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Review

Antibiotic Resistance in Bacteria Associated with Food Animals: A United States Perspective of Livestock Production

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ABSTRACT

The use of antimicrobial compounds in food animal production provides demonstrated benefits, including improved animal health, higher production and, in some cases, reduction in foodborne pathogens. However, use of antibiotics for agricultural purposes, particularly for growth enhancement, has come under much scrutiny, as it has been shown to contribute to the increased prevalence of antibiotic-resistant bacteria of human significance. The transfer of antibiotic resistance genes and selection for resistant bacteria can occur through a variety of mechanisms, which may not always be linked to specific antibiotic use. Prevalence data may provide some perspective on occurrence and changes in resistance over time; however, the reasons are diverse and complex. Much consideration has been given this issue on both domestic and international fronts, and various countries have enacted or are considering tighter restrictions or bans on some types of antibiotic use in food animal production. In some cases, banning the use of growth-promoting antibiotics appears to have resulted in decreases in prevalence of some drug resistant bacteria; however, subsequent increases in animal morbidity and mortality, particularly in young animals, have sometimes resulted in higher use of therapeutic antibiotics, which often come from drug families of greater relevance to human medicine. While it is clear that use of antibiotics can over time result in significant pools of resistance genes among bacteria, including human pathogens, the risk posed to humans by resistant organisms from farms and livestock has not been clearly defined. As livestock producers, animal health experts, the medical community, and government agencies consider effective strategies for control, it is critical that science-based information provide the basis for such considerations, and that the risks, benefits, and feasibility of such strategies are fully considered, so that human and animal health can be maintained while at the same time limiting the risks from antibiotic-resistant bacteria.

INTRODUCTION

ANTIMICROBIAL AGENTS HAVE BEEN widely used in livestock and poultry since the 1950s. Since that time, food animal production has increasingly included larger farms and greater animal densities, requiring a greater need for disease management. Improved health management in modern livestock production has occurred, in part, due to the introduction of antimicrobial products (NRC, 1999). At least 17 classes of antimicrobials are approved for use in food animals in the United States (Anderson et al., 2003). Table 1 provides a summary of antibiotics approved for the various livestock species in the United States. Antimicrobial prod-

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ucts work through a variety of bacteriostatic and bactericidal mechanisms that include inhibition of cell wall and cell membrane synthesis, protein synthesis, folate synthesis, and DNA synthesis (Barton, 2000; Khachatourians, 1998). Some antibiotics primarily target specific groups of bacteria, such as Gram positive, Gram negative, anaerobic, and aerobic organisms. Others may be used to treat a broad spectrum of organisms when it is not possible or economically feasible to determine the causative agent.

Antibiotics are used in food animals for four main purposes: therapeutic use to treat sick animals; metaphylaxis, or short-term medication to treat diseased animals and prevent infection in other animals; prophylactic use to prevent infections at times of risk, such as transport or weaning; and growth promotion to improve feed utilization and production (McEwen and Fedorka-Cray, 2002; Viola and DeVincent, 2006). The last of these uses originated from the early discovery that such compounds resulted in growth benefits for livestock beyond those directly associated with treatment for disease (Hardy, 2002; Kiser, 1976). Researchers noted that chickens administered vitamin B_{12}, in the form of crude *Streptomyces aureofaciens* fermentations, experienced increased growth compared to birds that were fed a diet containing purified B_{12} (Stokstad et al., 1949). It was concluded that the crude fermentations contained an unidentified growth factor, later identified as chlortetracycline (Stokstad and Jukes, 1950). Shortly after the report by Stocktad and Jukes, the United States Food and Drug Administration (FDA) approved the inclusion of certain antibiotics in livestock diets for growth promotion and disease prevention (Kiser, 1976). Of all types of antibiotic application, use for growth enhancement in livestock has been most controversial (Viola and DeVincent, 2006).

The total quantity of antimicrobial agents used in animals in the United States is not known with certainty. The Animal Health Institute (AHI) reported that member companies sold more than 9,170 tonnes (10,108 tons) of antimicrobial products in the United States in 2003, with ionophores/arsenicals, tetracyclines, cephalosporins and macrolides, sulfonamides and penicillins, aminoglycosides, and fluoroquinolones representing 42.8%, 32.6%, 18.9%, 3.7.%, 1.8%, and 0.2% of the total, respectively. It was also estimated that 92% of the total use was for therapeutic purposes (AHI, 2005). However, estimates have varied widely with regard to the relative amounts of antimicrobials used in agriculture. For example, Carnevale (2000) reported that of the 22.7 million kg of antibiotics produced in the United States, approximately 17.8% were used in animal production. In contrast, Mellon et al. (2001) indicated that approximately 70% of antibiotics produced were used in livestock. Confounding this issue are the various ways in which quantities of antibiotics are defined for various estimates—as weight of active ingredients, as total weight of feed additive (including carriers and/or complexes) and, in some cases, as total weight of feed supplements that may contain antimicrobial products.

