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Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data

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The use of antibiotics in food animals selects for bacteria resistant to antibiotics used in humans, and these might spread via the food to humans and cause human infection, hence the banning of growth-promoters. The actual danger seems small, and there might be disadvantages to human and to animal health. The low dosages used for growth promotion are an unquantified hazard. Although some antibiotics are used both in animals and humans, most of the resistance problem in humans has arisen from human use. Resistance can be selected in food animals, and resistant bacteria can contaminate animal-derived food, but adequate cooking destroys them. How often they colonize the human gut, and transfer resistance genes is not known. In zoonotic salmonellosis, resistance may arise in animals or humans, but human cross-infection is common. The case of campylobacter infection is less clear. The normal human faecal flora can contain resistant enterococci, but indistinguishable strains in animals and man are uncommon, possibly because most animal enterococci do not establish themselves in the human intestine. There is no correlation between the carriage of resistant enterococci of possible animal origin and human infection with resistant strains. Commensal *Escherichia coli* also exhibits host-animal preferences. Anti-Gram-positive growth promoters would be expected to have little effect on most Gram-negative organisms. Even if resistant pathogens do reach man, the clinical consequences of resistance may be small. The application of the 'precautionary principle' is a non-scientific approach that assumes that risk assessments will be carried out.

Keywords: antibiotic resistance and food animals, animal antibiotic use and human health risk

Introduction

Antibiotics—naturally-occurring, semi-synthetic and synthetic compounds with antimicrobial activity that can be administered orally, parenterally or topically—are used in human and veterinary medicine to treat and prevent disease, and for other purposes including growth promotion in food animals. Antibiotic resistance is as ancient as antibiotics, protecting antibiotic-producing organisms from their own products, and other originally susceptible organisms from their competitive attack in nature. All antibiotics can select spontaneous resistant mutants and bacteria that have acquired resistance by transfer from other bacteria. These resistant variants, as well as species that are inherently resistant, can become dominant and spread in host-animal populations. The more an antibiotic is used, the more likely are resistant populations to develop among pathogens and among commensal bacteria of an increasing number of animals in an exposed population. However, there is great diversity: whereas some bacteria very rapidly develop resistance in the individual treated, others remain susceptible.

Antibiotic resistance defined in this way is a microbiological phenomenon, which may or may not have clinical implications depending on pharmacokinetic and pharmacodynamic parameters as they apply to specific antibiotics. Nevertheless, even low-level resistance (diminished antibiotic potency within the clinically susceptible range) is noteworthy since it may be a first step towards clinical resistance. These considerations have always been important in definitions of rational antimicrobial therapy,[1] and have been re-emphasized by recent calls for prudent therapy in human and veterinary medicine.

The campaign against what has been considered excessive clinical use has been generally evenly directed at human and animal medicine, but there has been a concerted attack on the agricultural use of antibiotics, based on the assumption that all such usage is imprudent since it might act as an important source of resistance in bacteria

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affecting humans.[2–8] In Europe, this has led to the banning of several antibiotic growth promoters as a precaution, despite the advice of the European Union's own Scientific Committee on Animal Nutrition (SCAN) that there were insufficient data to support a ban,[9,10] and it is proposed to withdraw the rest in 2006. There are calls for a wider application of the ban. Pieterman & Hanekamp have drawn attention to the logical, legal and moral flaws inherent in the 'precautionary principle', taking as an example the banning of growth-promoting antibiotics in Europe.[11] In the words of the National Research Council and Institute of Medicine, 'given some limited facts, authoritative opinions, and some projections on possible although not necessarily probable biological events, scenarios can be quickly woven to paint a bleak picture of the future'.[12] The potentially adverse effects of bans are often ignored.

Whereas a theoretical hazard to human health arises from the use of growth-promoting antibiotics, an independent examination of the facts, free from commercial or political influence, shows that the actual risk is extremely small and may be zero in many cases. For this reason, and in order to try to redress what we perceive as an imbalance, we accepted the invitation of the Animal Health Institute (AHI) to meet colleagues in human and veterinary medicine, to attempt to draw out the facts among much misinformation, with an independent agenda chosen by ourselves. Throughout, we have tried to draw a distinction between events that do happen, that may happen, that might happen, or that do not happen.

The authors were initially convened as an advisory board by the Animal Health Institute (AHI), an association of manufacturers of animal health-care products in the USA. They decided, as independent scientists and practitioners, to produce this review. Drafts were produced by Prof. I. Phillips as co-ordinating author. The paper was not commissioned by AHI nor were its contents influenced or approved by AHI or by any of its members.

The use of antibiotics in food animals

Definitions of use

The National Committee for Clinical Laboratory Standards (NCCLS) has defined terms to describe herd or flock antibiotic use.[13] Therapy is the administration of an antimicrobial to an animal, or group of animals, which exhibit frank clinical disease. Control is the administration of an antimicrobial to animals, usually as a herd or flock, in which morbidity and/or mortality has exceeded baseline norms. Prevention/prophylaxis is the administration of an antimicrobial to exposed healthy animals considered to be at risk, but before expected onset of disease and for which no aetiological agent has yet been cultured. (Metaphylaxis is a term sometimes used when there is clinical disease in some animals, but all are treated.) Growth promotion is the administration of an antimicrobial, usually as a feed additive, over a period of time, to growing animals that results in improved physiological performance.

Therapy, control and prevention: When antibiotic treatment is necessary, it often has to be administered to food animals in feed or water. Individual animal treatment is almost never practical for poultry, but may be practical for cattle and swine.

In livestock production, the objective is to limit progression of disease in the population, since illness decreases animal performance. Herd or flock treatment is often indicated when illness is first recognized in a small proportion of the animals. For example, one of the indications for the use of antibiotics in animals is physical stress involved, for example, in the movement of animals in large numbers. Whereas mass regimens can improve animal performance and the general welfare of the treated animals, such regimens do result in increased antimicrobial usage.[14] Mass treatment programmes generally err on the side of administering treatment to individuals that do not need it (as occurs in prophylaxis in human medicine), whereas limitation of therapy to recognized clinical cases errs on the side of withholding treatment from some individuals that would benefit. Attempts to limit mass metaphylaxis to those individual animals most likely to benefit, using rectal temperature as a clinical indicator for treatment, have usually been unsuccessful.[15] More sophisticated measures of disease status are being investigated as one means to improve treatment selection criteria.

Growth promotion: The growth promoting effects of antibiotics were first discovered in the 1940s when chickens fed by-products of tetracycline fermentation were found to grow faster than those that were not fed those by-products.[16] Since then, many antimicrobials have been found to improve average daily weight gain and feed efficiency in livestock in a variety of applications,[17–19] and this is known as 'growth promotion'. Whereas the precise mechanisms of growth-promoting effects were, and are still, often unknown, knowledge is improving,[19,20] the net benefit of antibiotic feeding to food-producing livestock was, and still is, measurable.[11] Such measurable benefit coupled with demonstrable target animal safety, edible tissue clearance and residue avoidance, and environmental safety is the basis for regulatory approval of growth promoting applications of antibiotics in livestock production.[17] Whereas some growth-promoting effects are mediated through alterations of the normal intestinal microbiota resulting in more efficient digestion of feed and metabolism of nutrients,[21,22] others are mediated through pathogen and disease suppression and immune system release. For example, rates of post-weaning scours increased following antimicrobial growth promoter restrictions in Sweden.[23,24] Similar problems have been experienced in many parts of Europe following the growth-promoter ban, requiring the increased use of therapeutic antibiotics (for references, see Casewell *et al.*[25]), making it clear that infectious disease suppression is an important effect of growth promoters.

Antibiotic use

In 2001, 23 products with antibacterial activity, excluding coccidiostats, had US regulatory approval and were marketed for feed additive applications.[26] Fifteen of those 23 antibacterial compounds had growth promotion label claims. Of those 15, only two (bambermycins and laidlomycin) did not have additional claims for therapeutic feed additive uses. Thus, distinctions between growth promotion and prophylactic applications are sometimes difficult. For example, whereas control and treatment dosages of lincomycin and tylosin are higher than those for growth promotion, it is clear from the Danish experience after the banning of growth promoters that the compounds at the lower growth promotion doses appear to help swine ward off the pathogenic effects of *Lawsonia intracellularis* and decrease the incidence and severity of ileitis and diarrhoea.[27] A recent publication reviews the current usage of antibiotics in livestock in the US, explaining the complex interaction of antimicrobials with dietary factors.[28]

Whereas many products used for growth promotion and prophylaxis such as bacitracin, bambermycins and carbadox have little or no application in human medicine, products used for prophylaxis and therapy are often closely related to antibiotics used in human medicine. The classes used include: β-lactams (penicillins and cepha-

losporins); sulphonamides with and without trimethoprim; tetracyclines; macrolides, lincosamides and streptogramins; and quinolones (including fluoroquinolones).[27] These have a variety of therapeutic and preventive applications in food animals. A few examples will suffice: in pigs, therapeutic antibiotics are used in the weaning period for the treatment of gastrointestinal disorders and later in life for the treatment of pneumonia (penicillins and fluoroquinolones for *Actinobacillus pleuropneumoniae*) and intestinal infections such as those as a result of *L. intracellularis* (macrolides, pleuromutilins) and swine dysentery (pleuromutilins). Tetracyclines, macrolides and pleuromutilins are frequently used in pigs for stabilization of the gut flora during the weaning phase. In cattle, antibiotics are used mainly to treat respiratory infections in calves and mastitis in cows. A full account may be found in *Antimicrobial Therapy in Veterinary Medicine*.[29]

Benefits of antibiotic use in animal agriculture

While controversy regarding the value of animal products in healthy diets and the overall contribution of livestock production to human and environmental well-being is beyond the scope of this report, animal product contributions to human diets are documented,[30] as are net contributions of livestock production to human health and nutrition over strictly horticultural systems.[31]

It is a common misconception that subsistence agriculture fosters a higher plane of animal health than the industrial agriculture currently practised in developed countries. Yet epidemics of infectious animal diseases such as rudderpost, anthrax and tick fever are recorded in ancient writings from India.[32] Similarly, livestock epizootics are prominent in the history of the Middle Ages.[33] Hog cholera, trichinosis, babesiosis, and especially contagious bovine pleuropneumonia resulted in the establishment of the Bureau of Animal Industry as part of what became the United States Department of Agriculture.[34] Before the major advances in animal science and veterinary medicine of the 19th and 20th centuries, livestock production was an uncertain venture encumbered by catastrophic animal health risk.

Veterinary medical advances, of which antimicrobials are part, made possible the specialization and division of labour critical to advancement of the various sectors of the agricultural economy. Some bacterial diseases such as lamb dysentery (intoxication by intraintestinal growth of *Clostridium perfringens* Type D[35]) and black leg of cattle (intramuscular infection with *Clostridium chauvoei* or *Clostridium novyi*[36]) cause great loss but are readily amenable to immunization. Some diseases, such as contagious bovine pleuropneumonia and foot-and-mouth disease are so devastating that large-scale, expensive efforts are justified to eradicate them from livestock populations and then protect livestock from their reintroduction.[37,38] Expensive eradication efforts are justified for still other livestock diseases such as brucellosis[34] and tuberculosis[34,39] because of their serious zoonotic consequences when left unchecked in food-producing livestock. A very few diseases, such as bovine babesiosis, have life cycles that make their eradication practical and cost effective by eradication of an intermediate host.[40]

However, many bacterial diseases are not readily amenable to vaccination and have a near-commensal association with either their food-animal hosts or a broad range of other reservoir species, either of which make eradication impossible. *Pasteurella multocida* is an example of an organism that causes disease in a wide variety of species[39] and can often be cultured from clinically normal animals. *Streptococcus suis*,[41] *Mannheimia (Pasteurella) haemolytica, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae, Escherichia coli* and *Haemophilus somnus*[42] are other organisms with close host association. Diseases caused by such agents are endemic, sporadic and multifactorial. As a result, control measures are often unclear or difficult to achieve in practical settings. Vaccines have been developed for many of these pathogens but clinical efficacy is generally disappointing.

