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Food Animals and Antimicrobials: Impacts on Human Health

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INTRODUCTION

For many decades, antibiotic resistance has been recognized as a global health problem. It has now been escalated by major world health organizations to one of the top health challenges facing the 21st century (40, 65). Some of its causes are widely accepted, for example, the overuse and inappropriate use of antibiotics for nonbacterial infections such as colds and other viral infections and inadequate antibiotic stewardship in the clinical arena (109). But the relationship of drug-resistant bacteria in people to antibiotic use in food animals continues to be debated, particularly in the United States (11, 14, 38, 44, 48, 96, 124).

Many have delved into this question, producing volumes of direct and indirect evidence linking animal use to antibiotic resistance confronting people. Among these are a number of studies which unequivocally support the concern that use of antibiotics in food animals (particularly nontherapeutic use) impacts the health of people on farms and, more distantly, via the food chain (69, 88, 90, 111). While it was hoped by many that the years of experience following the bans on nontherapeutic use of antimicrobials in Europe would clearly signal an end to this practice, arguments continue, largely along the lines of a cost/benefit ratio and perceived deficits in solid scientific evidence. Action in the United States continues to lag far behind that of the European Union, which has chosen to operate proactively based on the "precautionary principle," a guiding tenet of public health. This principle states that "when evidence points toward the potential of an activity to cause significant widespread or irreparable harm to public health or the environment, options for avoiding that harm should be examined and pursued even if the harm is not yet fully understood or proven" (103).

This communication summarizes a large number of studies on the links between antimicrobials used for growth promotion, in particular, as well as other nontherapeutic antimicrobial (NTA) use in animal husbandry and aquaculture, and the emergence of antibiotic-resistant bacteria in humans. The *FAAIR Report* (*Facts about Antibiotics in Animals and the Impact on Resistance*) of the Alliance for the Prudent Use of Antibiotics (APUA) cites areas where antibiotic use can be curtailed and proposes several viable recommendations that could be utilized to reduce the burden of resistance genes created by nontherapeutic antibiotic use in animals (22).

Lastly, we consider whether knowledge gaps exist that need addressing in order to answer persisting questions that fuel the controversy over NTA use in food animals.

ANTIMICROBIAL USE IN ANIMALS: EFFECTS ON ANTIBIOTIC RESISTANCE EMERGENCE

Antimicrobials are delivered to animals for a variety of reasons, including disease treatment, prevention, control, and growth promotion/feed efficiency. Antimicrobial growth promotants (AGPs) were first advocated in the mid-1950s, when it was discovered that small, subtherapeutic quantities of antibiotics such as procaine penicillin and tetracycline (1/10 to 1/100 the amount of a therapeutic dose), delivered to animals in feed, could enhance the feed-to-weight ratio for poultry, swine, and beef cattle (142). For many years, the positive effects of this practice were championed, while the negative consequences went undetected. But microbiologists and infectious disease experts facing antibiotic resistance questioned the possible harm from this use (74, 89, 109, 136). They found that farms using AGPs had more resistant bacteria in the intestinal

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floras of the farm workers and farm animals than in those for similar people and animals on farms not using AGPs. A prospective *in vivo/in situ* study in 1975 was performed to evaluate the effect of introducing low-dose in-feed oxytetracycline as an AGP on the intestinal floras of chickens and farm dwellers (111). The results showed not only colonization of the chickens with tetracycline-resistant and other drug-resistant *Escherichia coli* strains but also acquisition of resistance in *E. coli* in the intestinal flora of the farm family. Other studies over the ensuing 3 decades further elucidated the quantitative and qualitative relationships between the practice of in-feed antimicrobials for animals and the mounting problem of hard-to-treat, drug-resistant bacterial infections in humans (83, 162).

Nontherapeutic Agents and Practices

The chief agricultural NTAs, used extensively in the United States and also used in Europe until the 1970s, include drugs that have likewise been employed widely in human medicine. In the absence of complete, unbiased data, this NTA use in the United States is estimated to be equal to (159) or as much as eight times greater than (67, 117) the quantity administered for therapeutic use.

More recently, concerns have arisen over the extensive use of antimicrobials in the burgeoning aquaculture industry, which more than doubled between 1994 and 2004 (36, 84). Eighty to 90 percent of total production occurs in Asia, with 67% occurring in China alone (64). In many parts of the world, fish farming is integrated with sewage or industrial wastewater or with land agriculture, as manure and other agricultural residues are commonly employed in fish feed (123). The overcrowding, unhygienic measures, and other manipulations in this intensive, industrial-scale production act as stressors to the fish and promote an increased use of antibiotic prophylaxis, particularly in the shrimp and carnivorous fish (such as salmon) industries. Moreover, even though the aquaculture use of AGPs in Western Europe and North America has been discontinued, therapeutic treatment of fish generally occurs *en masse* via inclusion in fish food, which results in exposure of the entire body of water to the antibiotic. The broad application of antibiotics in fish food leads to leaching from unconsumed food and feces into the water and pond sediments, where it not only exerts selective pressures on the sediment and water microflora but also can be washed to more distant sites, exposing wild fish and shellfish to trace antimicrobials (36). In this environment, the role of transduction (infection by bacterial phages) is considered highly important in facilitating lateral gene transfer (71). Sorum suggested that, historically, the transfer and emergence of resistance have occurred faster from aquatic bacteria to humans than from terrestrial animal bacteria to humans (141).

In the United States, the total fish industry use of antibiotics was estimated to be 204,000 to 433,000 pounds in the mid-1990s (25) (about 2% of the nonmedical use in cattle, swine, and poultry [117]). In much of the world, however, antibiotics are unregulated and used indiscriminately, and use statistics are rarely collected (25, 157). Although the total quantities of antibiotics employed in aquaculture are estimated to be smaller than those used in land animal husbandry, there is much greater use of antibiotic families that are also used in

human medicine (Table 1). In Chile, for example, ~100 metric tons of quinolones are used annually (10-fold greater than the amount used in human medicine), mostly in aquaculture (35). At least 13 different antimicrobials are reportedly used by farmers along the Thai coast (75).

Salmonella and the *Swann Report*

Alarmed by the rise in multidrug-resistant *Salmonella* in the 1960s, the United Kingdom's *Swann Report* of 1969 recognized the possibility that AGPs were contributing largely to the problem of drug-resistant infections (144). It concluded that growth promotion with antibiotics used for human therapy should be banned. The recommendation was implemented first in England and then in other European countries and Canada. The practice continued unchanged, however, in the United States and ultimately also continued in Europe, but with agents that were not used therapeutically in humans. Antibiotics such as bacitracin, avoparcin, bambermycins, virginiamycin, and tylosin gained in popularity as narrower-spectrum substitutes that had a smaller impact on the broad range of gut flora. Unforeseen, however, was the structural relationship between some of these agents and agents used clinically in humans (Table 1). This similarity meant that they shared a single bacterial target and that use of one agent could produce cross-resistance to the other.