THE ORIGIN OF ANTIBIOTIC RESISTANCE

A number of bacterial and fungal species possess the ability to produce antimicrobial compounds, typically to gain a competitive advantage in microorganism-rich environments, including soils and biofilms (Amabile-Cuevas and Chicurel, 1992). It is thus likely that naturally occurring antibiotics have been in the environment since before higher organisms evolved. Many antimicrobial products used in human and animal medicine today have their origins in antibacterial compounds produced by organisms such as *Streptomyces*, *Bacillus*, *Pennicillium*, *Cephalosporium*, and *Pleurotus*. As researchers have identified these compounds and their active components, development of more potent analogs has become possible (Schwarz et al., 2006).

Antibiotic resistance likely also emerged in nature prior to human use of drugs, as organisms producing antibiotic compounds required the means to survive in the presence of their own products, and competing species also found ways to counteract effects of those compounds (Davies, 1997). Thus, some resistance genes likely originated long before the advent of man, modern medicine, and agricultural use

Table 1. Antibacterial Products Approved for Use in Livestock in the United States[a]

Drug	*Antibiotic family*	*Animals used in*	*May be used in feed*	*Used in human medicine*
Amoxicillin	β-lactam	B, D, P, S	No	Yes
Ampicillin	β-lactam	B, D, P, S	No	Yes
Apramycin	Aminoglycoside	S	Yes	No[b]
Arsanilic acid	Arsenical	P	Yes	No
Avilamycin	Orthosomycin	S	Yes	No
Bacitracin	Bacitracin	B, D, P, S	Yes	Yes
Bambermycin	Bambermycin	B, D, P, S	Yes	No
Carbadox	Quinoxaline	P, S	Yes	No
Ceftiofur	Cephalosporin	B, D, P, S	No	No[b]
Chlortetracycline	Tetracycline	B, D, P, S	Yes	No[b]
Cloxacillin	β-lactam	B, D	No	Yes
Colistin	Polypeptide	P	Yes	Yes
Danofloxicin	Fluoroquinolone	D	No	No[b]
Efrotomycin	Elfamycin	S	No	No
Enrofloxacin	Fluoroquinolone	B	No	No[b]
Erythromycin	Macrolide	B, D, P, S	No	Yes
Florfenicol	Phenicol	B, D, P	No	No[b]
Gentamicin	Aminoglycoside	B, D, P, S	No	Yes
Hygromycin	Aminoglycoside	P, S	Yes	No
Lincomycin	Lincosamine	B, D, P, S	Yes	Yes
Neomycin	Aminoglycoside	B, D, P, S	Yes	Yes
Novobiocin	Novobiocin	B, D, P	Yes	No
Oleandomycin	Macrolide	B, D	No	No
Oxytetracycline	Tetracycline	B, D, P, S	Yes	Yes
Penicillin	β-lactam	B, D, P, S	Yes	Yes
Pirlimycin	Lincosamine	B, D	No	No[b]
Polymyxin	Polypeptide	B, D	Yes	Yes
Roxarsone	Arsenical	B, D, P, S	Yes	No
Spectinomycin	Aminocyclitol	B, D, P, S	No	Yes
Sulfachlorpyridizine	Sulfonamide	B, D, S	No	No[b]
Sulfadimethoxine	Sulfonamide	B, D, P, S	No	No[b]
Sulfaethoxypyridazine	Sulfonamide	B, D, P, S	No	No[b]
Sulfamethazine	Sulfonamide	B, D, P, S	Yes	No[b]
Sulfathiazole	Sulfonamide	B, D, S	Yes	No[b]
Tetracycline	Tetracycline	B, D, P, S	No	Yes
Tiamulin	Diterpene	S	Yes	No
Tilmicosin	Macrolide	P, S	Yes	No[b]
Tylosin	Macrolide	B, D, P, S	Yes	No[b]
Tulathromycin	Triamilide	B, D, S	No	No
Virginiamycin	Streptogrammin	P, S	Yes	No[b]

[a]Adapted from CVP, 2006; Guardabassi and Couravalin, 2006; FDA, 2006b.
[b]Closely related analogs are used in and are of importance to human medicine.
B, beef cattle; D, dairy cattle; P, poultry; S, swine.