For bacterial diseases with complex aetiologies, or which have not responded to alternative measures, control of subclinical disease and therapeutic intervention for recognized clinical disease using antimicrobials is frequently the only practical option. When disease-prevention measures fail, therapy is indicated from both economic and humane perspectives. Antibiotic use in animal agriculture results in healthier animals, and we believe that the health-promoting effects, from which at least some of the growth-promoting effects arise, deserve more attention.

Confinement livestock

Intensive livestock production has arisen to utilize the plentiful supplies of grain and energy effectively, while conserving the more highly valued resources of land and labour.

The logistical advantages arising from animal population concentration translate to reduced variable costs, of which the largest in livestock production is feed. Livestock concentration makes formulation and delivery of high-quality, consistent, nutrient-dense diets feasible. High-quality diets formulated to meet all of the animals' nutrient requirements not only raise animals using the lowest possible level of feed input, they do it using the least time. Out of these constraints on agricultural production arise the motivations to use antimicrobials in livestock. The feed conserving attributes of antimicrobial growth promoters are well documented,[17] even if their precise mechanisms are not completely elucidated.[19–22,43]

Poultry production is in the hands of integrated producers with extensive data-management and analytical expertise. The current benefits of antibiotic use in such integrated production systems are not publicly known, but their continued use in commercial production indicates improvement in mortality, morbidity, growth and feed efficiency. The benefits of metabolic modulation of the intestinal microbiota of cattle are comparatively well defined.

Controversy exists over the effects of feed grade antimicrobials in swine production. Recent advances in swine housing and management, including diets[20] may have supplanted some of the effects formerly attributed to growth promoting antimicrobials in swine rations. In a recent publication,[44] it was reported that antimicrobials administered in feed to pigs reared in multi-site production systems resulted in improved performance in nursery pigs but not in finisher pigs. However, the study pooled results from trials with different protocols and did not evaluate the contribution of therapeutic antibiotic use. Furthermore its results are applicable only to one type of management practice 'and are not necessarily generalizable to the entire US swine population',[44] and probably even less so universally. However, they are consistent with results reported following antimicrobial growth promoter restrictions in Europe.[23,25] Thus, advances in swine management appear to have made reduced reliance on growth-promoting antimicrobials possible, but not eliminated their requirement for some phases of efficient, humane and profitable livestock production.

Whereas precise quantification of the impact of therapeutic antimicrobial use on livestock production is difficult, in part because of the imprecision of clinical case definitions for livestock diseases, it is

clear that various therapeutic applications of antimicrobials are vital to profitable and humane livestock production. The distinction between prudent and overzealous use is more difficult.

In all, antimicrobials are an integral part of efficient and humane livestock production. Current livestock production practices have developed, along with their reliance on the various applications of antimicrobials, in response to broad economic forces ultimately driven by the price elasticity of consumer demand for protein over the last century.

Whereas the microeconomic considerations of antimicrobial use in livestock are compelling from the perspective of the livestock producer as well as from the standpoint of past consumer behaviour, they are threatened by current consumer and activist group attitudes toward risk. Estimates of the financial impact on consumers of withdrawal of growth-promoting antimicrobial applications range from US$5 to US$10 per capita per annum[45] to possibly as high as US$40 per capita per annum.[46]

Environmental considerations are less striking than economic considerations. The increased demand for cropland as a result of decreased food efficiency without antibiotics could be met, in the USA, by an additional 2 million acres.[47] That is 0.6 standard deviations of the harvested acres over the past 11 growing seasons. It is hard to imagine that the environmental effects of such a change would be noticeable among the myriad other factors typically having greater impact on this industry. However, it can be argued that a ban on certain types of antibiotic use in animal agriculture, because of reduced feed efficiency would also increase the amount of animal waste per unit of animal product.

Pharmacodynamics of antibiotic use

The principal goal in the use of antimicrobial agents for the treatment of infections is eradication of the pathogen as quickly as possible with minimal adverse effects on the recipient. In order to accomplish this goal, three basic conditions must exist.[48] First, the antibiotic must bind to a specific target-binding site or 'active site' on the microorganism. Although the active sites are different for different classes of antibiotics, the principle is the same, namely to disrupt a point of biochemical reaction that the bacterium must undergo as part of its life cycle. If the biochemical reaction is critical to the life of the bacteria, then the antibiotic will have a deleterious effect on the life of the microorganism. The second condition is that the concentration of the antimicrobial is sufficient to occupy a critical number of these specific active sites on the microorganism. Finally, it is important that the agent occupies a sufficient number of active sites for an adequate period of time.

The relationship between the antibiotic concentration and the time that the concentration remains at these active sites, termed the area under the concentration–time curve (Cp × time = AUC), is important to the life and death of the bacteria.[49,50] Unfortunately, we do not know the concentration of antibiotics (AUC) at the active site of bacteria. The surrogate concentration (AUC) that is easily measured and commonly used is the blood AUC.[48,49] Although this is a good surrogate in the majority of situations, certain infections may require different body sites as more accurate surrogates.[48,49] For example, in the case of lung infections, the epithelial lining fluid (ELF) has been employed as a surrogate marker.[51] The appropriate marker for growth-promoting antibiotics is unknown.

Pharmacodynamics is simply the indexing of the total drug exposure in the serum or other body sites (AUC) to a measure of microbiological activity of the agent against the organism.[48,49,52] The measure of microbiological activity that is commonly used is the minimum inhibitory concentration (MIC). Therefore, the AUC/MIC is the fundamental pharmacodynamic parameter.[49,52] This parameter represents the degree to which the serum concentration and time exposure of the antimicrobial exceed the minimum needed to interfere with the bacterial life cycle. The higher the AUC/MIC ratio, the greater the probability of maximum eradication of the organism.[49] Resistance can occur as a result of using low doses, selecting organisms in a population that have higher MIC values.[53] As a result, the use of higher AUC/MIC ratios not only maximizes eradication but can also minimize the risk of selection of resistant organisms.

These basic pharmacodynamic principles can be applied to practices involving the use of antibiotics in animal food production.[54] As discussed above, there are four major practices in animal food production that involve the use of antibiotics: therapy, control, prevention/prophylaxis and growth promotion. It is necessary to determine for each use whether sufficient AUC/MIC ratios are obtained to achieve maximum effectiveness and prevent the development of resistance.

In the case of antimicrobial therapy for treatment of infections in animals, it is likely that doses will be appropriate, with adequate AUC/MIC concentrations. As a result, therapeutic antibiotic use should lead to maximum eradication and prevention of the emergence of resistant microorganisms because the antibiotic concentration is high relative to the MIC of the organism. This, however, might not be the case when antimicrobials are used to control/prevent infections or promote growth. In these situations, where the antimicrobial is introduced into the feed or water, factors such as the given dose of antibiotic as well as the quantity of feed and water consumed by the animal must be considered as a function of the AUC/MIC. Again, the important antibiotic concentration is that where the bacteria reside and it may not be the blood. If the AUC/MIC is not maximized, these practices may lead to the emergence of resistance.

For orally administered antibiotics, little work has been done identifying whether sufficient AUC/MIC ratios have been achieved in the animal's gut when these agents are used in animal food production. Complicating reasons include the number of animals needed for such studies, intestinal content that makes analysis more difficult, issues of dosing, duration of intake, site of sample acquisition, and differences in elimination for different animal species. Furthermore, the doses used must not cause toxicity in the animals. Finally, a withdrawal period (length of time needed to allow the antibiotic to be removed from edible tissue) is necessary and the impact of this on the development of resistant bacteria is not known. Considering the paucity of data related to the actual concentrations over time that the animal's gut flora is exposed to antibiotic, it is obvious that more work is needed before one can come to any scientific conclusion regarding the negative effect of the use of antibiotics in animal feed or water.

Unfortunately except for the data from a few studies, we are left only with general principles that indicate that low doses of antibiotic tend to select for bacterial resistance and high doses tend to kill the microorganism rapidly. We do know, however, that the low doses of antibiotics used for growth promotion continue to be effective, and that this includes the suppression of some infectious diseases (see above). It thus seems possible that AUC/MIC ratios might be adequate in the gut.

It is thus, inappropriate to conclude that the use of antibiotics in animal food production always results in the emergence of resistant bacteria. Those practices that target adequate exposures (AUC/MIC) of antimicrobials should continue, whereas those practices that might

produce low exposures should be investigated more rigorously. Sufficient data are not available to make a definitive conclusion about these issues.

Antibiotic use in humans and the problem of resistance

Antibiotics are widely used to treat and to prevent infection in humans. There are many guidelines for their rational use, and these have always considered the likelihood of the emergence of resistance as a parameter.[1] Such guidelines have been further developed as policies for antibiotic use within given communities, ranging from individual hospitals to whole nations. Most antibiotic prescription in developed nations is in the hands of community medical practitioners, of whom there is less control than is possible in hospitals. In some countries, it is still possible for a patient to buy potent antibiotics directly from the pharmacist without a medical prescription.

The antibiotics used in human medicine belong to the same general classes as those used in animals, and in many cases even if they are not exactly the same compounds their mode of action is the same. In most parts of the world, β-lactam agents (ranging from penicillin G to fourth-generation cephalosporins and carbapenems) play a major role, but sulphonamides (with or without trimethoprim), macrolides, lincosamides and streptogramins (the MLS group), fluoroquinolones, tetracyclines, aminoglycosides and glycopeptides are widely used, some mainly in the community and some mainly in hospitals.

With the range of antibiotics available, it is possible to treat infection with a high expectation of success. The benefits of use are clear both in the community and in hospitals, and failures of therapy are likely to be because of such factors as misdiagnosis (for example of viral respiratory infections, or exacerbations of chronic bronchitis not caused by bacteria) or serious underlying disease (as in the treatment of sepsis) or use when clinical experience shows it to be inappropriate (as in most gastrointestinal infections caused by salmonellae and campylobacters). There has been considerable emphasis on the avoidance of such pitfalls in the pursuit of rational and prudent antibiotic therapy.

This is not to say that resistance is not a clinical problem, but when it developed to the first antibiotics introduced, the pharmaceutical industry responded by producing semi-synthetic derivatives and a range of new compounds to deal with the problem. However, the flow of truly new agents slowed during the last two decades. This has clearly affected our ability to treat serious nosocomial infection caused by Gram-negative pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter* spp. and Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs), but more recently the focus has shifted to multiply-resistant Gram-positive pathogens such as *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci, pneumococci, enterococci and even viridans group streptococci, some of which cause common infections in the community outside hospitals.

There have until recently been few adequate international antibiotic resistance surveillance systems, and those that do exist have been driven by the interests of the pharmaceutical industry and are limited in scope.[55] Nonetheless, such systems as SENTRY, SMART, The Alexander Project and several others listed by Bax *et al.*,[55] have yielded valuable information on antibiotic resistance patterns in clinical isolates of resistant pathogens in different parts of the world.

The Danish National System, DANMAP, has now been reporting for 6 years, and has been unique in trying (with varying success) to bring together in coordinated reports, DANMAP 97, DANMAP 98, DANMAP 99, DANMAP 2000, DANMAP 2001 and DANMAP 2002 reliable data on the usage of antibiotics and on antibiotic resistance from human and veterinary medicine and food hygiene.[27,56–60] It is unfortunate that there have been no comparable systems in other countries of Europe since the Danish experience is clearly not representative of them all. In the USA, the National Antimicrobial Resistance Monitoring System (NARMS) is an attempt to do much the same kind of study as DANMAP, and is already yielding valuable data.[61–63] The CDC's FoodNet is another source of information on the prevalence and resistance of food-borne pathogens,[64] as are a variety of national systems that concentrate on the same area. Efforts are being made to coordinate the different national and international systems.[65]

Correlation between antibiotic use in animals and antibiotic resistance in humans

Much of the evidence relating to the potential for transfer of a resistance problem from animals to man comes from a consideration of the epidemiology of zoonoses, mainly salmonella and campylobacter infection, and of what have become known as 'indicator organisms'—enterococci and *Escherichia coli*, which cause no disease in animals (the animal-pathogenic *E. coli* are excluded) but can cause disease in man and which might be zoonotic. The epidemiology of these diseases is far from simple since there are many possible sources other than food animals and many routes of transmission other than food of animal origin (Figure 1).