Impacts of Nontherapeutic Use

Therapeutics applied properly for the treatment of individual animals tend to control the emergence and propagation of antimicrobial-resistant strains, in large part due to their relatively short-term application and relatively small numbers of animals treated. The resistant strains which may appear are generally diluted out by the return of normal, drug-susceptible commensal competitors (110). In contrast, any extended antibiotic applications, such as the use of AGPs, which are supplied for continuous, low-dose application, select for increasing resistance to the agent. Their use in large numbers of animals, as in concentrated animal feeding operations (CAFOs), augments the "selection density" of the antibiotic, namely, the number (density) of animals producing resistant bacteria. An ecological imbalance results—one that favors emergence and propagation of large numbers of resistance genes (113). The selection is not linked merely to the total amount of antibiotic used in a particular environment but to how many individuals are consuming the drug. Each animal feeding on an antibiotic becomes a "factory" for the production and subsequent dispersion of antibiotic-resistant bacteria. NTA uses are also clearly linked to the propagation of multidrug resistance (MDR), including resistance against drugs that were never used on the farm (10, 52, 59, 60, 92, 107, 111, 132, 141, 153, 154, 164). The chronic use of a single antibiotic selects for resistance to multiple structurally unrelated antibiotics via linkage of genes on plasmids and transposons (111, 143).

Studies on the impact of NTA use on resistance in land food animals have focused primarily on three bacterial genera—*Enterococcus*, *Escherichia*, and *Campylobacter*—and, to a lesser extent, on *Salmonella* and *Clostridium*. All of the above may be members of the normal gut flora (commensals) of food animals

TABLE 1. Antimicrobials used in food animal production[a]

Antibiotic	Purpose	Antimicrobial class	Spectrum of activity	Use in human medicine	Structurally related antibiotic(s)/antibiotic(s) with shared cross-resistance	Comments[c]
Ardacin	Bovine AGP	Glycopeptides	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 1997; not licensed in U.S.
Amoxicillin,[b] ampicillin[b]	Aquaculture, oral treatment of swine colibacillosis, treatment of bovine bacterial enteritis and subclinical mastitis	Aminopenicillins	Moderate	Yes	All penicillins	
Avilamycin	AGP for broilers	Orthosomycins	Gram-positive organisms	No	Everninomycin	Withdrawn from EU; not licensed in U.S.
Avoparcin[b]	AGP	Glycopeptides	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 1997; not licensed in U.S.
Bacitracin/zinc bacitracin	AGP for poultry, beef cattle, and swine; control of swine dysentery and bacterial enteritis; control of poultry enteritis	Polypeptides	Gram-positive organisms	Yes (zinc bacitracin)	Actinomycin, colistin, polymyxin B	Withdrawn from EU in 1999; available in U.S.; used in Japan
Bambermycin	AGP for poultry, swine, and cattle	Phosphoglycolipids	Gram-positive organisms	No	Vancomycin, teicoplanin	Withdrawn from EU in 2006; available in U.S.
Carbadox	Control of swine dysentery	Quinoxalines	Gram-positive and -negative organisms	No	Other quinoxolines	Withdrawn due to worker toxicity in EU and Canada; available in U.S. and Mexico
Carbomycin[b]	Respiratory disease prevention and treatment in poultry	Macrolides	Gram-positive organisms	No	Other macrolides	
Chloramphenicol	Aquaculture (oral/bath/injection)	Amphenicols	Broad	Yes	All amphenicols	Chloramphenicol approved in U.S. for dogs only
Colistin	Broiler, swine, and cattle feed	Cyclopolypeptides	Gram-negative organisms	Yes	All polymyxins	Used in Japan
Efrotomycin	AGP for swine	Elfamycins	Gram-positive organisms	No	Other elfamycins only	Not marketed
Enrofloxacin[b]	Therapy for bovine and swine respiratory disease, use in aquaculture (oral/bath)	Fluoroquinolones	Broad	No	All quinolones	Banned for use in poultry by FDA in 2005; not approved for aquaculture in U.S.
Erythromycin[b]	Aquaculture (oral/bath/injection); AGP for poultry, cattle, and swine; therapy for poultry respiratory disease and bovine mastitis	Macrolides	Gram-positive organisms	Yes	Oleandomycin and other macrolides and lincosamides	
Florfenicol	Respiratory disease treatment of cattle and swine	Amphenicols	Broad	No	All amphenicols	
Flumequin[b]	Aquaculture (oral)	Fluoroquinolones	Broad	No	Fluoroquinolones and quinolones	Not approved in U.S.
Furazolidone	Aquaculture (oral/bath)	Nitrofurans	Broad	Yes		Banned in U.S. food animals in 2005
Gentamicin[b]	Therapy for swine colibacillosis and dysentery, prevention of early poultry mortality, turkey egg dip	Aminoglycosides	Gram-positive and -negative organisms	Yes	Other aminoglycosides	
Lasalocid	AGP for cattle, poultry, sheep, and rabbits; coccidiosis prevention in poultry and sheep	Ionophores	Gram-positive organisms	No	Not demonstrated	Approved in EU and U.S.
Lincomycin	AGP for chickens and swine; therapy for swine dysentery, pneumonia, chicken necrotic enteritis, and respiratory disease	Lincosamides	Gram-positive organisms	Rare	Erythromycin and other macrolides and lincosamides, clindamycin	Approved in U.S.
Maduramycin	Poultry coccidiostat	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Approved in EU and U.S.
Monensin	Bovine AGP; prevention/control of coccidiosis in bovines, poultry, and goats	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Withdrawn from EU as bovine AGP but authorized as poultry coccidiostat