of antibiotics. As antibiotic use became commonplace in human medicine and food animal production, selection pressure increased the advantage of maintaining resistance genes in diverse groups of bacteria, and bacterial evolutionary progress eventually included mechanisms to retain, accumulate, and disperse resistance genes among bacterial populations (Aarestrup, 2006). However, use of antibiotics in human medicine and animal production may promote dissemination of resistance genes beyond that expected as a direct consequence of selective pressure on target organisms. For example, D'Costa et al. (2006) suggest that soil microbes provide a large reservoir of antibiotic resistance genes that can be quickly mobilized into other microbial communities, including enteric bacteria and pathogens, under the selection of antibiotic use. Other research suggests that dissemination of resistance genes has

been accelerated due to the presence of bacterial DNA carrying resistance genes in antibiotic preparations (Benveniste and Davies. 1973). Webb and Davies (1993), using PCR amplification of known resistance genes, demonstrated that contaminating DNA from *Streptomyces* spp. used in industrial production of antibiotics resulted in a source of resistance genes in commercial antibiotic preparations.

Among the first reports to suggest that antibiotic use in livestock promoted resistance was that of Starr and Reynolds (1951), who noted streptomycin resistance in coliform bacteria from turkeys that had been fed that antibiotic. Since that time, numerous studies have demonstrated a link between antibiotic use in livestock and increased prevalence of antibiotic-resistant organisms associated with those animals, the farm environment and, in some cases, agricultural products (McEwen and Fedorka-Cray, 2002; Witte, 2000).

THE DEBATE OVER ANTIBIOTIC USE IN AGRICULTURE

With concern over antibiotic resistance growing in the 1960s, a number of organized deliberations on the issue occurred, including the Netherthorpe committee (Netherthorpe Committee, 1962) and the Swann committee (Swann et al., 1969). Both groups focused specifically on antibiotic use in food animals, and came to different conclusions regarding the risk to human health. The Netherthorpe report concluded there was no evidence that agricultural use posed a risk to humans, whereas the Swann committee concluded otherwise and indicated that the administration of antibiotics to livestock, particularly at nontherapeutic levels, posed a significant hazard to human and animal health.

Among the recommendations of the Swann committee were that antibiotics used for livestock production be available by prescription only and in-feed antibiotics should be limited to 100 ppm. Additionally, the committee recommended that surveillance programs be established to monitor antibiotic resistance in bacteria of concern. The Swann report spawned much debate among the scientific community, as some noted the findings were partly based on anecdotal evidence or studies with little scientific rigor, and in some cases were more presumptive than substantive. This debate resulted in an increase in studies to investigate the issue of antibiotic use in livestock and the risks associated with such use.

Over subsequent decades, other organizations became active in the debate. Among the most notable was the American Society of Microbiology (ASM), which formed the Task Force on Antibiotic Resistance, consisting of scientists from academia, the government, and industry. Their initial report focused on critical issues and risks posed by the widespread and growing use of antibiotics in human medicine and agricultural production. This report provided a comprehensive set of recommendations for surveillance programs, as well as recommendations to address emerging resistant organisms and the development of new drugs and nonantibiotic therapies (ASM, 1995). In 1997, the World Health Organization (WHO) released a report that provided a strong statement against the use of antibiotics for growth enhancement, indicating that such use is particularly conducive to selection for resistant bacteria (WHO, 1997). Later reports included the WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food, which was formulated jointly with the Office of International des Epizooties (OIE) and the United Nations Food and Agricultural Organization (FAO) (WHO, 2000). These principles were intended to provide a framework of recommendations to reduce the overuse and misuse of antibiotics for the protection of human health, including recommendations for pre-approval, manufacturing, distribution, sales, and prudent use of drugs; surveillance of resistance; and the education of veterinarians and producers regarding the use and hazards of food animal antibiotics. The report also recommended that, in the absence of risk assessments, growth promoting antibiotics that are also used in human medicine should be rapidly phased out, preferably through voluntarily programs but, if necessary, by legislation. In 2002, the American Academy of Microbiology, representing the highest leadership within the ASM, issued a report titled *The Role*

of Antibiotics in Agriculture (Isaacson and Torrence, 2002). Among its recommendations were a call for better estimates of antibiotic use in livestock and aquaculture production, the need for research into the economics of growth promoting antibiotics, a call for wider dissemination and education of the principles of judicious use among veterinarians and producers, and more research into reservoirs of resistance, resistance transfer, and quantitative risk assessments.