The important antibiotic-resistant strains in this context are the multiply antibiotic-resistant salmonellae, macrolide- or fluoroquinolone-resistant campylobacters, glycopeptide- or streptogramin-resistant enterococci and multiply antibiotic-resistant *E. coli.* In all cases, the hypothesis is that the food chain is the main means of transmission. The hypothesis is intuitively attractive, and there can be no doubt of the existence of a hazard, but neither of these considerations means that the hypothesis is correct or of universal significance.

Emergence and disappearance of resistance in bacteria from food animals

When antibiotics are used in animals, resistance is likely to be selected in the normal and pathogenic intestinal flora (and in other colonized or infected body sites) and to increase in prevalence.[27,56–59] For example, in the USA, where virginiamycin is widely used as a growth promoter, resistance to streptogramins is common in animal *Enterococcus faecium*,[66] whereas avoparcin has not been used and appropriately mediated acquired resistance to glycopeptides is virtually non-existent in animal enterococci.[67–69] Resistance is equally likely to diminish in prevalence when antibiotic use is decreased or discontinued, since although individual strains may retain resistance genes,[70,71] they are often replaced by susceptible strains when the selective pressure is removed. There is now evidence that both of these phenomena have occurred in enterococci in Europe in relation to the use and discontinuation of use of growth-promoting antibiotics.[59,72–74] As is shown in Table 1, some 75% of *E. faecium* isolates from broiler chickens in Denmark were resistant to avoparcin (and thus also to vancomycin) and some 65% resistant to virginiamycin (and thus to quinupristin–dalfopristin). In addition, some 75% were resistant to avilamycin which has no current counterpart used in

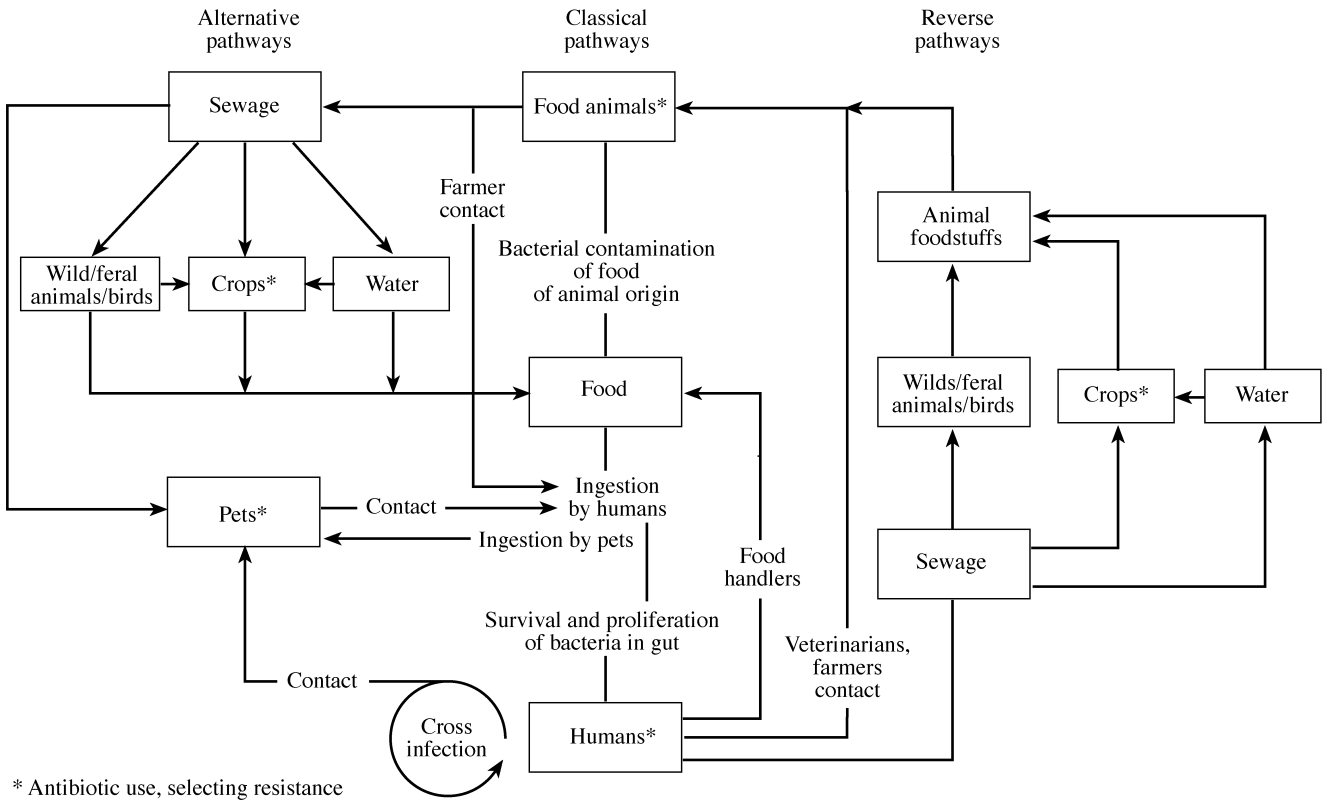

Figure 1. Some routes of transmission of antibiotic-susceptible or -resistant gastrointestinal pathogens or normal intestinal flora between animals and humans.

human medicine. In 2000, after the growth-promoter ban, the resistance rates were less than 5% for avoparcin and avilamycin, but remained at around 30% for virginiamycin.[59] There is evidence from the USA and from Norway that some resistance may persist long after the use of an antibiotic has been discontinued.[90,91] The persistence of virginiamycin resistance after its ban has been attributed to the use of penicillin selecting for associated resistance to virginiamycin,[59] but it has recently been suggested that the use of copper as a feed supplement might also co-select antibiotic resistance in *E. faecium*.[92] Such associated resistance is of general importance since the use of one antibacterial substance can select for resistance to another that is unrelated because the two resistance determinants are genetically linked on the same plasmid or transposon.

Transfer of resistant bacteria from animals to man by the food chain and other means

It is well known that antibiotic-resistant bacteria that have been selected in animals may contaminate meat derived from those animals and that such contamination also declines when the selecting antibiotics are not used: Table 1 gives examples. However, most of the studies of the food chain ignore the fact, already noted, that there are potential sources of resistant enterococci and Enterobacteriaceae other than farm animals given antibiotics (Figure 1). Humans themselves as well as other animals may be a source of resistant bacteria subsequently isolated from food animals, since commensals and pathogens (including resistant strains) can reach the general environment via sewage.[69] Wild animals, especially rodents, and birds, especially gulls, can acquire these environmental contaminants and pass them on via their excreta to grazing land or to the foodstuffs of food animals. VRE have been found in wild rodents[93,94] and in pet animals.[94] Vegetables may also be contaminated from sewage, especially in countries in which human faeces is used as a fertilizer. Multiply antibiotic-resistant *E. coli* strains were found to be widespread contaminants of market vegetables in London during the investigation of a community outbreak of *E. coli* O15 infection, although we failed to find the epidemic strain among them.[95,96] Fish farming involves the use of antibiotics (although this is diminishing in Europe), and fish as food may be contaminated with resistant bacteria.[59] Furthermore, antibiotics are widely used to prevent bacterial diseases in plants: tetracyclines and aminoglycosides are used to protect fruit trees from fire blight.[97] Streptogramin-resistant *E. faecium* have been isolated from bean sprouts from sources yet to be identified.[56,57] Genetic engineering in plants involves the use of a variety of antibiotics including vancomycin.[98] We are aware of no rigorous epidemiological studies of such potential reservoirs, and the assumption that they make negligible contributions to human enteric pathogen resistance is unfounded.

Animals that carry, or in certain cases are infected by, resistant organisms are a hazard to those who work with them since the organisms can be transferred by direct contact. This is the probable explanation of the rare but well publicized finding of indistinguishable glycopeptide-resistant enterococci—for example, in the faeces of a Dutch turkey farmer and his flock,[99] and of streptogramin-resistant *E. faecium* in the faeces of a Dutch chicken farmer and his chickens.[100] Even in these cases, we cannot exclude the possibility that both animals and humans acquired the strains from a common source, or even that the organisms were transferred from man to his animals.

Table 1. Use of growth-promoting and therapeutic antibiotics in animals and antibiotic susceptibility of enterococci from animal faeces, human faeces, animal-derived food, and human infection

	Use of antibiotics (tonnes) in animals in Denmark:		VRE (%) [reference] in:					Streptogramin-resistant *E. faecium* (%) [reference] in:				
						human clinical isolates					human clinical isolates	
	for growth promotion	for therapy	broiler faeces	broiler meat	human faeces	Europe	USA	broiler faeces	broiler meat	human faeces	Europe	USA
Pre-1994					12 [72,75][a]	3–4 [76][b]	0.3–8 [77]					
1994	116	90				14 [76][b]						
1995[d]			75			20 [76][b], 3.8 [76][b]		28				
1996	106	48	45	20		20 [76][b]		60	53	30 [73][c]	0 [78]	
1997			20	10	3.3 [75][a],4 [72][a]	22 [76][b]	18 [77]	65	58			0, 0.2 [78,79]
1998	49	57	10	9	1.4 [80][c]	24 [76][b]		60	50	14 [81][a]	0.3 [78]	0.9 [78]
1999[e]	12	62	10	12	5–6 [82] [c]	21 [76][b], 3.8 [83]		40	10	12 [73][c]		3.8 [78], 5 [84]
2000	0	81	8	2		3–4 [85,86]	37–60 [77,87,88]	35,58 [84]	15	1 [84]	1.8 [78]	
2001	0.01	94	5	5	11.3 [89]	19 [76][b]		30	5		16+[88]	

Data are derived from DANMAP[27,59] unless otherwise noted.
[a]Germany.
[b]England and Wales bacteraemia.
[c]Netherlands.
[d]Year of avoparcin ban in Denmark.
[e]Year of ban of avoparcin, bacitracin, spiramycin, tylosin and virginiamycin in whole of EU.

The recent description of an outbreak in China of virulent but not antibiotic-resistant *E. faecium* infection in pigs and those in close contact with them seems too unusual for us to learn much about the epidemiology of 'normal' enterococci.[101] Isolates of enterococci from human and animal faeces that have no evidence of close conventional epidemiological links are often different on molecular testing, depending on the sensitivity of the method used, although in these studies, indistinguishable strains have sometimes been found among human and animal faecal enterococci.[102–106] Recent work from Bruinsma *et al.*[82] suggests that whereas human and pig faecal isolates of *E. faecium* have genetic similarities, those from poultry faeces are different. Others have not found such similarities,[81] and clearly more work needs to be done.

It is generally accepted that adequate cooking destroys bacteria in food. No evidence indicates that antibiotic-resistant strains are more refractory to cooking than are the largely susceptible strains on which the original research was conducted. Although most of the work was done on salmonellae, we are aware of no specific investigation of antibiotic-resistant campylobacters or the 'indicator organisms' *E. coli* and enterococci. We must also assume that as with salmonellae, inadequate cooking fails to decontaminate food. We also know that salmonella cross-contamination between uncooked and cooked food may occur if hygiene measures are inadequate in food outlets, and it may be that such cross-contamination occurs with other bacteria as well, including resistant strains, but again there is no direct information. We know nothing of the degree, if any, of contamination of food on the plate just before its ingestion, by any of these organisms.