Antimicrobial	Use	Class	Spectrum		Cross-resistance	Status in U.S.
Neomycin[b]	AGP for swine and poultry; treatment/control of swine enteritis and pneumonia; control of mortality from *E. coli* in turkeys, bovines, swine, sheep, and goats; control of respiratory and other poultry diseases; aquaculture (bath)	Aminoglycosides	Gram-positive and -negative organisms	Yes	Gentamicin and other aminoglycosides	Approved in U.S.
Narasin	Poultry feed coccidiostat, prevention of necrotic enteritis in chickens, AGP for cattle	Ionophores	Coccidia, Gram-positive organisms	No	Not demonstrated	Approved in U.S.
Nourseothricin	Swine AGP	Streptothricins	Gram-negative organisms	No	None	Withdrawn from EU; never used in U.S.
Novobiocin	Treatment of staph infections, treatment and control of fowl cholera, treatment of bovine mastitis	Aminocoumarins	Gram-positive organism	Yes		Withdrawn due to worker toxicity in EU and Canada; available in U.S.
Olaquindox	Swine AGP, control of swine dysentery/enteritis	Quinoxalines	Gram-positive and -negative organisms	No	Other quinoxolines	
Oleandomycin[b]	Poultry and swine AGP	Macrolides	Gram-positive organisms	Yes	Erythromycin and other macrolides	
Ormetoprim	Poultry AGP, prevention of fowl cholera and other infections	Diaminopyrimidines	Broad	No	Trimethoprim	
Oxolinic acid[b]	Aquaculture (oral)	Quinolones	Broad	No	Other quinolones	Not approved in U.S.
Pristinamycin	AGP	Streptogramins	Gram-positive organisms	Yes	Other streptogramins (virginiamycin, quinupristin/dalfopristin)	Withdrawn in EU; available in U.S.
Procaine penicillin[b]	AGP in poultry and swine	Beta-lactams	Gram-positive organisms	Yes	Other beta-lactams	
Roxarsone	AGP for poultry and swine, poultry coccidiostat, treatment of swine dysentery	Arsenicals	Coccidia	No	Other arsenicals	
Salinomycin	Swine AGP, prevention/control of swine dysentery and porcine intestinal adenomatosis, control of *Clostridium perfringens* in growers	Ionophores	Gram-positive organisms	No	Not demonstrated	Withdrawn from EU
Spiramycin[b]	Swine AGP; treatment of bovine mastitis	Macrolides	Gram-positive organisms	Yes	Erythromycin and other macrolides and lincosamides	AGP use withdrawn from EU in 1999; not approved in U.S.
Streptomycin[b]	Aquaculture (bath)	Aminoglycosides	Broad	Yes	All aminoglycosides	
Sulfonamides	Aquaculture (sulfamerazine [oral] and sulfadimethoxine [oral]), swine AGP (sulfamethazine), chicken AGP (sulfadimethoxine)	Sulfonamides	Broad	Yes	All sulfonamides	Sulfamerazine authorized for U.S. aquaculture, but not marketed
Tetracyclines (oxy- and chlor-)[b]	AGP for poultry, swine, and cattle; treatment and control of multiple livestock diseases; aquaculture (oral/bath/injection); control of fish and lobster disease	Tetracylines	Broad	Yes	All tetracyclines	Withdrawn from EU; authorized in U.S.
Tiamulin	Swine AGP; treatment of swine enteritis, dysentery, and pneumonia	Pleuromutilins	Gram-positive organisms, mycoplasmas, spirochetes	No	Tylosin, erythromycin, and other macrolides	Used for disease prevention and treatment in chickens outside the U.S.
Tylosin[b]	Swine AGP, therapeutic treatment of mastitis	Macrolides	Gram-positive organisms	No	Erythromycin and other macrolides and lincosamides	AGP use withdrawn from EU; available in U.S.
Virginiamycin	AGP for broilers	Streptogramins	Gram-positive organisms	Yes	Quinupristin/dalfopristin and other streptogramins	Withdrawn from EU; available in U.S.

[a] Based on data from references 7, 32, 34, 53, 84, 96, 98, 133, and 162.
[b] Highly important in human medicine or belongs to critically important class of human antimicrobials.
[c] EU, European Union.

but possess the potential to become serious human pathogens. The prospective farm study by Levy in 1975 (111) and studies of others in the following decades clearly demonstrated the selective nature of low-dose, nontherapeutic AGPs on both the pathogenic and commensal flora of food animals such as poultry, swine, and cattle (8, 16, 18, 90, 98, 146, 149). Likewise, in the past decade, studies have demonstrated the selective nature of mass treatment with antimicrobials in aquaculture (36, 62, 84). In the latter, studies have focused on *Aeromonas* pathogens of both fish and humans and the subsequent high-frequency transfer of their resistance plasmids to *E. coli* and *Salmonella* (36).

Aarestrup and Carstensen found that resistance derived from use of one NTA (tylosin) was not confined to swine gut bacteria only but could cross species and appear in staphylococci isolated from the skin. While the conversion of gut enterococci to erythromycin (a related human therapeutic) resistance occurred rapidly (within 1 week) the skin-derived resistant organism *Staphylococcus hyicus* appeared more gradually, escalating to a 5-fold increase over 20 days (5).

The finding of bacterial cross-resistance between NTAs used in food animals and human drugs was aptly demonstrated with avoparcin (an AGP) and its close relative vancomycin (an important human therapeutic) when vancomycin-resistant enterococci (VRE) emerged as a serious human pathogen. A connecting link between resistance in animals and humans was revealed when Bates et al. found avoparcin- and vancomycin-coresistant enterococci in pigs and small animals from two separate farms. Ribotyping methods showed that some of the patterns from farms and sewage exactly matched those of *Enterococcus* spp. from the hospital (24). The structures of the two drugs are similar: they are both members of the glycopeptide family (24).

Since that time, numerous studies have examined the impacts of newer NTAs on the floras of animals. The use of tylosin and virginiamycin in Norwegian swine and poultry led to high prevalences of resistance to both these agents in *Enterococcus faecium* (75% to 82% for tylosin and 49 to 70% for virginiamycin) (1). Avilamycin resistance, while significantly associated with avilamycin use, has been observed on both exposed and unexposed farms and was significantly higher in isolates from poultry than in those from swine, despite its use in both these species (4). These findings suggest that other selective agents may be present in the environment or that substances related to avilamycin were not recognized. As described above, not only the drug choice and amount but also the number of animals treated can affect the consequence of its use.

Other findings suggest that complex ecologic and genetic factors may play a role in perpetuating resistance (63). Resistance (particularly to tetracycline, erythromycin, and ampicillin) has been found inherently in some antibiotic-free animals, (10, 45, 93, 130), suggesting that its emergence is related to other factors, such as diet, animal age, specific farm type, cohort variables, and environmental pressures (26). While Alexander et al. found MDR (tetracycline plus ampicillin resistance) in bacteria in control animals, the strains that emerged after AGP use were not related to these (10). In addition, resistance to tetracycline was higher for a grain-based diet than a silage-based one. Costa et al. found non-AGP-related resis-

tances in enterococci, most likely derived from previous flocks, i.e., the farm environment and the feed source appeared to be responsible for the emergence of the unrelated resistances (45). Khachatryan et al. found an MDR phenotype (streptomycin, sulfonamide, and tetracycline [SSuT] resistance phenotype) propagated by oxytetracycline in a feed supplement, but upon removal of the drug, the phenotype appeared to be maintained by some unknown component of the unmedicated feed supplement, possibly one that selects for another gene that is linked to a plasmid bearing the SSuT resistance phenotype (100). The persistence may also relate to the stability of the plasmid in its host and the fact that expression of tetracycline resistance is normally silent until it is induced by tetracycline. Thus, the energy demands exerted on the host by tetracycline resistance are lower. One can conclude that removal of the antibiotic may not lead to rapid loss of the resistant strain or plasmid.

EFFECTS OF BANNING GROWTH PROMOTANTS IN ANIMAL FEEDS IN EUROPE

One of the first bans on AGP use was that imposed on tetracycline by the European Common Market in the mid-1970s (39). Prior to institution of the ban in the Netherlands (1961 to 1974), Van Leeuwen et al. had tracked a rise in tetracycline-resistant *Salmonella* spp. Following the ban, however, they observed a decline in tetracycline resistance in both swine and humans (150).

More than 10 years have passed since the final 1999 European Union ban, during which a plethora of studies from multiple European countries, Canada, and Taiwan have examined antibiotic use and resistance trends subsequent to the removal of key AGP drugs, especially avoparcin, and the consequences on vancomycin resistance in *Enterococcus* (7, 15, 17, 21, 29, 30, 76, 85, 97, 102, 107, 121, 148, 150, 156a). Its structural relationship to and cross-resistance with avoparcin render vancomycin a drug of prime interest for determining the impact of avoparcin in triggering and promoting resistance in human infection.

