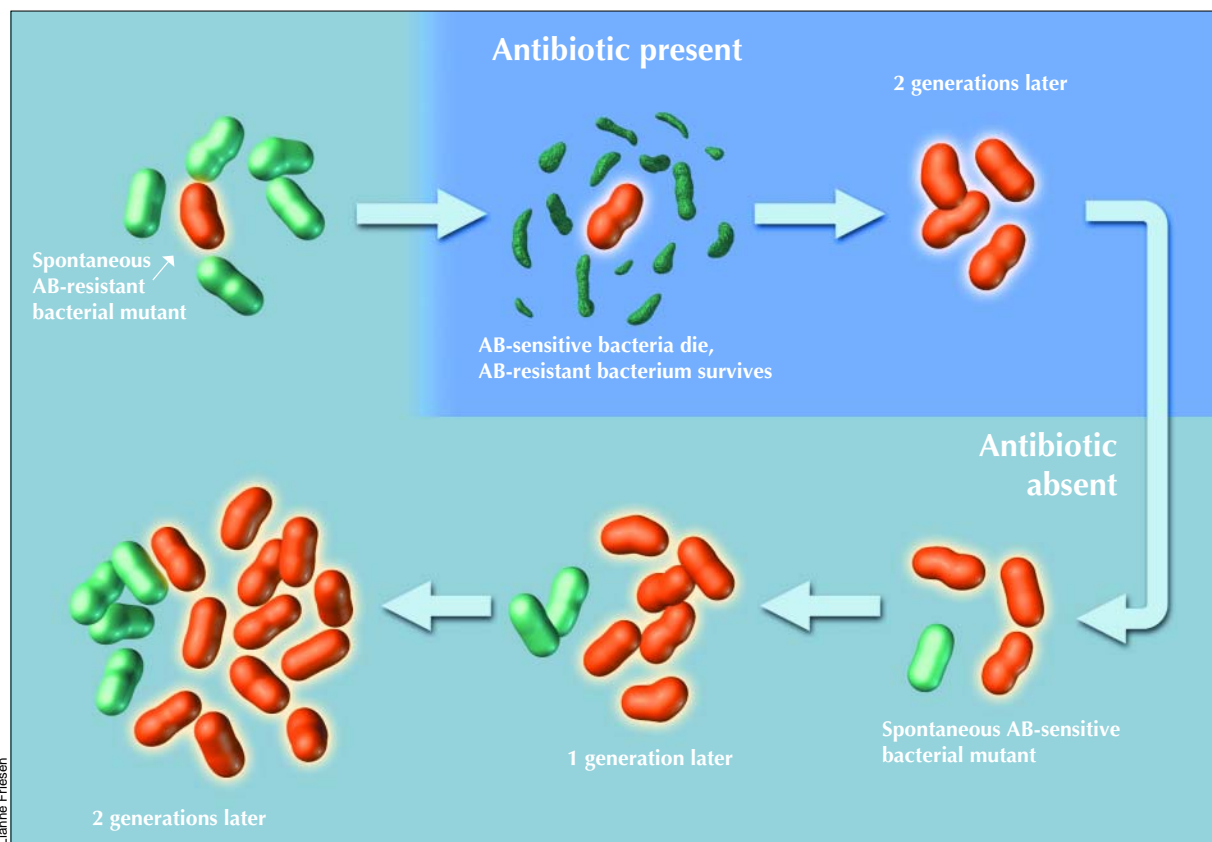


Fig. 5: Diagram illustrating the development of antibiotic resistance in bacteria and the re-establishment of a balance between antibiotic resistance and sensitivity. Beginning at the top left, a spontaneous mutation in a population of antibiotic-sensitive bacteria leads to antibiotic resistance in one bacterium. In the presence of antibiotic, the antibiotic-sensitive bacteria die, but any resistant bacteria survive and reproduce. Continued presence of antibiotic ensures that only resistant bacteria survive. However, if the antibiotic is removed (bottom right), spontaneous mutation to sensitivity may occur. After many generations, the proportions of sensitive and resistant bacteria reach a new balance.



otic use is discontinued, the ecological balance will be re-established, at least in terms of the presence of antibiotic sensitivity^{3,7-9} (Fig. 5). In addition, novel applications of lactic acid bacteria and their antibacterial peptides as probiotics in the gastrointestinal tract of animals and humans and as combatants against food pathogens offer new avenues for the appropriate use of antibiotics in animal feed, human health and food safety.^{45,46}

We now need to convert our concern into action. Canada and other nations must support and implement the recommendations that have been made. It is time to recognize the true costs of antibiotic use in agricultural practice in terms of antibiotic resistance and its consequences on the sustainability of susceptible bacterial flora in the environment and to act accordingly.

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Reprint requests to: Dr. George G. Khachatourians, Department of Applied Microbiology and Food Science, College of Agriculture, University of Saskatchewan, Saskatoon SK S7N 5A8; fax 306 966-8898; khachatouria@sask.usask.ca