There is experimental evidence for host-species specificity among enterococci: ingestion of heavy inocula of strains from humans by animals[107] or of animal strains by humans[108] does not result in their permanent establishment. In the experiment of Sørensen *et al.*,[108] ingestion of pig or chicken strains resulted in their excretion for a very limited period of time: in only one experimental subject out of 12 was the same organism detected at 15 days after ingestion but in none thereafter. As already noted, enterococci from chickens do not closely resemble those in human faeces, although those from pigs may have similar molecular characteristics to those from humans,[82] but this does not mean that humans acquire their faecal enterococci from pigs. However, on the basis of analyses of *vanX* variants on Tn*1546* in *E. faecium* from chickens and pigs and humans, Jensen *et al.*[109] argue that spread is indeed from animals to man and not vice versa. The frequency of inter-host-species spread of faecal enterococci remains unknown.

The same host–animal specificity appears to apply to *E. coli*: van den Bogaard *et al.*[110] give a good account of the history of the disagreement as to whether or not resistant *E. coli* from animals colonize and infect humans. In a study carried out by Parsonnet & Kass,[111] women working in a chicken abattoir, when they developed urinary tract infections (UTI), rarely yielded isolates that resembled (in terms of antibiotic resistance patterns) those from the chicken carcasses unless the woman developing UTI had been treated with antibiotics. A recent study from the Netherlands reported that among three poultry and five farmer/slaughterer populations, the PFGE patterns of ciprofloxacin-resistant *E. coli* in the faecal flora were 'quite heterogeneous', but three farmers each had a faecal isolate of *E. coli* with PFGE patterns that were indistinguishable from those of some of the poultry isolates.[110] As with enterococci in farmers and their animals, it seems likely that transmission was not via animal-derived food.

Zoonoses such as salmonella and campylobacter infection, undoubtedly can reach humans via the food chain, but their immediate source may not be the animal faecal flora. In each case, reports of infection traced from a farm to a human non-epidemic infection are uncommon. Furthermore, campylobacter strains from chickens, their commonly assumed source for humans, are often genetically different from strains isolated from humans (see *Campylobacter* below).

The evidence that 'indicator' bacteria reach and persist in the human faecal flora via the food chain is increasingly contradictory. Although it may seem highly plausible that the VRE or streptogramin-resistant *E. faecium* found in animal faeces, on meat derived from them and in human faeces in non-hospitalized patients (the prevalence varying widely in part because of differences in microbiological technique) are the same,[112] the fact is that isolates from human faeces are usually different from those in animals (except occasionally in the case of the farmers mentioned above) and on food.[99,100] Even when those who report studies claim that all these enterococci belong to the same pool of organisms, there is evidence of segregation in their results, although some authors have not commented on this.[81] As already noted, a recent study shows that chicken enterococci do indeed belong to a different pool from those of humans and pigs.[82] Thus, in the absence of adequate conventional and molecular epidemiological studies, we are aware of no evidence of the extent to which resistant enterococci or *E. coli* from food animals are able to colonize the human intestinal tract.

Gene transfer

The ultimate defence of those who support the farm-to-clinic hypothesis is that provided animal organisms reach the human faeces, they need to survive only for brief periods to pass on their antibiotic-resistance genes to resident organisms. There is absolutely no doubt that transfer of resistance genes can occur, and countless *in vitro* experiments have characterized the event in endless variety, including among selected but by no means all strains of enterococci,[113] a phenomenon that may also be demonstrated experimentally in the germ-free animal gut.[114] However, there have been no observations to determine its frequency under natural conditions—or even if it occurs at all in the normal human gut with the 'indicator organisms' from animal sources. The clearest cases of *in vivo* natural transfer have involved gut pathogens such as salmonella and shigella, *E. coli,* and other Enterobacteriaceae. The transfer of vancomycin resistance from VRE to *Staphylococcus aureus* under experimental conditions a decade ago[115] has to date been reported to occur only twice in nature, in the USA, related to intensive vancomycin use in humans—the single case of *S. aureus* with VanA that was presumably acquired from a vancomycin-resistant *E. faecalis* strain from the same patient, recently reported,[116] and a second case of a similar nature.[117] However, it is without doubt true that although some genetic elements, such as the transposon Tn*1546*, are heterogeneous both in animal and human faecal enterococci, indistinguishable variants may be found. For example, Jensen found two variants of the *vanX* gene, T and G, in human faecal vancomycin-resistant *E. faecium*, but only T in pigs and G in poultry.[118] On this basis, they concluded that spread from animals to humans was the likely explanation. Jensen *et al.*[119] later reported that six human isolates (one of them from an infected patient) carried Tn*1546* variants that were indistinguishable from those in common pig isolates. In the UK, Woodford *et al.*[120] found 10 variants of Tn*1546* in human isolates, eight only in animals but six in both. We agree with them that 'non-human sources cannot be excluded as a reservoir'. However animal strains are not the only potential source of resistance since other species with the genes responsible for the VanA phenotype have been found, including

some in the normal intestinal flora,[121] but it cannot be assumed that the genes have passed from these organisms to enterococci rather than vice versa. It is a matter of great regret that molecular characterization of resistance genes has been allowed to relegate good 'shoe-leather' epidemiology in these cases. The simple (and it is now simple) demonstration that two genes are indistinguishable, or even truly identical, tells us nothing of the source of infection or its route of transmission or the dynamics of carriage without a study of temporal and spatial relationships. In many reported studies, such considerations are totally absent.

The truth about gene transfer from animal isolates of indicator organisms to human isolates in the human intestine (or even in other relevant sites) thus remains beyond our grasp. The results of the Danish ingestion experiment in which no human faecal isolates were other than the animal strains swallowed by the experimental subjects, and in which no permanent carriage was demonstrated, suggest that it is not a common event *in vivo*.[108]

Evidence of animal origin of strains colonizing or infecting humans

The case for or against the animal origin of strains of resistant bacteria colonizing or infecting humans depends on a full analysis of each antibiotic and bacterial species involved—clearly an impossible task in a paper such as this. However, we can illustrate the range of possibilities.

Salmonellae: Human infection with salmonellae is common but generally declining in incidence in Europe:[122] documented infection occurred at a rate of 54.5 cases per 100 000 inhabitants in Denmark in 2001, and it increased in prevalence during that year.[59] In the USA, the incidence of documented infection was 15.1 per 100 000 inhabitants and declined by some 15% between 1996 and 2001.[123] The major pathogens are *Salmonella* Enteritidis and *Salmonella* Typhimurium, the first accounting for half of the cases and the second for 20% in Denmark in 2001,[59] whereas in the USA, the prevalence of these two serovars is more nearly equal. Among 1332 *Salmonella* isolates typed in the NARMS in 2000, 24% were *Salmonella* Enteritidis and 23% *Salmonella* Typhimurium,[61] whereas in the CDC National Surveillance System involving 25 878 human isolates, 22% were *Salmonella* Typhimurium and 19% *Salmonella* Enteritidis.[124]

Despite efforts to control them, salmonellae, including resistant strains, have still been common in animal-derived foods: a recent study in the United States reported that 20% of samples of ground meats yielded salmonellae,[125] whereas others have found salmonellae in chicken, turkey, pork, beef and shellfish.[126,127] *Salmonella* Enteritidis PT4 has been particularly associated with eggs.[128,129] Hancock *et al.*[130] have recently reviewed the multifaceted epidemiology of *Salmonella* Typhimurium DT104.

In general, when the appropriate studies have been carried out—as in Denmark—the resistance patterns of animal, food and human strains are similar, especially when imported strains, which are sometimes more resistant, are taken into account.[59]

Clearly, resistance may be selected in salmonellae in animals given antibiotics,[131–133] but this does not necessarily mean that the resistance arose in animals (Figure 1).

Salmonellosis, an undoubted zoonosis, is far from simple epidemiologically and microbiologically, but sophisticated methods of phenotyping and genotyping make it possible to conduct particularly accurate epidemiological studies. Although an animal origin is likely or can be proved for many outbreaks of infection, in which genotypically and phenotypically indistinguishable salmonellae are found in animals and in patients or carriers,[134] the route by which an infection can reach an individual is complex. The simple hypotheses that raw animal products are the principal source of human salmonellosis, that the risk of transmission to humans is equal for all food products, and that all *Salmonella* serotypes have an equal ability to cause human illness, are not sustained by mathematically modelled predictions of serotype distribution.[135] Direct transfer is possible, not only from farm animals in contact with farmers or veterinarians but also from domestic animals and pets in variety,[136–138] and—as with *Salmonella* Typhi—from one human being to another, especially when hygiene measures are inadequate. Human to human transfer is the rule in some tropical and other contexts, such as nursing homes.[139] Furthermore, salmonellae can persist in biofilms in the domestic toilets of those who have gastroenteritis[140] and in the more general environment of infected children.[141] In a study in Ohio, salmonellae were commonly present in human sewage sludge applied to farmland, and on the basis of serological evidence, may have infected humans living in the vicinity.[142] Similar salmonella contamination of sewage, feral animals and chickens in a nearby flock was found in southern California.[143] Indirect transfer via food not only arises from primarily contaminated food but also from cross-contaminated food and from food contaminated by food-handler carriers. Thus even in an undoubted zoonosis, the immediate origin in an outbreak or in a sporadic infection can be remote from any food-animal source.

It is neither necessary nor sufficient for an epidemiologically successful salmonella to be antibiotic-resistant, although they may have an advantage when antibiotics to which they are resistant are being used for other purposes.[144] In Denmark, among human isolates, normally antibiotic-susceptible *Salmonella* Enteritidis is 2.5-fold more common than *Salmonella* Typhimurium, which is often multiply resistant to agents such as ampicillin/amoxicillin, tetracycline, sulphonamides and aminoglycosides.[59] In the USA, *Salmonella* Typhimurium, often multiply antibiotic-resistant, is no more common than the usually susceptible *Salmonella* Enteritidis.[61] Since different types of *Salmonella* Typhimurium often behave as epidemic pathogens—variants such as DT104 come and go—the resistance prevalence varies from time to time and place to place with no obvious relationship to current antibiotic usage patterns in humans or animals.[145] On the other hand, although genetic analyses of salmonellae with reduced susceptibility to fluoroquinolones show some degree of clonality, resistance in most isolates appears to have resulted from *de novo* mutations.[146]

It might be thought that antibiotic-resistant salmonellae would have a devastating clinical effect, but this is rarely the case in developed countries.[147,148] In most cases of salmonella infection, the organism is confined to the gut and antibiotics are thought by many to be contraindicated since they can do little good and potentially considerable harm. In a minority of cases, the patient suffers from systemic infection, for which antibiotic therapy *is* indicated. In a recent international study of bacteraemia isolates, salmonellae were 13th in frequency and accounted for only 0.4% of bacteraemia episodes in the USA.[149] Furthermore, resistance rates to fluoroquinolones and ceftriaxone among blood isolates were less than 1%. Many patients with systemic infection have underlying diseases, and a fatal outcome may occur whether the causative organism is resistant or not. However, some recent preliminary reports document increased morbidity or mortality associated with antibiotic resistance in salmonellosis,[150–152] but Travers & Barza[153] conclude that this 'probably reflects a somewhat higher virulence of the (resistant) infecting organism'.

Growth-promoting antibiotics with a predominantly Gram-positive spectrum, have little, if any, effect on the antibiotic resistance of the salmonellae, and thus on human infection caused by them. However, some of the antibiotics commonly used to prevent or treat disease in animals, and used for growth promotion in some parts of the world, could be expected to have an adverse effect, especially when associated resistance is taken into account, since the very same antibiotics are used in human therapy. On the other hand, Piddock[154] has recently concluded that 'clear evidence that antibiotic-resistant bacteria from animals caused human infections which were difficult to treat, is extremely difficult to find' and that 'it is not widely accepted that quinolone-resistant strains (of *Salmonella* Typhimurium DT104) are transmitted through the food chain'.