Avoparcin

In many European countries, the use of avoparcin as a feed additive led to frequent isolation of VRE from farm animals and healthy ambulatory people (3, 18, 102). Since the emergence of the enterococcus as a major MDR pathogen, vancomycin has evolved as a key therapy, often as the drug of last resort. Following the 1995 ban on avoparcin, several investigators reported a decline in animal VRE. In Denmark, frequencies peaked at 73 to 80% and fell to 5 to 6% (7, 18) in poultry. In Italy, VRE prevalence in poultry carcasses and cuts decreased from 14.6% to 8% within 18 months of the 1997 ban (121), and in Hungary, a 4-year study showed not only a decline in prevalence of VRE among slaughtered cattle, swine, and poultry after removal of avoparcin but also a decrease in vancomycin MICs (97). In surveillance studies both before and after the German ban in 1996, Klare et al. showed a high frequency of VRE in 1994, followed by a very low frequency of just 25% of poultry food products in 1999 (102). Similar de-

clines were reported in broiler farms following a ban on avoparcin in Taiwan in 2000 (107).

A dramatic reduction in human carriage of VRE also followed the ban on avoparcin. Parallel surveillances of the gut floras of healthy ambulatory people showed that VRE colonization in Germany declined from 13% in 1994 to 4% in 1998 (102), and in Belgium, it declined from 5.7% in 1996 to ~0.7% in 2001 (68).

Virginiamycin and Other Antibiotics

Increased virginiamycin use in Danish broilers during the mid-1990s correlated with a rise in resistant *E. faecium* prevalence, from 27% to ~70% (7). Following the ban, resistance declined to 34% in 2000. Likewise, in Denmark, the 1998 ban on the use of tylosin in swine resulted in a decline in erythromycin (a structurally related macrolide) resistance, from 66% to 30% (49). Avilamycin use in 1995 and 1996 increased resistance in broiler *E. faecium* strains, from 64% to 77%, while declining applications after 1996 lowered the prevalence to 5% in 2000 (7).

Some of these studies revealed a genetic linkage between bacterial macrolide and glycopeptide resistances in swine, such that neither resistance declined in prevalence until both avoparcin (a glycopeptide) and tylosin (a macrolide) use was limited. With a reduction in tylosin use, the prevalence of glycopeptide-resistant enterococci fell to 6% and macrolide resistance fell from nearly 90% to 47% in *E. faecium* and to 28% in *Enterococcus faecalis* (7). Notably, the first report of transfer of vancomycin resistance from *Enterococcus* to *Staphylococcus aureus* was demonstrated in laboratory mice because of its linkage to macrolide resistance on the same plasmid (119).

One concern voiced following the banning of NTAs was that the incidence of disease in animals would rise and result in a parallel increase in therapeutic use. This has become the subject of some debate. Some countries encountered rises in necrotic enteritis in chickens and colitis in swine soon after the institution of AGP bans (33, 159). In Norway, an abrupt increase in necrotizing enteritis (NE) in poultry broilers was reported following the removal of avoparcin, with a coincident rise in antibiotic therapy. When the ionophore feed additive narasin was approved, NE declined once again (77). It was concluded that the ban on avoparcin consumption produced a negligible effect on the need for antibiotic therapy (76). Likewise, in Switzerland, Arnold et al. reported a postban increase in overall antibiotic quantities used in swine husbandry but observed a stable therapy intensity (prescribed daily dose) (15). By 2003, total animal use of antibiotics in Denmark, Norway, and Sweden had declined by 36%, 45%, and 69%, respectively (76). The most thorough postban analysis of this phenomenon comes from Denmark. In a careful review of swine disease emergence, animal production, and antibiotic use patterns over the years 1992 to 2008, Aarestrup et al. reported no overall deleterious effects from the ban on finishers and weaners in the years 1998 and 2000, respectively. Despite an increase in total therapeutic antibiotic consumption immediately following the ban, no lasting negative effects were detected on mortality rate, average daily weight gain, or animal production (6). Moreover, even if therapeutic use increased, the numbers of animals treated would be reduced compared to those with growth promotion use, so selection density would be decreased (113).

In summary, the in-depth, retrospective analyses in Denmark shed a different perspective on postban concerns over increased therapeutic use. Over time, it appears that the negative after-effects of the ban have waned. As farmers modified their animal husbandry practices to accommodate the loss of banned NTAs, these disease outbreaks became less prominent. Improved immunity and reduced infection rates led to fewer demands for therapeutic antibiotics.

Interestingly, recent studies have shown that the original beneficial aspects observed with AGP use (i.e., weight gain and feed efficiency) appear to have diminished, although the results are mixed and depend upon the kind of animals and type of antibiotic involved. Diarra et al. found no effect on body weight or feed intake in poultry from five different AGPs, and feed efficiency was improved with penicillin only (52). In contrast, Dumonceaux et al. reported a significantly increased body weight (10%) and a 7% increase in feed efficiency with the AGP virginiamycin, but only for the first 15 days (55a). In short, improved farming practices and breeding programs, which may include reduced animal density, better hygiene, targeted therapy, and the use of enzymes, prebiotics, probiotics, and vaccines, appear to have at least partially replaced the beneficial aspects of antibiotic growth promoters (27, 158, 160).

EVIDENCE FOR ANIMAL-TO-HUMAN SPREAD OF ANTIBIOTIC RESISTANCE

Any use of antibiotics will select for drug-resistant bacteria. Among the various uses for antibiotics, low-dose, prolonged courses of antibiotics among food animals create ideal selective pressures for the propagation of resistant strains. Spread of resistance may occur by direct contact or indirectly, through food, water, and animal waste application to farm fields. It can be augmented greatly by the horizontal transfer of genetic elements such as plasmids via bacterial mating (conjugation). We summarize here the evidence for animal-to-human transfer of resistant bacteria on farms using antibiotics for treatment and/or nontherapeutic use.

Resistance Acquisition through Direct Contact with Animals

Farm and slaughterhouse workers, veterinarians, and those in close contact with farm workers are directly at risk of being colonized or infected with resistant bacteria through close contact with colonized or infected animals (Table 2). Although this limited transmission does not initially appear to pose a population-level health threat, occupational workers and their families provide a conduit for the entry of resistance genes into the community and hospital environments, where further spread into pathogens is possible (118, 155).

The majority of studies examining the transmission of antibiotic-resistant bacteria from animals to farm workers document the prevalence of resistance among farmers and their contacts or among farmers before and after the introduction of antibiotics at their workplace. Direct spread of bacteria from animals to people was first reported by Levy et al., who found

TABLE 2. Key evidence for transfer of antibiotic resistance from animals to humans