Federal agencies also addressed the issue of antibiotic resistance. In 1997, surveillance, educational, and research initiatives to address antimicrobial resistance in foodborne pathogens were expanded through funds provided by the President's Food Safety Initiative (Torrence, 2001). In 1999, an interagency task force on antimicrobial resistance was formed by the Centers for Disease Control (CDC), the National Institutes of Health (NIH), and the FDA. In 2001, the task force released the Public Health Action Plan to Combat Antimicrobial Resistance, whose main aspects included surveillance, prevention and control, research, and product development related to antimicrobial resistance (CDC, 2001). The task force continues to expand in scope, and in partnership with other national and international agencies, addresses high priority issues relevant to antibiotic resistance (CDC, 2006). Soon after establishment of the task force, the FDA directly addressed the issue of risks caused by use of antibiotics in food animals with the release of the Guidance for Industry 152, which outlined steps for risk assessment in the evaluation of new animal drugs in terms of microbial food safety (FDA, 2003). While not mandated, the steps suggested by Guidance 152 have provided clear direction for pharmaceutical companies to assess the potential for emergence and selection of resistant foodborne pathogens as a result of use of the drug.

SURVEILLANCE PROGRAMS

In response to calls for more rigorous monitoring of antibiotic resistance, a number of countries have established surveillance programs (McEwen and Fedorka-Cray, 2002). Among the primary goals of such programs are improved detection of emerging antimicrobial resistance, prolonging the useful life of antimicrobial drugs, and providing guidance for the development and use of new drugs. The Danish Ministry of Food, Agriculture, and Fisheries and the Danish Ministry of Health provided one of the first models of a national surveillance program with the establishment in 1995 of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). The objectives of this program include monitoring antibiotic use, resistance prevalence, and changes over time, and investigating associations between antibiotic use and resistance prevalence among bacteria associated with animals and humans (DANMAP, 2005). A similar program was initiated in the United States in 1996 with the establishment of the National Antimicrobial Resistance Monitoring System (NARMS), a cooperative effort of the FDA, the United States Department of Agriculture, and the CDC. The stated objectives of the program are to provide data on the prevalence and temporal trends of drug resistance in enteric bacteria of concern; to facilitate identification of antibiotic resistance as it arises; and to provide timely information to veterinarians and physicians regarding resistance patterns of pathogens. NARMS has provided much data regarding antibiotic resistance prevalence and temporal changes in clinical isolates and isolates from healthy specimens. In 2002, surveillance of retail meat isolates was initiated as a part of NARMS, as were pilot studies of animal feed ingredients.

While surveillance data have provided a basis to assess trends in antibiotic resistance and those data have been used in the development of resistance risk assessments (Hurd et al., 2004), current surveillance programs do not yet provide adequate information regarding the defined hazard of antibiotic resistance, which is the failure of a therapy due to a specific drug resistance in humans. An analysis of prevalence and temporal trends of treatment applications, complications, and failures, and how these relate to antibiotic resistance in bacteria of concern might provide more definitive information regarding the true risks of agricultural, companion animal, and human use of an-

tibiotics; particularly as antibiotic resistance by a pathogen, as measured by current standards, does not always translate into treatment failure (Phillips et al., 2003). Garnering appropriate and adequate information of this type would be a considerable task and require much greater participation from the medical community.

Information gained from surveillance programs has continued to fuel the debate regarding risks and benefits of antibiotic use in animal agriculture. While there is general agreement that the prevalence of some antibiotic resistance is increasing on farms due to antibiotic use, specific examples to the contrary are also evident. For example, NARMS data indicate that *Campylobacter jejuni* veterinary isolates from chickens are becoming increasingly resistant to ciprofloxacin (9.4% of isolates in 1998 versus 15% of isolates in 2005), while resistance to azithromycin, chloramphenicol, clindomycin, erythromycin, gentamicin, and tetracycline has not increased, or in some cases has even decreased, over that same time period (NARMS, 2005).

In Denmark, where growth promoting antibiotics were curtailed in 1998 and eventually banned in 2000, glycopeptide and avilamycin resistance in *Enterococci faecium* from broilers, and macrolide resistance in *E. faecium* from pigs, have declined markedly. However, tetracycline resistance in *E. faecium* from pigs is similar to levels prior to the ban. Sulfonamide and tetracycline resistance in *Escherichia coli* from pigs has also remained comparable to levels before the ban was imposed, and ampicillin resistance has increased (DANMAP, 2005). During the same period, *Salmonella* Typhimurium isolated from pigs demonstrated increased resistance to chloramphenicol, ampicillin, tetracycline, and sulfonamides. The percentage of multi-resistant *Salmonella* strains also increased from 6.3% in 1999 to 21.4% in 2005: this increase could not be fully explained by the occurrence of phage types DT104, 104b, or DTU302, which comprised only 6.5% of the total. The existence of multiple resistance genes in clusters, or in integrons, could partially explain this phenomenon, as closely linked resistance genes may result in selection for resistance to one antibiotic while causing the persistence of genes conferring resistance to unrelated drugs, including antibiotics that are no longer used. However, these data point to the need for a selective approach towards curtailment of antibiotic use, based on antibiotic type, bacteria of concern, and perceived risks and benefits of implementing greater restrictions or bans on drug use.