Campylobacter: Thermophilic *Campylobacter* spp., mostly *Campylobacter jejuni* but less often *Campylobacter coli* are among the commonest causes of gastroenteritis in developed countries. In Denmark there were 86.4 documented cases per 100 000 inhabitants in 2001, and the incidence is increasing,[59,155,156] as also in many other countries in Europe,[157] whereas in the USA there were only 13.8 documented cases per 100 000 inhabitants in 2001, a decrease of 27% since 1996.[123] Phenotyping and genotyping methods have been developed more recently than for salmonellae and knowledge of the epidemiology of these organisms is still developing. Farm animals and companion animals commonly carry campylobacters, and chicken and turkey meat is commonly contaminated when it reaches retail outlets—34% of raw chicken and 22% of raw turkey samples in a recent Danish study,[59] and similar contamination is reported in the USA.[126]

It is easy to assume that chicken meat particularly is the most important source of human campylobacter infection,[158] and the evidence seems strong in a recent report of efforts to control campylobacter infection in Iceland.[159] However, the simultaneous introduction of a variety of control measures and the interplay of unexplained variations in campylobacter load in food with variations in the incidence of campylobacter infection call for caution, especially since investigations continue there. Case–control studies that fail to consider alternative hypotheses frequently find chicken consumption to be a major risk factor.[160] Furthermore, many past studies have used strong parametric modelling assumptions in which the modeller's choice of variables can strongly affect findings. Most early studies that defined chicken as a risk factor did not consider restaurant dining and commercial food preparation as an explanation or as a confounder. In relatively large, well designed recent case–control studies,[161–164] it has become clear that chicken prepared and eaten at home has a statistically negative association with campylobacter risk, whereas chicken and other meats eaten in restaurants are risk factors. Once venue is taken into account, chicken is no longer a risk factor (Tables 2 and 3).[165,166] The usefulness of typing, including genotyping, as an aid to the understanding of epidemiology, depends on its having an appropriate discriminating power. The case of *C. jejuni* is further complicated by the plasticity of certain types. Most investigators report some overlap, varying widely in extent, in types between isolates from chickens and from patients (see Smith *et al.*[158] and Piddock[167] for references) leading them to the conclusion that chicken is the main source of human campylobacteriosis. In the absence of full epidemiological investigations, such a conclusion cannot be valid. Even if types are identical, they could have been acquired by both from a third unidentified source. We commend the cautious conclusions of Hänninen *et al.*[168] in their paper on campylobacter types in Helsinki.

Table 2. International evidence on protective factors for *C. jejuni* illness

Protective factor	Odds ratio	Country
Eating chicken	<1	USA
Eating chicken at home	0.36	New Zealand
Whole chicken	0.59	New Zealand
Chicken prepared at home	0.67	New Zealand
Baked/roasted chicken	0.75	New Zealand
Chicken purchased frozen	0.61	New Zealand
Chicken leg	0.55	Denmark
Preparing main meals	0.9	UK
Handling raw chicken	0.41	UK

Based on Neimann[165] and Engberg *et al.*[166]

Table 3. International evidence on chicken and human *C. jejuni* risk

Findings	Country	Reference
'Risk of campylobacteriosis was strongly associated with recent consumption of raw or undercooked chicken (matched odds ratio 4.52, 95% confidence intervals 2.88, 7.10). There was also an increased risk with chicken eaten in restaurants (matched odds ratio 3.85; 2.52, 5.88)'	New Zealand	Eberhart-Phillips *et al.*[161]
'Recent consumption of baked or roasted chicken seemed to be protective'	New Zealand	Eberhart-Phillips *et al.*[161]
'Handling any whole chicken in the domestic kitchen that had been bought raw with giblets [was] significantly associated with a decrease in the risk of becoming ill with campylobacter'	England	Adaak *et al.*[162]
'Eating any dish cooked from chicken of this type in the home (OR 0.41–0.44; CI 0.24, 0.79) [was] significantly associated with a decrease in the risk of becoming ill with campylobacter'	England	Adaak *et al.*[162]
'Eating poultry at a friend's house (OR = 3.18, CI 1.0, 10.73, $P = 0.03$), at a barbecue (OR = 3.00, CI 0.99, 9.34, $P = 0.03$) or eating undercooked chicken (OR = 4.94, CI 1.03, 23.62, $P = 0.05$) was a risk [for CP illness]'	New Zealand	Ikram *et al.*[163]
'Eating at home was protective (OR = 0.36, CI 0.14, 0.9, $P = 0.02$)'	New Zealand	Ikram *et al.*[163]

Antibiotic resistance is increasingly common in *C. jejuni* (and even more so in *C. coli*). In human cases of infection occurring in Denmark in 2001, 25% of *C. jejuni* isolates were resistant to tetracycline, 21% to ciprofloxacin and 7% to erythromycin, and resistance rates were even higher, except in the case of erythromycin, in the relatively few cases studied that had been acquired abroad (33%, 53%, 0%).[59] Isolates from chicken meat tended to be more antibiotic susceptible (2%, 13% and 2%, resistance, respectively, and 8%, 0%, 0% if imported), and isolates from chickens at slaughter even more so (1%, 6%, 0%). Such a differential in susceptibility gives further support to the view that chicken is not the major source of campylobacter infections in humans.[169] In another study of *Campylobacter* spp. isolated from human infections in 2001, resistance rates were 23.5% for ciprofloxacin and erythromycin in Europe, whereas they were 9.1% and 1.5%, respectively in the USA, another example of a major difference between the two areas.[170]

It has been suggested that the use of fluoroquinolones for the treatment and prevention of disease in chickens (the fluoroquinolones have not been used for growth promotion) is responsible for resistance in human isolates.[171–173] Engberg *et al*.[166] continue to insist that 'fresh raw meat, especially poultry, is a major source of infection', and despite doubts in relation to the complex chain of transmission conclude that resistance in isolates from humans can be related to the exposure of animal strains to antibiotics used in farming. However, there are conflicting findings. Resistance commonly emerges when campylobacter infection is treated in humans (for references, see Piddock[167]). There are no baseline figures in the USA for resistance rates in animal isolates before the introduction of ciprofloxacin in human medicine in 1988. Enrofloxacin was not introduced for animal therapy until 1995, by which time 21% of human isolates in one Pennsylvania study were resistant to ciprofloxacin, none having been resistant between 1982 and 1992,[174] and by 2001, 40% of human isolates were resistant to fluoroquinolones in this study. Furthermore, fluoroquinolone resistance has been encountered in human isolates in countries in which fluoroquinolones are not approved for use in food animals, such as Sweden,[175] Finland[176] and Canada.[177] Finally, it has been observed in Sweden that animal isolates may be fluoroquinolone-resistant in the absence of animal use of the fluoroquinolones.[178]

The case for erythromycin resistance being selected in animals is even more difficult to assess since macrolides have been used for therapy and growth promotion in animals and in human therapy over decades. Macrolide use in human medicine is generally increasing since the realization that many pathogens in community-acquired respiratory tract infection are unlikely to respond to β-lactam drugs because of intrinsic or acquired resistance. It seems possible that the pressures arising from the use of macrolides in human medicine, driving resistance in purely human pathogens, notably *Streptococcus pneumoniae* and *Streptococcus pyogenes*, and in the normal flora[179] might also affect campylobacters.

Human campylobacter infection is usually confined to the intestine, and antibiotic therapy is usually not needed. Systemic infection and campylobacter dysentery in children are very uncommon but do require antibiotic therapy. However, there is no reliable evidence to suggest that erythromycin resistance is associated with higher failure rates. Piddock[167,180] has commented that patients infected with fluoroquinolone-resistant strains often appear to respond to treatment with fluoroquinolones. More recently, Marano *et al*.[181] reported a 4 day decrease in the duration of diarrhoea (from 12 to 8 days) for patients infected with fluoroquinolone-resistant strains treated with ciprofloxacin (but paradoxically no decrease for susceptible strains—6 days for both treated and untreated patients). Travers & Barza[153] have commented on the apparent difference in virulence between susceptible and resistant strains. As with salmonellosis, although the hazard is obvious, the risk to human health from campylobacters that might have acquired their resistance in animals is probably very small.

All these considerations suggest to us that the banning of growth promoters, including macrolides, is likely to have little effect on resistance in campylobacters from humans, and no effect on human medicine. The fluoroquinolones used therapeutically in animals also appear to pose little threat to human health.

Enterococci: The case against growth-promoting antibiotics has relied very heavily on antibiotic-resistant enterococci. Various species form part of the normal faecal flora of animals and man, but *Enterococcus faecalis* and *Enterococcus faecium* are responsible for most human infections. Historically, enterococci caused a minority of urinary tract infections, and there were signs of an increase in prevalence, particularly of *E. faecium* before acquired resistance became an issue. Enterococci have also long been known as a cause of endocarditis in pregnant women and elderly men. More recently, enterococci have increasingly been isolated from vulnerable patients in intensive care, renal and oncology units, often associated with intravascular catheters, and it is largely in relation to such infections that acquired resistance has become a problem. Cross-infection with strains of normal susceptibility is not uncommon.[182] For *E. faecalis*, ampicillin/amoxicillin remains active against most isolates, but high-level aminoglycoside resistance, the incidence of which has increased over the past decades,[183] and which reverses the normal synergy between penicillins and aminoglycosides, has diverted therapy to the glycopeptides vancomycin and teicoplanin. *E. faecium* from human sources is almost always resistant to the β-lactam antibiotics,[184] and vancomycin has become the drug of choice for serious infections. This shift to vancomycin therapy, which has been particularly marked in the USA,[185,186] has added to its existing increased usage for pseudomembranous colitis and methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Not unsurprisingly vancomycin-resistant enterococci (VRE) have become prevalent as agents of human infection (Table 1), particularly in the USA, increasing in prevalence from 0.3% in 1989 to 7.9% by 1993, 17.7% in 1997, and up to 50% in some studies in 1998 and 1999.[77,78] In contrast, in Europe, in a multicentre study of clinical isolates in 1999–2000, VRE accounted for only 0.6% of *E. faecalis* and 3% of *E. faecium* isolates overall—5.9% in the UK, 3.9% in Italy, 3% in Austria, 2.5% in Ireland, 0.7% in Germany but none in Belgium, Denmark, France, Iceland, The Netherlands, Spain, Sweden, or Switzerland.[88] Other studies have confirmed this variability and general rarity, adding Greece, Israel and Portugal to the higher prevalence areas, but overall results indicate that the incidence may be increasing.[85,86,187]