Transfer type	Species tracked	Animal host(s)	Recipient host(s)	Resistance transferred	Evidence	Reference
Human colonization via direct or indirect animal contact	E. coli	U.S. chickens	Animal caretakers, farm family	Tetracycline	Following introduction of tetracycline on a farm, resistant E. coli strains with transferable plasmids were found in caretakers' gut floras, with subsequent spread to the farm family	111
	S. aureus, Streptococcus spp., E. coli and other enterobacteria	French swine	Swine farmers	Erythromycin, penicillins, nalidixic acid, chloramphenicol, tetracycline, streptomycin, cotrimoxazole	Phenotypic antibiotic resistance was significantly higher in the commensal floras (nasal, pharyngeal, and fecal) of swine farmers than in those of nonfarmers	16
	E. coli	U.S. chickens	Poultry workers	Gentamicin	Increase in phenotypic gentamicin resistance in workers through direct contact with chickens receiving gentamicin prophylactically	126
	E. coli	Chinese swine and chickens	Farm workers	Apramycin (not used in human medicine)	Detection of aac(3)-IV apramycin resistance gene in humans, with 99.3% homology to that in animal strains	164
	MRSA ST398	Dutch veal calves	Veal farmers	MDR	Human nasal carriage of the mecA gene was strongly associated with (i) greater intensity of animal contact and (ii) the number of MRSA-positive animals; animal carriage was related to animal antibiotic treatment	78
Human infection via direct or indirect animal contact	Salmonella Newport	Beef cattle (ground beef) receiving chlortetracycline AGP	Salmonella-infected patients with diarrhea	Ampicillin, carbenicillin, tetracycline	Direct genetic tracking of resistance plasmid from hamburger meat to infected patients	87
	E. coli	German swine (ill)	Swine farmers, family members, community members, UTI patients	Streptothricin	Identification of transferable resistance plasmids found only in human gut and UTI bacteria when nourseothricin was used as swine AGP	90
	E. coli, Salmonella enterica (serovar Typhimurium)	Belgian cattle (ill)	Hospital inpatients	Apramycin, gentamicin	Plasmid-based transfer of aac(3)-IV gene bearing resistance to a drug used only in animals (apramycin)	42
	Enterococcus faecium	Danish swine and chickens	Hospital patients with diarrhea	Vancomycin	Clonal spread of E. faecium and horizontal transmission of the vanA gene cluster (Tn1546) found between animals and humans	80
	E. coli	Spanish chickens (slaughtered)	Bacteremic hospital patients	Ciprofloxacin	Multiple molecular and epidemiological typing modalities demonstrated avian source of resistant E. coli	95

the same tetracycline-resistant *E. coli* strains in the gut flora of chicken caretakers as in the chickens receiving tetracycline-laced feed (112). The observation extended to the farm family as well and showed an increased frequency of tetracycline-resistant and multidrug-resistant *E. coli* after several months of use of AGP-laden feed. Studies such as this (which examined a variety of antibiotic classes and assorted pathogens) have consistently shown a higher prevalence of resistant gut bacteria among farm workers than in the general public or among workers on farms not using antibiotics (16, 90, 98, 149).

While gentamicin is not approved for growth promotion in the United States, it remains the most commonly used antibiotic in broiler production, being employed for prevention of early poultry mortality (115). A revelatory 2007 study found that the risk for carrying gentamicin-resistant *E. coli* was 32 times higher in poultry workers than in other members of the community: half of all poultry workers were colonized with gentamicin-resistant *E. coli*, while just 3% of nonpoultry workers were colonized. Moreover, the occupationally exposed population was at significantly greater risk for carriage of multidrug-resistant bacteria (126).

New gene-based methods of analysis provide even stronger evidence for the animal origin of bacteria that colonize or infect humans. Homologous relationships between bacterial resistance genes in humans and farm animals have been identified most commonly for food-borne pathogens such as *Escherichia coli* and *Salmonella* (see below) but have also been recorded for various species of *Enterococcus* and for methicillin-resistant *Staphylococcus aureus* (MRSA). Zhang and colleagues found *E. coli* strains resistant to apramycin (an antibiotic used in agriculture but not in human medicine) in a study of Chinese farm workers. All farms in the study that used apramycin as an AGP had workers that carried apramycin resistance genes. The same resistance gene, *aac*(*3*)-*IV*, was present in each swine, poultry, and human isolate, with some resistance profiles also matching across species (164). A group of French scientists found the same resistance gene [*aac*(*3*)-*IV*] in cow, pig, and human *E. coli* strains that bore resistance to apramycin and gentamicin (42). In another study, similar resistance patterns and genes were detected in *E. faecalis* and *E. faecium* strains from humans, broilers, and swine in Denmark (2). Lee sampled MRSA isolates from cattle, pigs, chickens, and people in Korea and found that 6 of the 15 animal isolates containing *mecA* (the gene responsible for methicillin resistance in *S. aureus*) were identical to human isolates (108).

Antibiotic Resistance Transmission through the Food Chain

Consumers may be exposed to resistant bacteria via contact with or consumption of animal products—a far-reaching and more complex route of transmission. There is undeniable evidence that foods from many different animal sources and in all stages of processing contain abundant quantities of resistant bacteria and their resistance genes. The rise of antibiotic-resistant bacteria among farm animals and consumer meat and fish products has been well documented (36, 108, 122, 162). Demonstrating whether such reservoirs of resistance pose a risk to humans has been more challenging as a consequence of the complex transmission routes between farms and consumers and the frequent transfer of resistance genes among host bacteria. Such correlations are becoming more compelling with the advent of molecular techniques which can demonstrate the same gene (or plasmid) in animal or human strains, even if the isolates are of different species.

For example, Alexander et al. showed that drug-resistant *Escherichia coli* was present on beef carcasses after evisceration and after 24 h in the chiller and in ground beef stored for 1 to 8 days (9). Others isolated ciprofloxacin-resistant *Campylobacter* spp. from 10% to 14% of consumer chicken products (79, 137). MRSA has been reported to be present in 12% of beef, veal, lamb, mutton, pork, turkey, fowl, and game samples purchased in the consumer market in the Netherlands (50), as well as in cattle dairy products in Italy (120). Likewise, extensive antibiotic resistance has been reported for bacteria, including human pathogens, from farmed fish and market shrimp (56, 84, 140).

Some of the antibiotic resistance genes identified in food bacteria have also been identified in humans, providing indirect evidence for transfer by food handling and/or consumption. In 2001, Sorensen et al. confirmed the risk of consuming meat products colonized with resistant bacteria, showing that glycopeptide-resistant *Enterococcus faecium* of animal origin ingested via chicken or pork lasted in human stool for up to 14 days after ingestion (139). Donabedian et al. found overlap in the pulsed-field gel electrophoresis (PFGE) patterns of gentamicin-resistant isolates from humans and pork meat as well as in those of isolates from humans and grocery chicken (55). They identified that when a gene conferring antibiotic resistance was present in food animals, the same gene was present in retail food products from the same species. Most resistant enterococci possessed the same resistance gene, *aac*(*6′*)-*Ie-aph*(*2″*)-*Ia* (55).

Emergence of Resistance in Human Infections

There is likewise powerful evidence that human consumption of food carrying antibiotic-resistant bacteria has resulted, either directly or indirectly, in acquisition of antibiotic-resistant infections (Table 2). In 1985, scientists in Arizona traced an outbreak of multidrug-resistant *Salmonella enterica* serovar Typhimurium, which included the death of a 72-year-old woman, to consumption of raw milk. Isolates from most patients were identical to the milk isolates, and plasmid analysis showed that all harbored the same resistance plasmid (145). A 1998 *S.* Typhimurium outbreak in Denmark was caused by strains with nalidixic acid resistance and reduced fluoroquinolone susceptibility. PFGE revealed that a unique resistance pattern was common to *Salmonella* strains from all patients, two sampled pork isolates, the swine herds of origin, and the slaughterhouse (118).