ANTIBIOTIC RESISTANCE IN BACTERIA OF CONCERN

It has been reported that 76 million people contract a foodborne illness annually in the United States (Mead et al., 1999) at an annual cost of nearly $6.9 billion (Allos et al., 2004). In severe cases, treatment of foodborne illness may require the use of antibiotics, making antibiotic resistance in foodborne pathogens a considerable concern. Foodborne pathogens, including *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *Yersinia* spp., and certain strains of *E. coli*, may be harbored by livestock and passed to humans through the food chain. Because of their common occurrence in livestock and the importance of these organisms to food safety, pre-harvest food safety research has focused primarily on enteric foodborne organisms. However, other bacteria, such as *E. faecalis* and *E. faecium*, which are also natural residents of the gastrointestinal (GI) tract, can pose zoonotic risks via environmental routes, and can cause nosocomial infections upon entry into surgical incisions or wounds.

Resistant strains of *Enterococcus* have become a major concern for the medical community as the number of infections caused by these organisms has increased dramatically over the past two decades (Huycke et al., 1998; Treitman et al., 2005). Some have suggested that agricultural use of antibiotics is partially to blame for this phenomenon (Bruinsma et al., 2005), and such organisms are sometimes also included in surveillance programs.

The relevance of antibiotic resistance among commensal bacteria also continues to be debated. It has been suggested that selection for antibiotic resistance in the nonpathogenic enteric microflora may provide for an additional reservoir of resistance genes (Levy, 1987; Lip-

sitch et al., 2002). As nontarget enteric microflora are regularly exposed to a variety of antibiotics, this may lead to increased prevalence of resistance factors on plasmids, transposons, and integrons (Salyers and Shoemaker, 1996; Tenover, 2001), facilitating transfer to pathogenic bacteria, resulting in a greater global prevalence of resistant pathogens (Angulo et al., 2004). Blake et al. (2003) simulated conditions of the porcine ileum *in vitro* to culture multi-resistant commensal *E. coli* strains along with sensitive pathogenic strains of Enterobacteriaceae, and observed transfer of resistance between the two groups, demonstrating that such exchange of resistance genetic elements can occur in the gut of animals. However, *in vitro* systems cannot fully simulate the complex interaction and competition among the hundreds of bacterial species making up the natural flora of the gut, and may not accurately reflect transfer rates, selection for, and persistence of, resistance genes *in vivo*. Thus far, few investigations have conclusively shown that such transfers occur to a significant degree in the living animal.

FACTORS AFFECTING RESISTANCE PREVALENCE

Selection of antibiotic resistance has been attributed primarily, but not exclusively, to nontherapeutic and growth promoting uses of antibiotics in livestock feeds (McEwan and Fedorka-Cray, 2002). However, factors other than antibiotics may also be involved in the selection for or prevalence of resistant bacteria. Animal stressors, including weaning and transport, have been reported to increase prevalence of resistant bacteria in pigs, regardless of whether antibiotics are used. Arnett et al. (2003) collected fecal samples from pigs prior to and immediately following transport and observed a greater prevalence of resistant Gram negative bacteria following transport. Langlois and Dawson (1999) had earlier noted such transportation effects and speculated that temporary selective shedding of resistant isotypes might occur during stress. Other experiments indicate that *E. coli* express increased resistance to antibiotics following weaning in pigs, regardless of antibiotic use (Mathew et al., 2001). It is possible that changes in feed intake, gut physiology, or gut motility, as a result of stress episodes, may affect the GI environment, causing an increased ability of bacteria to acquire resistance genes, or allow resistant subtypes, which may naturally occur in low numbers, to gain an advantage and increase in number. Additionally, increased prevalence of resistance caused by changes in the microbial environment may be linked to stress-induced genes, which may occupy the same genetic elements in the bacterium as the resistance factors. Selecting for stress-resistant isolates may indirectly select for resistant organisms. Efflux systems, by which bacteria can pump offending compounds out of the cell, may also result in increased activity under such circumstances, providing greater resistance to a broad range of antibiotics (Webber and Piddock, 2003).