VRE, first described as causing a clinical problem in the UK,[188] was thus confidently associated with high human use of glycopeptides until it was noted that, as a result of the use of the glycopeptide avoparcin as a growth promoter, food-animal faecal enterococci were also often glycopeptide-resistant. When it was found that such enterococci contaminated meat in retail outlets[189] and that the faecal flora of humans in countries with a heavy use of avoparcin also often contained VRE,[56,190] it was quickly concluded by many that the case against growth-promoting antibiotics was proved.[191,192] However, as discussed above, it is now realized that there are many potential alternative sources. In specific relation to enterococci, animal feedstuffs may be contaminated with enterococci,[193] and contamination

of vegetables with enterococci,[56] and the fact that pets, wild rodents and badgers can carry VRE,[94,194,195] suggested that primary sources other than food animals might be involved. Vancomycin is among the numerous antibiotics used in plant tissue culture.[98] Attempts to demonstrate that vegetarians do not carry VRE have produced conflicting results. Shouten *et al.*[196] examined the faeces of 42 vegetarians and 62 meat eaters, finding no VRE in the first group but six in the second. van den Braak *et al*.[197] have pointed out that the investigations were confined to two homes for the elderly, and that cross-contamination in the meat-eating home was not excluded. They therefore examined faeces from the wider community in the Netherlands and found one isolate of *E. faecium* in meat eaters and none in vegetarians (although both had other resistant enterococcal species), concluding that there was no significant difference between the groups.[197] It is of great importance that, from the experience in the USA where glycopeptides were not used for growth promotion, it seems very probable that heavy human glycopeptide use alone can give rise to a major problem. In the NNIS survey of ICU patients in the USA, a quarter of all clinical enterococcal isolates were VRE, an increase of 43% over the mean for the previous 5 years.[198] In Europe, where glycopeptide use in humans is much less, and where VRE infection is generally uncommon,[75] the use of avoparcin in animals was held to be the source of the problem,[199] although when molecular typing methods were applied some doubts emerged.[200] The realization that VRE infections are largely confined to clinical units in which glycopeptides are heavily used, such as renal,[188] oncology or liver transplant units,[201,202] suggested that such usage in humans might be the driving factor, alongside cross-infection. Recent results from the European Antimicrobial Resistance Surveillance System (EARSS) show that countries in Europe with a higher incidence of infection with MRSA, and who presumably therefore use more glycopeptides for their treatment, have the highest incidence of VRE infection.[85] Several northern European countries, which have little MRSA have no VRE (Figure 2).[85]

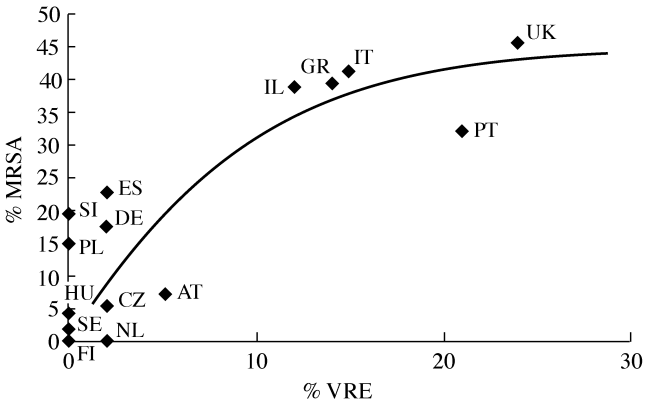

Figure 2. Prevalence of MRSA and VRE (*E. faecium*) in Europe based on EARSS report 2001.[85] Those countries that reported on the susceptibility of <10 isolates of *E. faecium* have been excluded. The UK figure for VRE is based on information from the UK ARMRL Newsletter (June 1999). AT, Austria; CZ, Czech Republic; DE, Germany; ES, Spain; FI, Finland; GR, Greece; HU, Hungary; IL, Israel; IT, Italy; NL, the Netherlands; PL, Poland; PT, Portugal; SE, Sweden; SI, Slovenia; UK, United Kingdom.

The case for the animal origin of resistant enterococci was further strengthened when a new streptogramin, quinupristin–dalfopristin was introduced into human medicine, specifically for the treatment of infection caused by resistant Gram-positive organisms, including *E. faecium,* but not *E. faecalis*, which is intrinsically resistant. During the early clinical development of the drug it was noted that *E. faecium* from documented human infection was universally susceptible to quinupristin–dalfopristin,[203,204] which was hailed as a unique hope for the treatment of VRE and other resistant Gram-positive organisms. However, it was soon noted that another streptogramin, virginiamycin, had been used for more than 30 years as a growth promoter, and was once again associated with a high prevalence of resistance in food-animal and food isolates of *E. faecium*,[56–58,84] and was associated with similar resistance genes in animal and some human isolates.[100] This streptogramin resistance in *E. faecium* clearly constituted a further theoretical hazard to human health, and many translated this into a real risk without further assessment. It was such considerations, and the application of the 'precautionary principle', that led the EU to ban the growth-promoting antibiotics avoparcin, virginiamycin, tylosin and spiramycin (macrolides also used therapeutically in animals) and bacitracin, ignoring the advice of their Scientific Advisory Committee (SCAN).[9,10]

A large number of factors militate against the assumption that the risk to humans arising from resistant animal enterococci is real.

(a) The intensive use of avoparcin and virginiamycin as growth promoters over a 30 year period, although associated with human faecal carriage of VRE and streptogramin-resistant *E. faecium*, had not resulted in a general clinical problem. Clinical isolates of VRE have been rare in most European countries:[75,83,205,207] the exception has been the UK,[208] where more than 20% of *E. faecium* blood isolates submitted to the Central Public Health Laboratory between 1995 and 2001 were VRE.[76] In the whole series of DANMAP reports, we have been able to find only one mention of VRE among human clinical isolates in Denmark—a single case reported in a special study of strains submitted to a reference centre in 1996[56]—despite the widespread use of avoparcin in animals in that country! The claim of Bruinsma *et al.*,[82] 'suggesting' that transmission of genetically related VRE 'can occur' and 'may' contribute to colonization and subsequent infection in humans is a classic case of the failure to use the words 'can', 'may' and 'might' appropriately. Streptogramin resistance among human clinical isolates of *E.* faecium has been rare until the introduction of quinupristin–dalfopristin into human practice (Table 1).

(b) In addition to the use of virginiamycin in animals, another streptogramin, pristinamycin, had been used for therapy in humans in francophone countries for many years without acquired resistance becoming a problem in the target pathogens (mostly staphylococci and streptococci),[209] or in enterococci.

(c) Resistance in *E. faecium* was soon shown to be selected in individual patients treated with quinupristin–dalfopristin, sometimes associated with clinical failure.[210–212] Superinfection was excluded, but in any case, MICs of non-susceptible human clinical isolates were invariably low (2–4 mg/L), unlike the isolates from animal and human faeces that produced streptogramin acetyltransferase.

(d) Successive surveillance studies associated with the SENTRY and SMART programmes reported an increasing prevalence of streptogramin resistance in clinical isolates of *E. faecium* in parts of the world in which quinupristin–dalfopristin was used for patients.[78,203,213] Such resistance was also associated with borderline MICs and an absence of the genetic mechanisms found in most animal strains,[78] and was thus less likely to be the result of selection of superinfecting strains of animal origin. During the period 1997–99, although 134/821 isolates of *E. faecium* collected from all parts of the world in the SENTRY programme were non-susceptible to quinu-

pristin–dalfopristin, only 1.8% of isolates had MICs of 8 mg/L or more.[78]

(e) Clinical isolates of vancomycin- or quinupristin–dalfopristin-resistant enterococci appear to be becoming more prevalent in Europe at a time when animal isolates as well as human faecal isolates in the community are becoming less prevalent following the growth-promoter ban (Table 1).

(f) A very important consideration is the degree of host-animal specificity among enterococci. Under experimental conditions, it has proved impossible to establish animal strains in humans who have swallowed large inocula of VRE[108] and conversely to establish strains of human origin in animals,[107] although, in experiments, animal strains may pass through the human intestine, multiply to a limited extent, and exceptionally be excreted for periods of up to 2 weeks.[108] The genotyping results of Willems *et al.*[214] provide striking support for the concept of host specificity for *E. faecium*. Using amplified length polymorphism analysis, they found that clinical isolates from hospitalized patients from several European centres resembled those from cats and dogs and some veal calves (subtype C), but, to their surprise, differed from faecal isolates from non-hospitalized human subjects, whose isolates resembled those of pigs (subtype A). Turkeys and chickens and their farmers had subtype B whereas most veal calves and their farmers had subtype D.

(g) Animal isolates of *E. faecium* often show differences in antibiotic resistance patterns from those of isolates from humans:[215,216] for example, they are usually as susceptible to ampicillin/amoxicillin as are *E. faecalis* isolates,[59,217] whereas human isolates of *E. faecium* are usually resistant.[183] Misidentification of enterococcal isolates is not uncommon and may blur this distinction.

(h) Epidemic strains of vancomycin-resistant *E. faecium* from the USA, Europe and Australia very often have an *esp* virulence gene variant[218] as well as a hyaluronidase gene[219] not found in non-epidemic or animal isolates.[218] Woodford *et al.*[220] found the *esp* gene in 61% of vancomycin-resistant *E. faecium* and 64% of vancomycin-susceptible *E. faecium* in a collection of mostly clinical isolates from the UK, but in no isolates from food or sewage. It has been suggested that virulent but antibiotic-susceptible strains might acquire resistance genes to become epidemic VRE.[221] However, it has also been suggested that avirulent resistant enterococci of animal origin might be protecting against the establishment of such strains in Europe.[219]

(i) In Australia, VanB is the predominant phenotype in resistant *E. faecium* from human cases, but has not been found in animals nor is it frequent in normal human faeces.[222] However, indistinguishable VanB elements have been found in anaerobic commensal bacteria in human faeces, and it is suggested that this might be the source of VanB resistance in enterococci.[223]

(j) Molecular genetic studies show that animal and human faecal strains usually segregate to a considerable degree in relationship studies[109,224–228] despite the claim of some authors that this is not the case. For example, in the study of Werner *et al.*,[81] some 80% of human isolates segregate in one-half of the tree. Bruinsma *et al.*[82] have recently shown that enterococci from chickens, whether VRE or not, form a genetically distinct group, rarely encountered in humans, whereas isolates from pigs and healthy humans belong to the same genetically diverse group. They believe that their results 'suggest that pigs are a more important VREF source for humans', although they have conducted no conventional epidemiological studies. To this may be added the rarity of reports of indistinguishable isolates from turkeys or chickens and their farmers alluded to above.

It is still possible that animal strains passing transiently through the human gut might transfer their resistance to human strains. Resistance transfer is clearly possible between selected strains *in vitro*[113] and in an animal model,[114] but it was not detected in the only experiment that might have detected it—the ingestion study of Sørensen *et al.*[108] Bruinsma *et al.*[82] argue that the occasional finding of indistinguishable Tn*1546* transposons suggests horizontal spread from animals to man, but did no studies of temporal or spatial relationships. Resistance transfer might also be the explanation of the finding of VRE in a wound resulting from an accident with a fork-lift truck in a chicken-processing plant.[229] Unfortunately, it seems that no one has yet reported or attempted experiments specifically designed to elucidate the matter, although we believe that such experiments are feasible.

Finally, we observe that a number of antibiotics with activity against resistant enterococci are under development or have been recently introduced. These include linezolid and other oxazolidinones, daptomycin, oritavancin and new classes such as peptide dehydrogenase inhibitors. It is thus no longer possible to invoke the 'antibiotic of last resort' argument in relation to quinupristin–dalfopristin.

On the basis of these considerations, we believe, along with many others,[9,10,230–233] that there is little or no evidence that resistant enterococci from animals are a risk to human health, and that a ban of growth promoting antibiotics was not justified on this basis, and will have no impact on the prevalence of VRE in human infections.

Escherichia coli: *E. coli* is a species with many serotypes found in the intestine of many animals including humans. Some of these serotypes have particular pathogenicity for man, including O1, which may cause meningitis in infants, O157:H7 and some other types, which cause haemolytic–uraemic syndrome, a variety of serotypes causing gastroenteritis in children or travellers, and a further group associated with urinary tract infection. Other serotypes are associated with gastrointestinal disease in animals, including O2 and O78 in poultry, F5 from calves, and O149 from pigs. In most cases, the genetic determinants for an array of virulence factors have now been identified. Despite this depth of knowledge, little is known of the epidemiology of these organisms, and only one of them is a recognized zoonotic infection in man—O157:H7, which originates in cattle and contaminates beef. 'Non-pathogenic' strains of *E. coli* contaminate foods of animal origin. It is assumed that at least some of the strains colonizing the human intestine come from animals, but whether this includes the common human uropathogens is not clear since there has been little recent epidemiological work. Dupont & Steele[230] have summarized some of the earlier findings, concluding that colonization with animal *E. coli* is transient and that animals are not an important source of resistant coliforms. In a large epidemic in London, no source was found for the *E. coli* O15, which was highly antibiotic-resistant and virulent, which caused an excess of urinary tract infections in the community, and at the height of the epidemic colonized some 10% of the citizens of south London.[95] Four years later the strain had completely disappeared,[96] but it was later found in Spain.[234] Similar strains have recently been found in the USA,[235] and although there has been speculation in relation to a possible animal source, none has been found.[236] It seems likely that *E. coli* often behaves in this way, but in the absence of pathogenicity and resistance markers it would not be noticed, and would thus fail to generate epidemiological investigations.