Samples from gentamicin-resistant urinary tract infections (UTIs) and fecal *E. coli* isolates from humans and food animal sources in China showed that 84.1% of human samples and 75.5% of animal samples contained the *aaaC2* gene for gentamicin resistance (86). Johnson et al. used PFGE and random amplified polymorphic DNA (RAPD) profiles of fluoroquinolone-resistant *E. coli* strains in human blood and fecal samples and in slaughtered chickens to determine that the two were virtually identical to resistant isolates from geographically linked chickens. Drug-susceptible human *E. coli* strains, how-

ever, were genetically distinct from poultry bacteria, suggesting that the ciprofloxacin-resistant *E. coli* strains in humans were imported from poultry rather than originating from susceptible human *E. coli* (94, 95).

Other reports demonstrate a broader linkage of resistance genes through the farm-to-fork food chain. A resistance-specifying *bla*$_{CMY}$ gene was found in all resistant isolates of *Salmonella enterica* serotype Newport originating from humans, swine, cattle, and poultry. The host plasmid, which conferred resistance to nine or more antimicrobials, was capable of transmission via conjugation to *E. coli* as well (165). An observed homology between CMY-2 genes in cephalosporin-resistant *E. coli* and *Salmonella* suggested that plasmids conferring resistance had moved between the two bacterial species. The authors found higher rates of CMY-2 in strains from animals than in those from humans, supporting an animal origin for the human pathogen (161). A 2000 study found matching PFGE profiles among vancomycin-resistant *Enterococcus faecium* isolates from hospitalized humans, chickens, and pigs in Denmark. Molecular epidemiology studies have also linked tetracycline resistance genes from *Aeromonas* pathogens in a hospital effluent to *Aeromonas* strains from a fish farm (127). These results support the clonal spread of resistant isolates among different populations (80).

Chronologic studies of the emergence of resistance across the food chain also strongly imply that reservoirs of resistance among animals may lead to increased resistance in consumers of animal food products. Bertrand et al. chronicled the appearance of the extended-spectrum beta-lactamase (ESBL) gene *CTX-M-2* in *Salmonella enterica* in Belgium. This resistance element was identified first in poultry flocks and then in poultry meat and, finally, human isolates (28). A recent Canadian study also noted a strong correlation between ceftiofur-resistant bacteria (the pathogen *Salmonella enterica* serovar Heidelberg and the commensal *E. coli*) from retail chicken and human infections across Canada. The temporary withdrawal of ceftiofur injection from eggs and chicks dramatically reduced resistance in the chicken strains and the human *Salmonella* isolates, but the trend reversed when the antibiotic use was subsequently resumed (57).

In three countries (United States, Spain, and the Netherlands), a close temporal relationship has been documented between the introduction of fluoroquinolone (sarafloxacin and enrofloxacin) therapy in poultry and the emergence of fluoroquinolone-resistant *Campylobacter* in human infections. An 8- to 16-fold increase in resistance frequency was observed—from 0 to 3% prior to introduction to ~10% in the United States and the Netherlands and to ~50% in Spain—within 1 to 3 years of the licensure (61, 128, 137). In the Netherlands, this frequency closely paralleled an increase in resistant isolates from retail poultry products (61), while the U.S. study used molecular subtyping to demonstrate an association between the clinical human isolates and those from retail chicken products (137).

It is now theorized, from molecular and epidemiological tracking, that the resistance determinants found in salmonella outbreaks (strain DT104) in humans and animals in Europe and the United States likely originated in aquaculture farms of the Far East. The transmissible genetic element contains the florfenicol gene (*floR*) and the tetracycline class G gene, both of which were traced to *Vibrio* fish pathogens (*Vibrio damsel* and *Vibrio anguillarum*, respectively). Both drugs are used extensively in aquaculture (36).

In the above examples, the link to nontherapeutic antibiotic use in the farm animals is still circumstantial and largely implied, often because the authors do not report any statistics on farm use of antibiotics. Interpreting these studies is also difficult because of the widespread resistance to some drugs in bacteria of both animals and humans and the ubiquitous nature of resistance genes. Moreover, the same farmer may use antibiotics for both therapeutic and nontherapeutic purposes.

The complexities of the modern food chain make it challenging to perform controlled studies that provide unequivocal evidence for a direct link between antibiotic use in animals and the emergence of antibiotic resistance in food-borne bacteria associated with human disease. While this concrete evidence is limited, a small number of studies have been able to link antibiotic-resistant infection in people with bacteria from antibiotic-treated animals. While not necessarily involving NTAs, these studies substantiate the considerable ease with which bacteria in animals move to people. For example, a multidrug-resistant *Salmonella enterica* strain in a 12-year-old Nebraska boy was traced to his father's calves, which had recently been treated for diarrhea. Isolates from the child and one of the cows were determined to be the same strain of CMY-2-mediated ceftriaxone-resistant *S. enterica* (69). It is now believed that the 1992 multiresistant *Vibrio cholerae* epidemic in Latin America was linked to the acquisition of antibiotic-resistant bacteria arising from heavy antibiotic use in the shrimp industry of Ecuador (13, 156).

By comparing the plasmid profiles of MDR *Salmonella* Newport isolates from human and animal sources, Holmberg et al. provided powerful evidence that salmonella infections in 18 persons from 4 Midwestern states were linked directly to the consumption of hamburger meat from cattle fed subtherapeutic chlortetracycline. A plasmid which bore tetracycline and ampicillin resistance genes was present in the organisms causing serious illness in those persons who ate the hamburger meat and who were also consuming penicillin derivatives for other reasons (87).

One of the most compelling studies to date is still Hummel's tracking of the spread of nourseothricin resistance, reported in 1986. In Germany, nourseothricin (a streptogramin antibiotic) was used solely for growth promotion in swine. Resistance to it was rarely found and was never plasmid mediated. Following 2 years of its use as a growth promotant, however, resistance specified by plasmids appeared in *E. coli*, not only from the treated pigs (33%) but also in manure, river water, food, and the gut floras of farm employees (18%), their family members (17%), and healthy outpatients (16%) and, importantly, in 1% of urinary tract infections (90). Ultimately, the resistance determinant was detected in *Salmonella* and *Shigella* strains isolated from human diarrhea cases (146).

The movement of antibiotic resistance genes and bacteria from food animals and fish to people—both directly and indirectly—is increasingly reported. While nontherapeutic use of antibiotics is not directly implicated in some of these studies, there is concern that pervasive use of antimicrobials in farming and widespread antimicrobial contamination of the environment in general may be indirectly responsible. For instance,

within the past 5 years, MRSA and MDR *Staphylococcus aureus* have been reported in 25 to 50% of swine and veal calves in Europe, Canada, and the United States (51, 78, 101, 114). Graveland et al. noted that this frequency was higher in veal calves fed antibiotics (78). Studies also show that colonization among farmers correlates significantly with MRSA colonization among their livestock (78, 101, 114, 138). In the Netherlands, colonization of swine farmers was found to be more than 760 times greater than that of patients admitted to Dutch hospitals (155). In a study of nasal swabs from veal and veal calf growers, family members, and employees at 102 veal calf farms in the Netherlands, Graveland et al. found that human MRSA sequence type ST398 carriage among the farmers was strongly associated with the degree of animal contact and the frequency of MRSA-colonized animals on the farm. When <20% of calves were carriers, the estimated prevalence in humans was ~1%—similar to that in the general public. With >20% carriage in calves, the prevalence in humans was >10% (78).