Luo et al. (2005), using an experimental model in chickens, noted that a point mutation in the *gyrA* gene, which imparts high resistance to fluoroquinolones in *Campylobactor jejuni*, may also provide for survival fitness of that organism in the absence of antibiotic use. When co-inoculated with nonmutated strains into chickens, the *gyrA* mutant was able to outcompete some fluoroquinolone-sensitive strains in the absence of antibiotic application. Groh et al. (2007) noted that the presence of a multidrug resistance pump coded by the *MexF* gene can enhance ecological fitness of nonpathogenic *Shewanella oneidensis* MR-1 in sediments. As this bacterium was isolated from an environment without pharmaceutical impact, it was suggested that the *Mex* system increases survival fitness by preventing toxic accumulation of naturally occurring antibiotics released by competing microorganism in the same environment, or protects from humic acid or other toxic chemicals in that environment. This would suggest that, in some cases, resistance genes also impart unknown advantages beyond those associated with selective advantage under antibiotic use. Others have suggested that clonal dissemination of multi-resistant organisms such as *Salmonella* may be more important in regional trends in resistance than selective pressure of antibiotic use (Davis et al., 2002).

Other factors, including dose and duration of antibiotic application, bacterial energetics, metabolic costs, and yet unknown factors may play roles in selection for and persistence of resistant populations, and such factors may be antibiotic-specific due to explicit mechanisms of the antibacterial effects. Shojaee AliAbadi and Lees (2000) indicated that development of antibiotic dosing regimens to minimize selection for resistance requires knowledge of the specific microbe biochemistry, structure, resistance mechanisms, mode(s) of transfer, population dynamics, and pharmacodynamics and pharmacokinetics. To determine optimal drug efficacy while minimizing the risk of resistance also requires complex mathematical modeling and robust statistical analysis to account for variations in response across the genetic range of microbial populations targeted (Shojaee AliAbadi and Lees, 2000; Toutain, 2003). There remains a great need for applied studies to determine how specific dosing regimens, drug rotations, timing, and method of antibiotic application affect resistance prevalence, and those studies will need to be conducted over the wide range of antibiotics currently in use. Without such information, it will be difficult to formulate effective strategies for control of resistant organisms in livestock production.

MECHANISMS OF RESISTANCE DEVELOPMENT AND TRANSFER

Microbial populations develop resistance through several mechanisms, including mutation, the rate of which may be determined by environmental factors, cell physiology, bacterial genetics, and population dynamics (Martinez and Baquero, 2000). Antimicrobial resistance in bacteria may also be acquired laterally or horizontally through several gene transfer mechanisms. Transformation occurs via the uptake from the immediate surroundings of exogenous DNA that becomes incorporated into the host genome (Roe and Pillai, 2003). Conjugation results from the transfer of plasmids, which are typically exchanged between bacteria through physical contact. Such exchange may occur between related and unrelated bacteria (Yan et al, 2003). Transduction, another gene transfer mechanism, is facilitated by bacteriophages that inject their DNA into the genome of a host bacterium, after which replication and re-packaging of the bacteriophage DNA occurs. In that process, bacterial DNA may be incorporated into the viral DNA and after dispersion of new bacteriophages and injection of repackaged DNA into new hosts, resistance genes from the original host may be disseminated into a new population (Dzidic and Bedekovic, 2003). Transposons are genetic elements that promote selfexcision and reinsertion into the genetic code of new hosts, providing another means of horizontal gene transfer (Roe and Pillai, 2003). It has been suggested that transposons may play a significant role in the development of antimicrobial resistance because they often contain resistance genes, including integrons (Stokes and Hall, 1989).

Integrons

Integrons are believed to play a major role in the rapid dissemination of multi-drug resistance (MDR) among bacteria (Ochman et al., 2000). Two major groups of integrons exist. Super integrons (SI) are located on chromosomes and may contain hundreds of gene cassettes encoding for a variety of functions (Rowe-Magnus et al., 2001). Resistance integrons (RI) typically carry gene cassettes encoding resistance to antibiotics and disinfectants and are currently divided into three classes, based on variations in sequence of primary elements, gene cassettes, and associations with transposons (Ahmed et al., 2005; Collis et al, 2002; Fluit and Schmitz, 2004). RI can occur on bacterial chromosomes or plasmids, and most common are class 1 integrons, which possess two conserved segments separated by a variable region that often includes resistance genes. The construct of class 1 integrons includes a conserved *intI* (integrase) gene and a complementary strand containing a common promoter region that is directed toward the site of integration (Levesque et al., 1995; Ouellette and Roy, 1987). Site-specific recombinations occur within integrons, enabling promotorless cassettes encoding for a wide range of resistance genes to be inserted downstream of the integron promoter region, providing for simultaneous resistance to multiple antibiotics (Fluit and Schmitz, 2004). Integrons can be exchanged indiscrimi-

nately between similar bacteria as well as among bacteria of different taxa (Johnson et al., 1994; Zhao et al., 2001), thus causing concern for wide dissemination of these genetic resistance elements.