The evidence that 'non-pathogenic' *E. coli* may be zoonotic is scanty. One of the few pieces of direct evidence relates to the use of

the antibiotic nourseothricin, an antibiotic unrelated to any others marketed, in animals in the former German Democratic Republic.[237] *E. coli* resistant to the drug were isolated from animals and eventually human faeces and infection. The case is fully discussed by Sundsfjord *et al*.[121] An animal source either for the organism or the resistance determinant seems highly likely. Similar resistance in human isolates followed the use of the novel aminoglycoside apramycin in animals,[238] but the issue is clouded by cross-resistance with other aminoglycosides. A study by van den Bogaard *et al*.[110] suggests that although genotypes of *E. coli* isolated from animals and humans may be similar, they are infrequently indistinguishable. This is supported by the evidence of Parsonnet & Kass,[111] quoted above. Furthermore, work carried out in the 1970s suggests that animal strains usually do not readily establish carriage in humans, although Levy *et al*.[239] found that plasmids disseminated readily in chicken and human isolates of *E. coli*. Finally, DANMAP 2001 reports that, in Denmark, 'non-pathogenic' *E. coli* from animals is much less often resistant to ampicillin than is *E. coli* causing infection in humans—16%, 0% and 10% resistant in chickens, cattle and pigs, respectively versus 30–45% in humans.[59] Animal strains are also more susceptible to sulphonamides although the margin is less, and, in this study, never resistant to ciprofloxacin, although up to 5% of isolates from chicken meat were resistant, as were 2–3% of human isolates. These susceptibility patterns support the hypothesis that resistance in *E. coli* is more likely to be driven by human antibiotic use, although an animal origin for at least some clinical isolates cannot be excluded (leaving aside the case of O157). It is intriguing that antibiotic-resistant *E. coli* has been isolated in rural areas from wild rodents in the UK.[240]

Human to animal transfer of resistance (Figure 1): It has been reported that MRSA can be transferred from humans to dogs, horses and cats in veterinary hospitals and in the community,[241,242] but there have been very few studies on the subject in general.[243] Host species specificity might be expected to play a major role in preventing the phenomenon in the 'indicator organisms' *E. coli* and enterococci, but salmonellae and campylobacters would be expected to be transferable from humans to animals.

The role of human sewage as a vehicle of salmonella infection has been identified on a number of occasions.[142,244] Environmental contamination from this source can lead to gut colonization in wild and feral animals, including gulls, which then enter animal houses and contaminate feed and grazing land, colonize chicken faeces and eggs, thus returning to humans. Such events might account for the upsurge of *Salmonella enteritidis* infections noted a decade ago.[245] Similar cycling might occur in other contexts involving resistant salmonellae and other bacteria of faecal origin (see enterococci above).

Much more work needs to be done to define the role of the spread of infection from man to animals, and especially on the possibility that therapy in humans might be responsible for resistance that appears to arise following therapy with the same antibiotics in animals—as, for example, with fluoroquinolone resistance in campylobacters.

Risk assessment

What is the probability of animal antibiotic-resistant bacteria causing treatment failures in human medicine? In order to affect human health, resistant bacteria selected by antibiotic use in animals must be transmitted to man and either themselves cause disease or transfer their resistance to other bacteria that cause disease. This is in the context of use of antibiotics in humans that are identical or so similar to those used in animals as to select identical resistance, and that are conceded to make the major contribution to the problem of resistance in human therapy.

For many important human pathogens, antibiotic use in humans is sufficient to create a major problem. The problem of resistant *S. aureus* was created by the successive use of antibiotics as they were introduced, from penicillin on, and we do not need to postulate any involvement from the use of antibiotics in animals. Likewise, penicillin- and macrolide-resistant *S. pneumoniae*, and macrolide-resistant *S. pyogenes* need no contribution from animal use. The normal oral streptococcal flora offers a ready source of seemingly relevant genes.[246,247] As discussed above, VRE can become a problem in man without animal glycopeptide use—as in the USA—whereas the presence of VRE, or more clearly streptogramin-resistant *E. faecium,* in animals, on food, and in the human intestine does not necessarily constitute a significant risk factor for human infection. When the case of *E. coli* is studied further, it may well be found to be similar to that for enterococci. Any impact is further mitigated by the fact that for the undoubted zoonoses, even if resistance in *Salmonella* or *Campylobacter* does originate to an important extent in animals (notably involving antibiotics used for therapy or prevention of disease and, except in the case of macrolides, not recently used for growth promotion in Europe), antibiotic therapy is seldom indicated. Even when therapy is needed, *in vitro* resistance is not always a barrier to success—as with fluoroquinolone resistance in campylobacters (see above).

Risk assessment conventionally involves the separate stages of hazard identification, exposure assessment, exposure–response modelling, risk characterization and uncertainty characterization. To date, as far as we know, no risk assessment has identified an actual (as opposed to conjectured) distinct clinically significant role for antibiotic-resistant bacteria from poultry causing adverse human health consequences. The hazard identification step thus has not been completed for fluoroquinolone-resistant campylobacter or for streptogramin-resistant *E. faecium*. This makes it necessary either to hypothesize a special hazard or assume that the risks are the same as for susceptible bacteria. The WHO has drafted a risk assessment of the impact of campylobacter from poultry on human health[248] and the US FDA's Center for Veterinary Medicine (CVM) has estimated the quantitative human health impact of fluoroquinolone-resistant campylobacters.[249] Neither process represents an adequate risk assessment, since each relies heavily on unsupported assumptions. CVM's assumptions, especially that human health harm is proportional to chicken consumed, seem to be directly contradicted by available data on the protective effects of chicken consumption in reducing risks of campylobacteriosis. Nor can we accept the conclusion of Travers & Barza[153] that fluoroquinolone resistance in campylobacters 'leads to >400 000 excess days of diarrhoea in the United States per year' since it too is based on unverified assumptions—that each patient infected with a resistant campylobacter and treated with a fluoroquinolone suffers two extra days of diarrhoea, and that what applies in Minnesota[158] applies to the rest of the USA. More data-driven risk assessments conclude that the risk to human health from fluoroquinolone-resistant campylobacters is vanishingly small.

Quantitative information on campylobacters is available for nearly all steps in the farm-to-fork chain, making quantitative, data-driven risk modelling practical for this pathogen. Table 4 summarizes parameters and data sources for a recent quantitative simulation model of campylobacteriosis risks.[169,257] Table 5 summarizes key conclusions from the model. An important finding, for policy purposes, is that risk management strategies that focus on eliminating

Table 4. Examples of parameters and data for *Campylobacter jejuni* quantitative risk assessment

Data input	Values in simulation model	Notes and references
Seasonality of pre-processing incidence	Winter, Spring, Summer, Fall multipliers = 0.82, 0.63, 1.43, 1.13, respectively	Friedman *et al.*;[164] Stern[250]
Pre-processing incidence of surface contamination	Surface contamination multiplier × caecal colonization incidence. Surface contamination multiplier: $U[0.22, 0.62]$, $\mu = 0.42$. Caecal colonization incidence: binomial probability, $P = 0.9$	Stern *et al.*;[251] Jones *et al.*[252]
Pr (resistant infection)	Binomial probability, $P = 0.094$	FDA-CVM[249]
Surface microbial load on chickens	Triangular distribution for $\log_{10}$ of values at farm: T(0, 2.98, 6.38)	Stern *et al.*[251]
Transportation factor	Triangular distribution for $\log_{10}$ of factor values: T(1.32, 2.73, 4.24)	Stern *et al.*[251]
Processing factor	Triangular distribution for $\log_{10}$ of factor: T(1.0, 2.23, 3.0) by which rinsing, scalding, etc. decrease surface microbial load	Stern *et al.*;[251] Stern;[250] Izat *et al.*;[253] Lillard;[254] Mead *et al.*[255]
Proportion further processed	Binomial probability, $P = 0.4678$. cfu count reduced to 0	Describes prepared and frozen chicken foods
Frozen chicken factor	Select non-further processed chickens with binomial probability $P = 0.163$ to freeze. Reduces cfu count by 100 on selected chickens	Mead *et al.*[255]
Post-processing surface contamination incidence	0.735 approx. equal to (cross-contamination multiplier) × (surface multiplier) × (caecal colonization incidence) $1.934 \times 0.4222 \times 0.90$ (using means)	Cross contamination multiplier is uniformly distributed $U(1.368,2.5)$. FDA-CVM[249]
Post processing incidence without retail infection	Binomial probability, $P = 0.302$, for chickens showing infection after processing but not at retail outlet	USDA[256]
Storage and preparation factor	Implied value (~1E–5) was estimated by model calibration	No data are available to fully quantify this factor

resistance are expected to create less than 1% of the public health benefit of strategies that focus on reducing microbial loads (resistant or not).

An even more disturbing conclusion was that, if the banning of fluoroquinolones gave even a modest increase in the variance of microbial loads on chickens leaving the processing plant, it would create far more cases of human infection than cases of resistant infection that it might prevent. Could some such consideration help to explain the increase in human campylobacter infections seen in Europe?[157] An increase in variability of pathogen load could occur despite the decreasing mean loads recently reported from Denmark.[258] The possibility is something that advocates of the 'precautionary principle' should weigh carefully before recommending bans on animal antimicrobials. The evidence from Europe suggests that such bans may lead to a reduction of resistant bacteria in animals and perhaps in some healthy members of the community who eat those animals, while allowing human illness and food-borne disease burdens to reach new heights.[59,155]

In relation to virginiamycin-resistant *E. faecium*, Smith *et al.*,[259] making some crucial but questionably valid assumptions, suggested that epidemics of infection might occur in hospitals sooner if virginiamycin were used in animals. Cox & Popken,[260] in contrast, have calculated that an immediate ban on virginiamycin would be expected to prevent at most 0.3 statistical mortalities in the entire US population over the next 5 years, given that transfer of resistant organisms leading to infection and treatment failure actually exists—and there is no evidence that it does.

Not surprisingly, initial attempts at risk assessment in relation to campylobacter, albeit based on too many unverified assumptions, show the risk to be very small. For example, an assessment of the impact on human health of fluoroquinolone-resistant campylobacters originating in cattle (not one of the major sources of infection) suggests that among 16 000 individuals who might acquire infection from ground beef, 150 might be hospitalized and up to four might die. Quinolone resistance might be responsible for one extra death after 10 years.[261] A recent informal sounding of opinion by two of us among UK and other clinical microbiologists worldwide showed that impartial scientists, microbiologists and infectious disease physicians believe that the contribution of animal use of antibiotics to the problem of antibiotic resistance in man is minimal.[262] On the contrary, it is almost universally recognized that over-prescription of antibiotics in human medicine is the leading cause of resistance in humans. Seeking to focus on a hypothesized contribution from animal use may simply distract from the real issues that should be of concern to those responsible for public health.