Recently, MRSA ST398 has appeared in the community. A Dutch woman without any known risk factors was admitted to a hospital with endocarditis caused by MRSA ST398, suggesting a community reservoir which passed on to people (58). Voss et al. demonstrated animal-to-human and human-to-human transmission of MRSA between a pig and pig farmer, among the farmer's family members, and between a nurse and a patient in the hospital. All isolates had identical random amplified polymorphic DNA profiles (155). Examples of similar MRSA strains among animals and people are mounting (82, 108, 147, 151, 152, 163).

ADDRESSING KNOWLEDGE GAPS: RESERVOIRS OF ANTIBIOTIC RESISTANCE

Historically, considerable attention has been focused on a very small minority of bacterial species that actually cause disease. However, a vast "sea" of seemingly innocuous commensal and environmental bacteria continuously and promiscuously exchange genes, totally unnoticed (116). A staggeringly diverse group of species maintain a large capacity for carrying and mobilizing resistance genes. These bacteria constitute a largely ignored "reservoir" of resistance genes and provide multiple complex pathways by which resistance genes propagated in animals can directly, or more likely indirectly, make their way over time into human pathogens via food, water, and sludge and manure applied as fertilizer. Horizontal (or lateral) gene transfer studies have identified conjugal mating as the most common means of genetic exchange, and there appear to be few barriers that prevent this gene sharing across a multitude of dissimilar genera (104).

While colonic bacteria have received much focused study, water environments such as aquaculture, sludge, freshwater, and wastewaters are prime sites for gene exchange but have been examined minimally for their roles as "mixing pots" and transporters of genes from bacteria of antibiotic-fed animals to humans (116). Aside from the already described impacts of NTA use on bacterial resistance, food animal use of NTAs has broad and far-reaching impacts on these environmental bacteria. It is estimated that 75% to 90% of antibiotics used in food animals are excreted, largely unmetabolized, into the environment (43, 105). Antibiotics or resistant bacteria have been detected in farm dust (81), the air currents inside and emanating from swine feeding operations (41, 72, 129), the groundwater associated with feeding operations (31, 37), and the food crops of soils treated with antibiotic-containing manure (54). This leaching into the environment effectively exposes countless environmental organisms to minute quantities of antibiotic—enough to select bacteria with resistance mutations to promote the emergence and transfer of antibiotic resistance genes among diverse bacterial types (104). The potentially huge impact of all these residual antibiotics on the environmental bacteria that are directly or indirectly in contact with humans has scarcely been examined.

The multiple pathways and intricacies of gene exchange have so far thwarted attempts to qualitatively or quantitatively track the movement of these genes *in vivo*, and thus we are left with minimal direct evidence for linking resistance in animals to that in humans. With extensive gene movement between disparate hosts, it is less likely that the same bacterial hosts will be found in animals and humans and more probable that only the resistance genes themselves will be identifiable in the final pathogens that infect humans. Even these may be altered in their journey through multiple intermediate hosts (161) (Fig. 1). Mounting evidence exists in reports of complex gene "cassettes" which accumulate resistance genes and express multi-drug resistance (106, 125).

A few investigators have undertaken the challenging task of developing mathematical models in order to predict the impacts of NTAs on human disease (12, 19, 20, 46, 91, 99, 134, 135). Models can be very useful in attempting to define the types of diverse data sets that are seen in this field. Some explore the entire "farm-to-fork" transmission process, while others tackle only portions of this extremely complex chain or adopt a novel backwards approach which looks first at human infections and then calculates the fraction that are potentially caused by NTA use in animals. Most models are deliberately simplified and admittedly omit many aspects of transmission and persistence. Moreover, current models are frequently based on multiple assumptions and have been challenged on the basis of certain shortcomings, such as limitation to single pathogens only, the determination of lethality while ignoring morbidity, and dependence on estimates of probabilities (19). Chief among these, however, is the lack of a complete understanding of the contribution made by commensals, which may play an important role in augmenting the link between animals and humans. Some models are driven by findings of dissimilar strains in animals and humans and therefore arrive at very low probabilities for a causal link between the two (47). A finding of dissimilar strains, however, overlooks two possibilities. First, it does not exclude the existence of small subpopulations of homologous strains that have gone undetected within the gut floras of animals. These may have been amplified temporarily by antibiotic selection and transferred their mobile genetic elements in multiple complex pathways. Subsequently, they may have declined to nondetectable levels or merely been outcompeted by other variants. Second, it overlooks dissimilarities that evolve as genes and their hosts migrate in very complex ways through the environment. Figure 1 illustrates the difficulties in tracking a resistance gene, since these genes are frequently captured in bacteria of different species or strains
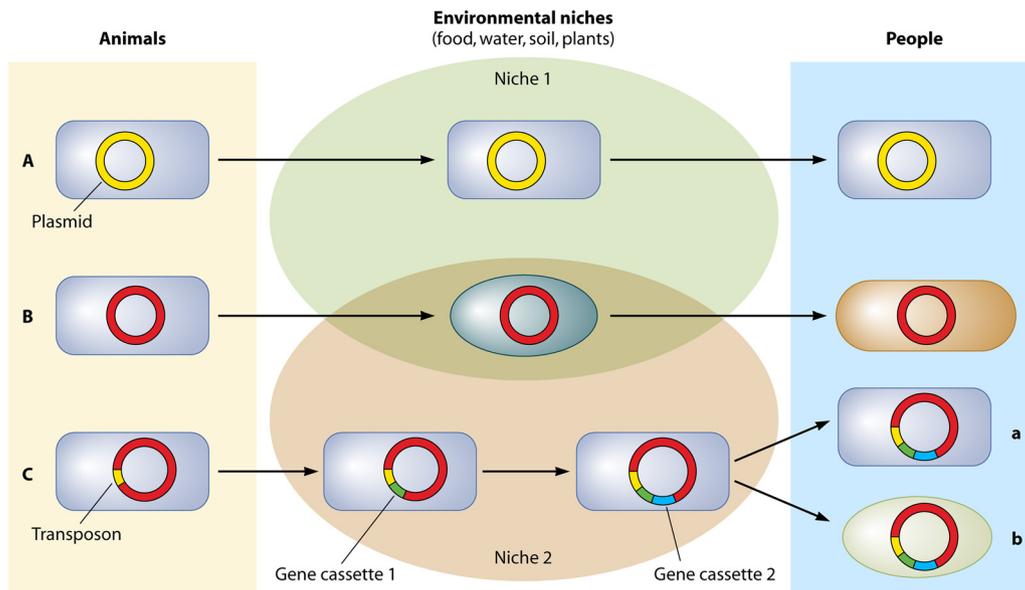
FIG. 1. Several scenarios may present themselves in the genetic transport that occurs as bacteria migrate from animal to human environments. (A) The same host and its indigenous genes in animals are transported unchanged to humans, with a resulting 100% match of the bacterial strain. (B) The genetic structure passes through one or more different hosts, ending in a new host (humans), with a resulting 100% match of DNA. (C) The host and its plasmid-borne genes pass through the environment, picking up gene cassettes en route, with a resulting 100% match for the host only (a) or a low-% match for DNA only (b). In both examples, the plasmid core remains the same.

which no longer resemble the original host. Over time, even the genes themselves may undergo mutations or become entrapped in gene cassettes that alter their genetic landscape. State-of-the-art technology and thoughtful investigation are often necessary to identify and track the actual strains that link animals and humans. These are facets of modeling that have yet to be explored, and obtaining direct evidence for the origins of specific genes can be highly challenging.