Class 1 integrons are often found in bacteria associated with livestock. In a study conducted by Singh and coworkers (2005), 16% of shiga-toxin producing *E. coli* isolated from poultry, cattle, swine, and humans possessed class 1 integrons conferring resistance to ampicillin, chloramphenicol, cephalothin, gentamicin, tetracycline, trimethoprim, sulfamethoxazole, and streptomycin. Other studies have also shown a high frequency of class 1 integrons in *E. coli* isolated from dairy cows, which conferred resistance to tetracycline, streptomycin, and sulfonamides (Lanz, et al., 2003; Murinda et al., 2005). Phongpaichit et al. (2007) collected *E. coli* fecal samples of pigs, pig farmers, and nonfarmers in southern Thailand and analyzed them for the presence of class 1 integrons: 56.8% of isolates from market pigs, 45.2% of isolates from pig farmers, and 35.7% of isolates from nonfarmers contained class 1 integrons. However, many of the resistance patterns could not be explained by the presence of integrons; in fact, all of the integron-negative isolates were found to be resistant to at least three antibiotics.

Class 1 integrons are also common in *Salmonella*. The 5-way antibiotic resistance pattern of S. Typhimurium DT104 is encoded by a sequence within the Salmonella genomic island 1 (SGI1) that includes a class 1 integron coding for resistance to sulfonamides, amipicillin, and streptomycin. The MFR associated with *S*. Typhimurium DT104 first brought attention to the role of integrons in persistent multi-resistant bacteria of animal origin. In recent years, other *S. enterica* serovars, including *S*. Newport, have gained prominence as multi-resistant foodborne pathogens harboring class 1 integrons (Angulo et al., 2004; Zhao et al., 2003).

ANTIBIOTIC USE AND RESISTANCE ASSOCIATED WITH DAIRY CATTLE

In dairy production, antimicrobial products are typically used for therapeutic purposes. Common diseases treated with antibiotics include respiratory and diarrheal diseases in unweaned heifers, respiratory disease in weaned heifers, and mastitis in cows. More than 57% and 59% of dairy operations in the United States reported treating respiratory and diarrheal disorders with antibiotics, respectively. Mammary infections in mature cows were the primary reason for antibiotic use on dairy farms, with more than 85% of operations reporting antibiotic use to treat this disorder. Antibiotics used on dairies include aminoglycosides, beta-lactams, cephalosporins, florfenicol, macrolides, sulfonamides, tetracyclines, and ionophores. For mastitis, beta-lactams and cephalosporins are the drugs of choice, with more than 55% and 36% of affected cattle being treated with those drugs, respectively. Florfenicol and tetracyclines are more commonly used for respiratory diseases in unweaned and weaned heifers.

Resistant pathogens have been noted at significant levels in dairy cattle. Rajala-Schultz et al. (2004) found that more than 43% of coagulase-negative *Staphylococci* isolated from infected dairy cattle were resistant to at least one antibiotic. Resistance to penicillin was most common (31.7%), followed by ampicillin (12.2%), sulfadimethoxine (12.2%), tetracycline (11.5%), and erythromycin (7.9%). More than 20% of isolates were resistant to at least two antibiotics, and 7.9% were resistant to three or more antibiotics. Combined data for clinical and nonclinical *Salmonella* from dairy cattle indicate that the percentage of resistance for most of the tested antibiotics increased from 2001 (NARMS, 2001) (the first year in which isolates from dairy cattle were considered separately) to 2003 (NARMS, 2003). In the earlier report, 26.8%, 12.5%, 17.1%, 37.2%, and 29.6% of isolates were resistant to ampicillin, ceftiofur, chloramphenicol, streptomycin, and tetracycline, respectively; whereas in 2003, 62.9%, 49.5%, 56.4%, 63.2%, and 65.5% of isolates were resistant to those same drugs, respectively.

Berge et al. (2005) noted an increasing prevalence of MDR isolates in fecal *E. coli* from dairy calves from the time of weaning through six weeks postweaning, and that increase was associated with antibiotic use and with more intensive calf-rearing facilities. However, the effects of individual therapies on resistance appeared to be transitory. The investigators hy-

pothesized that antibiotics provided in milk replacers selected for resistant *E. coli* in the calf gut. Roesch et al. (2006) noted that the prevalence of antibiotic resistance in udder pathogens from cows raised on organic farms, where antibiotic use was restricted, did not differ from that of pathogens from cows raised on conventional dairy farms. Such information points to the need for more definitive studies to determine risk factors beyond antibiotic use that may select for resistant bacteria in dairy cattle.