The impact of the growth promoter ban in Europe

The immediate effects of the growth-promoter ban in Europe have recently been discussed in some detail by Casewell *et al.*[25] Earlier experience of a ban of growth promoters in Sweden had already suggested the course of events to be expected.[23] In Denmark (Table 1), the overall use of antibiotics in animals fell from 206 000 kg in 1994 to 94 000 kg in 2001—over 50%—as the use of avoparcin, bacitracin,

Table 5. Results of quantitative risk simulation model: estimated health benefits per 100 000 person-years for different interventions

	Cases reduced				Illness days reduced[a]		
Risk management option	CP (%)	CP (cases)[b]	FQ resistant CP[c]	FQ resistant CP prescribed FQ[d]	CP[e]	FQ (Min/Max)[f]	Total
1. *Eliminate FQ at farm*	0	0	6.14	0.45	0	0.90/12.3	0.90
2. *Optimize withdrawal period*							
10 days	0	0	1.8	0.135	0	0.27/3.7	0.27
20 days	0	0	3.1	0.225	0	0.45/6.1	0.45
30 days	0	0	4.0	0.29	0	0.581/7.9	0.58
3. *Track FQ batches*	0	0	6.1	0.45	0	0.90/12.3	0.90
4. *Processing changes*							
↓cross-contam. (10%)	5.5%	5.3	0.34	0.025	31.8	0.05/0.68	31.85
↓cross-contam. (100%)	46.4%	44.5	2.8	0.21	267	0.42/5.7	267.4
↑cfu-reduction (10%)	12.8%	12.3	0.79	0.058	242.0	0.11/16	73.9
↑cfu-reduction (100%)	80.1%	76.8	4.9	0.36	460.8	0.72/9.8	461.2
both (10%)	20%	19.2	1.2	0.09	115.2	0.18/2.46	115.4
both (100%)	88%	84.4	5.4	0.395	506.4	0.79/10.8	507.2
5. *Restaurant changes*	18.6%	17.8	1.14	0.083	106.8	9.17/2.3	107.07
6. *Physicians prescribe FQ 10% less frequently*	0	0	0	0.42	0	0.848	0.85

CP, *Campylobacter jejuni*; FQ, fluoroquinolone; cfu, colony forming unit.
[a]Assumes that all excess days in a resistant case are attributed to resistant organisms from a causal point of view.
[b]Non-zero values are from 10 or more runs of the simulation.
[c]CP > 0 → CP × 0.064, CP = 0 → 95.9 × 0.064 × proportion of FQ relative to original.
[d]0.0732 × FQ resistant CP.
[e]6 × CP.
[f]0.10× (the number of cases of FQ resistant CP prescribed FQ—all sources).

spiramycin, tylosin and virginiamycin for growth promotion was abandoned. Furthermore, data from Denmark,[59,60,263] Germany,[72] and Holland[73] for example (Table 1), show that the ban has also had a marked effect on resistance rates in enterococci in the faecal flora of man and animals. DANMAP 2001 reports that resistance of enterococci to avoparcin has virtually disappeared from chickens and meat derived from them and from pigs and pork since avoparcin use was discontinued in 1995–6.[59] Unfortunately, there is no recent information on vancomycin resistance in enterococci colonizing or causing infection in humans in Denmark, but it is understood that VRE infections have always been very rare.[83] Similarly, the virginiamycin resistance rate in *E. faecium* has dropped from about 60% to 30% in chickens and to 5% in chicken meat since the ban in 1997–8: it is suggested that the rate has not fallen further because of associated resistance between streptogramins and penicillin or macrolides in *E. faecium* promoted by an increased therapeutic use of penicillin[59] and macrolides, or perhaps even copper,[92] in animal therapy. Thus, the prevalence of resistance appears to decline as resistant strains are replaced by susceptible strains when the use of the antibiotic selecting the resistance is completely discontinued. Streptogramin resistance rates for human *E. faecium* in Denmark are not reported. However, results from Germany and Holland indicate that vancomycin and quinupristin–dalfopristin resistance rates in human faecal enterococci (*E. faecium* for the latter) have also declined, supporting the hypothesis that at least some of these strains, or their resistance genes, are indeed of food animal origin.[72,73] Since VRE have rarely been reported in the past among clinical isolates in Europe other than in the UK (and it is important to distinguish between isolates that cause infection and isolates in the same clinical laboratories from the faecal flora or sites contaminated by the faecal flora—a crucial distinction not made in the study of Werner *et al.*[81]), we can only assume that there has been no direct impact on human health. Other sources suggest that both vancomycin resistance and quinupristin–dalfopristin resistance (in *E. faecium*) has increased in human enterococci in several countries in Europe since the growth-promoter ban, but coincident with increasing human glycopeptide and streptogramin use.[76,85–88]

One potentially highly undesirable effect of the growth-promoter ban has been the concomitant increase in the use of therapeutic antibiotics in animals clearly documented in Denmark,[27,56–60] and subsequently elsewhere.[25] The changes in use of growth-promoting and therapeutic antibiotics in animals in Denmark are outlined in Table 1. The antibiotics involved in these increases were tetracyclines, which almost doubled in use, penicillins with both narrow and broad spectra, sulphonamides plus trimethoprim, macrolides (also doubling) and aminoglycosides.[59] Thus although there was an overall 50% decrease in the total number of kilograms of antibiotic used in animals, there was a marked increase in the therapeutic use of antibiotics commonly used in veterinary and human medicine. It might be that this increased therapeutic use is contributing to the increases in tetra-

cycline resistance in pig and human isolates of *Salmonella* Typhimurium, the very phenomenon that led to the Swann report in 1969.[264] It will probably never be possible to determine whether this increase in therapeutic use will contribute to resistance in animal and human pathogens and thus to inadequacy of therapy. The same phenomenon of increased intestinal infection necessitating increased therapeutic use of antibiotics was observed in Sweden after their ban in 1986, and even after more than a decade, despite the development of better husbandry, better diets, and the use of zinc oxide dietary supplements 'losses in production parameters.... have not fully recovered on a national basis'.[23]

The case of bacitracin is of particular interest.[265] The antibiotic is little used (and not at all in Denmark) in human medicine, and then only topically, for which it is replaceable by a variety of other agents. A possible use for the clearing of VRE faecal carriage has been found to be unsustainable,[266–269] possibly since such a large proportion of isolates are resistant. Many doctors have agreed that if bacitracin were to be withdrawn totally from human medicine it would be little missed. On the other hand, bacitracin has been widely used as a growth promoter, with the additional advantage of suppression of clostridial necrotic enteritis of chickens,[270] and also *Lawsonia* infections,[59] for which a vaccine is now becoming available. Its use in either context has had no detectable deleterious effect on resistance in the staphylococci and streptococci that are the target species in man. It was predicted that if the agent were withdrawn as a growth promoter, gastrointestinal problems would emerge, requiring therapy, and this proved to be the case, contributing to the general increases in the therapeutic use of antibiotics in animals reported by the Danes[27,56–60] and now by others.[271] Furthermore, there has been no decrease in resistance to bacitracin among *E. faecium* since the ban.[59] The effect of the ban on bacitracin is thus entirely undesirable. Similar considerations may well apply to other growth promoters, but in each case the situation is more complex. Nonetheless, the EU has continued the ban.

The other theoretical hazard arising from the discontinuation of growth-promoting antibiotics is the possibility that the loads of salmonellae and campylobacters reaching man on food might vary more widely (see Risk assessment, above), increasing the risk of infection with these organisms. We note the increase in the incidence of campylobacter infection in Denmark, albeit starting before the antibiotic bans, and the recent temporary increase in salmonella infections there.[59] Microbiologically confirmed campylobacteriosis has also reached record levels in many other European countries,[155–157] while declining by over 25% in the USA.[123]

A WHO panel has recently reported on a review of the effects of the growth-promoter ban in Denmark, and concluded that it has attained its objectives, now defined as the reduction in the resistance-gene pool—with no consideration of actual beneficial effects on human health. It concluded that countries with 'similar conditions to Denmark' might expect similar results. We continue to believe that better human health should be the objective.[272]

Prudent use of antibiotics in food animals

The guidelines for the prudent use of antibiotics in animals, such as those produced by the World Veterinary Association[273] and the American Veterinary Medical Association,[274] are basically the same as those in human medicine. Essentially, antibiotics are used if they are known to be effective for their indicated purpose. They must cure or prevent infection, or in the case of growth promotion, must have a significant effect on food conversion parameters, and thereby improve the economic return to the animal producer, and they should not harm the animal. The target organisms must be known or shown to be susceptible, and adequate concentrations must be shown to reach the target. Furthermore, circumstances in which resistance is particularly likely to be selected should be avoided if possible, especially if this has clear clinical consequences. Cost is also a factor. Given all these considerations, it is not surprising that there is no perfect antibiotic, and antibiotic use always involves compromise. This is not always rational, and it is suggested that penicillin, for example, would not nowadays be approved for use in human medicine given its neurotoxicity, the high incidence of allergy and the common occurrence of resistance.

The case of the growth promoting antibiotics is no different. There is a need for assurance that they are still valuable in agriculture, contributing to the efficient rearing of food animals. If they prevent certain recognized infections this too should be taken into account. There is also a need for assurance that the usage will not harm animals or humans. All these call for the full range of the skills of the risk analysts, and when desirable and undesirable effects have been identified and quantified (and to be maximally helpful, compared with other more easily understood risks), it is time for the appropriate authorities, accountable to the population at large, to take action.

It may be that the effects of the campaign for prudent use of antibiotics are beginning to be observed in the USA, where, according to the Animal Health Institute, the amount of antibiotics used in animals has recently declined by almost 10%.[275] The effects on resistance arising from such prudence may be more difficult to discern.[276]

Conclusions

All the facts at our disposal persuade us that whereas resistance is undoubtedly selected in man and animals by the use of antibiotics, in organisms that are part of the normal flora as well as in pathogens, including zoonotic pathogens, and whereas some resistant organisms can be shown to reach man via the food chain, little additional harm results from resistance, even when infection supervenes. Only in the case of salmonellae and campylobacters do risk analyses, albeit still hampered by a lack of data, suggest that resistance possibly acquired in animals may add, albeit very little, to the burden of human disease. However, virulence is increasingly being identified as a factor in any adverse consequence of infection with strains that are also antibiotic-resistant. Almost every case made for or against antibiotics used in animals is complicated by the use of the same antibiotics in humans, which are equally able to give rise to resistance. This is particularly true of growth-promoting antibiotics with their Gram-positive spectrum of activity, and which have deleterious effects on enterococci in food animals, food and possibly the normal human faecal flora, but which do not seem to be responsible for infections in humans, nor, where adequate studies have been done, for the resistance determinants seen in true clinical isolates. What has not happened in 50 years of antibiotic use in animals and man seems unlikely to happen at a rapid rate now.

The banning of any antibiotic usage in animals based on the 'precautionary principle' in the absence of a full quantitative risk assessment is likely to be wasted at best and even harmful, both to animal and to human health. We believe that efforts should be concentrated instead on minimizing the transmission of all food-borne pathogens regardless of their antibiotic susceptibility, by insistence on good hygiene practices on farms, in abattoirs, during distribution and marketing of food, in food preparation, and, finally, by the consumer. It seems possible that the decreasing rates of important food-borne

diseases in the USA, in contrast to increases documented for some countries in Europe such as Denmark, might reflect differences in the recent pursuit of improved food hygiene, such as the use of Hazard Analysis Critical Control Point (HACCP) regulations and other hygiene measures. A lower overall incidence of disease means a diminished potential for such resistance as might arise in animals to cause any significant harm. The banning of the use of growth-promoting antibiotics has not been claimed even by its most ardent supporters to have had any detected beneficial effect on human health—and it might even have adverse effects.

We support truly rational and prudent use of antibiotics in all contexts—aided by the many guidelines that now exist. Emphasis on food hygiene is well founded historically and appears to have had an effect on the overall problem of resistance in food-borne pathogens. Whatever is done, competent surveillance of disease and antibiotic resistance as well as repeated refinement of risk analyses are a necessity, so that we may concentrate our efforts to limit the effects of antibiotic resistance on what is shown to work in practice.

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