In general, the weaknesses of present models lie in their simplicity and the lack of crucial knowledge of microbial loads at each stage of the "farm-to-fork" transmission chain. Many of the available studies that examine links between animals and humans suffer from a failure to examine the antibiotic use practices for the farm animals they investigate. More powerful evidence could have accumulated that would aid in modeling efforts if data on the quantities and uses of farm antibiotics had been reported. These oversights are often due to the lack of registries that record and report the utilization of antibiotics on food animal farms. It is widely advocated that surveillance studies of resistance frequencies at all levels of the transmission chain would aid greatly in reducing our knowledge deficits and would help to inform risk management deliberations (23, 34). A number of localized and international surveillance systems exist for the tracking of human pathogens. In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) has become instrumental in the monitoring of resistance trends in pathogens found in food animals, retail meats, and humans (73). However, at the level of commensals, resistance monitoring is still in its infancy. The Reservoirs of Antibiotic Resistance (ROAR) database (www.roarproject .org) is a fledgling endeavor to promote the accumulation of data that specifically focus on commensal and environmental strains as reservoirs of antibiotic resistance genes. With ad-

vances in detection at the genetic level, the potential for tracking the emergence and spread of horizontally transmissible genes is improving rapidly. By capturing geographic, phenotypic, and genotypic data from global isolates from animal, water, plant, and soil sources, the ROAR project documents the abundance, diversity, and distribution of resistance genes and utilizes commensals as "barometers" for the emergence of resistance in human pathogens.

CONCLUSIONS

Data gaps continue to fuel the debate over the use of NTAs in food animals, particularly regarding the contribution and quantitation of commensal reservoirs of resistance to resistance in human disease. Nonetheless, it has been argued reasonably that such deficits in surveillance or indisputable demonstrations of animal-human linkage should not hinder the implementation of a ban on the use of nontherapeutic antibiotics (23). Food animals produce an immense reservoir of resistance genes that can be regulated effectively and thus help to limit the negative impacts propagated by this one source. In the mathematical model of Smith et al., which specifically evaluates opportunistic infections by members of the commensal flora, such as enterococci, it was concluded that restricting antibiotic use in animals is most effective when antibiotic-resistant bacteria remain rare. They suggest that the timing of regulation is critical and that the optimum time for regulating animal antibiotic use is before the resistance problem arises in human medicine (134).

A ban on nontherapeutic antibiotic use not only would help to limit additional damage but also would open up an opportunity for better preservation of future antimicrobials in an era when their efficacy is gravely compromised and few new ones

are in the pipeline. Although the topic has been debated for several decades without definitive action, the FDA has recently made some strides in this direction. Officially, the organization now supports the conclusion that the use of medically important antimicrobials for nontherapeutic use in food animal production does not protect and promote public health (131). Although not binding, a guidance document was released in 2010 that recommended phasing in measures that would limit use of these drugs in animals and ultimately help to reduce the selection pressures that generate antimicrobial resistance (66).

The Danish experience demonstrated that any negative disease effects resulting from the ban of NTAs were short-lived and that altering animal husbandry practices could counter expected increases in disease frequency (6). For aquaculture, also, it has been demonstrated that alternative processes in industry management can be instituted that will reduce antibiotic use without detrimental financial effects (141). Still, it has been argued by some in animal husbandry that the different situation in the United States will result in increased morbidity and mortality, projected to cost $1 billion or more over 10 years. Again, however, the Danish postban evaluation found that costs of production increased by just 1% for swine and were largely negligible for poultry production due to the money saved on antibiotics themselves. Models also showed that Danish swine production decreased by just 1.4% (1.7% for exports), and poultry production actually increased, by 0.4% (0.5% for exports) (158). Such calculations still fail to consider the negative externalities that are added by the burden of antibiotic resistance and the antibiotic residue pollution generated by concentrated animal feeding operations.

Opponents of restriction of NTA use argue that a comprehensive risk assessment is lacking, but such an analysis is impossible without the kind of data that would come out of surveillance systems. Although surveillance systems have been advocated repeatedly (23, 70), such systems are sparse and extremely limited in their scope.

In 2002, working with the accumulated evidence and an assessment of knowledge deficits in the area of animal antibiotic use, the APUA developed a set of guidelines that are still viable today and can be used to guide both policy and research agendas. In summary, APUA recommended that antimicrobials should be used only in the presence of disease, and only when prescribed by a veterinarian; that quantitative data on antimicrobial use in agriculture should be made available; that the ecology of antimicrobial resistance in agriculture should be a research priority and should be considered by regulatory agencies in assessing associated human health risks; and that efforts should be invested in improving and expanding surveillance programs for antimicrobial resistance. Suitable alternatives to NTAs can be implemented, such as vaccination, alterations in herd management, and other changes, such as targeted use of antimicrobials with a more limited dosage and duration so as not to select for resistance to critical human therapeutics (23).

There is no doubt that human misuse and overuse of antibiotics are large contributors to resistance, particularly in relation to bacteria associated with human infection. Interventions in medical settings and the community are clearly needed to preserve the efficacy of antibiotics. Efforts in this area are being pursued by the Centers for Disease Control and Prevention, the Alliance for the Prudent Use of Antibiotics, the American Medical Association, the American Academy of Pediatrics, the Infectious Diseases Society of America, and other professional groups. Still, given the large quantity of antibiotics used in food animals for nontherapeutic reasons, some measure of control over a large segment of antibiotic use and misuse can be gained by establishing guidelines for animals that permit therapeutic use only and by then tracking use and health outcomes.

The current science provides overwhelming evidence that antibiotic use is a powerful selector of resistance that can appear not only at the point of origin but also nearly everywhere else (104). The latter phenomenon occurs because of the enormous ramifications of horizontal gene transfer. A mounting body of evidence shows that antimicrobial use in animals, including the nontherapeutic use of antimicrobials, leads to the propagation and shedding of substantial amounts of antimicrobial-resistant bacteria—both as pathogens, which can directly and indirectly infect humans, and as commensals, which may carry transferable resistance determinants across species borders and reach humans through multiple routes of transfer. These pathways include not only food but also water and sludge and manure applications to food crop soils. Continued nontherapeutic use of antimicrobials in food animals will increase the pool of resistance genes, as well as their density, as bacteria migrate into the environment at large. The lack of species barriers for gene transmission argues that the focus of research efforts should be directed toward the genetic infrastructure and that it is now imperative to take an ecological approach toward addressing the impacts of NTA use on human disease. The study of animal-to-human transmission of antibiotic resistance therefore requires a greater understanding of the genetic interaction and spread that occur in the larger arena of commensal and environmental bacteria.

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