ANTIBIOTIC USE AND RESISTANCE ASSOCIATED WITH BEEF CATTLE

Less research has been conducted in beef cattle with regard to antibiotic resistance than in swine, poultry, and dairy cattle. More than 2,000,000 kg of antimicrobial agents are said to be administered to beef cattle each year (Mellon et al., 2001). Antibiotics used in beef production typically include ionophores, chlortetracycline, sulfamethazine, tylosin, and virginiamycin (Inglis et al., 2005). Feedlot cattle typically receive antibiotics in feed for therapeutic purposes and increased performance. Medicated feed additives are sometimes applied during the weaning process of replacement heifer calves to prevent coccidiosis and increase feed efficiency. Chlortetracycline is often used to help maintain weight gain under conditions of respiratory challenge, such as in shipping, as well as aiding in the prevention of liver abscesses, diarrhea, and foot rot (Troxel and Gadberry, 2006). Ionophores are commonly applied to increase feed efficiency of cattle fed high concentrate diets, and tylosin and virginiamycin are also commonly used as feed additives (Inglis et al., 2005).

Animal age seems to be a defining factor for the prevalence of resistant *E. coli* in beef cattle. It has been shown that young animals have a higher prevalence of resistant fecal *E. coli* than older cattle, and carriage of ampicillin-resistant *E. coli* by young calves has been shown to decline with age (Hoyle et al., 2004a). A study performed by Hoyle et al. (2004b) found that calves rapidly acquired isolates resistant to nalidixic acid, apramycin, and ampicillin within a few weeks of birth. In another study, it was found that 13% of *E. coli* isolated from scouring calves was resistant to ceftiofur (Bradford et al., 1999). In feedlot cattle, nontherapeutic administration of tetracycline, alone or in combination with sulfamethazine, has been shown to select for resistant strains of *Campylobacter* spp. (Inglis et al., 2005). Vidovic and Korber (2006) conducted a survey of three feedlots in Saskatchewan, Canada to determine prevalence and resistance patterns of *E. coli* O157:H7. They detected the organism in 14.2% of 400 fecal samples and found that 65% of those isolates were resistant to at least one of the 17 antibiotics tested in the study. Most common was resistance to sulfisoxizole (61%), followed by tetracycline (12%). Resistance to chloramphenicol and streptomycin was noted in 2.3% of isolates. All isolates were sensitive to amikacin, cefoxitin, gentamicin, kanamycin, nalidixic acid, trimethoprim-sulfamethoxazole, and ceftiofur. MDR patterns included chloramphenicol-sulfisoxizole-streptomycin-tetracycline (2.3%) and sulfisoxizole-tetracycline (6%).

ANTIBIOTIC USE AND RESISTANCE ASSOCIATED WITH SWINE

NAHMS data provide some indication of the prevalence of antibiotic use in national swine production (USDA-NAHMS, 2005). It was found that 92% of farms surveyed had used antibiotics in the six months prior to the survey, with most delivered through the feed. More than 85% of sites used in-feed antibiotics in the grower/finisher phase. Most commonly used were tylosin, chlortetracycline, and bacitracin, with 56%, 43%, and 35% of sites using each, respectively. However, recent changes in the swine industry, a growing awareness of issues surrounding nontherapeutic uses of antimicrobial products, and changing trends in therapeutic and nontherapeutic regimens may have caused a change in the overall use of antibiotics, particularly in grower/finisher units and in high health herds where advantages of extended antibiotic use are less easily demonstrated (Cromwell, 2001; Dritz et al., 2002).

A link between antibiotic use in swine and increased prevalence of resistant bacteria has

been demonstrated through applied studies (Mathew et al., 2001; Mathew et al., 2005). While such studies have shown significant increases of resistance in the gut flora following use of antibiotics, it has also been shown that rapid reversion to susceptibility in commensal microflora following drug withdrawal may also occur, depending upon drug type. Studies with the aminoglycoside drug apramycin have shown that the general population of fecal *E. coli* demonstrate an increase in apramycin resistance soon after initiating use of that antibiotic; however, this increase was followed by a return to more normal susceptibility when the drug was withdrawn (Mathew et al., 2002; Mathew et al., 2005). As this antibiotic was used exclusively in young pigs, the impact of apramycin use would appear to be minimal with regard to resistance of *E. coli* in market animals.

A study reported by Gellin et al. (1989) examined antibiotic resistance in experimental swine herds and found that 36.4%, 74.3%, and 99.6% of *E. coli* isolates obtained from a herd regularly exposed to antibiotics were resistant to ampicillin, streptomycin, and tetracycline, respectively. The same study examined resistance levels in *E. coli* isolated from a different herd that had not been exposed to antibiotics in over 50 weeks and found that only 0.5%, 12.4%, and 26.7% of isolates were resistant to those same antibiotics, respectively. A study by Mathew et al. (1998) showed that 98%, 64.4%, 86%, and 29% of *E. coli* isolated from commercial swine farms where antibiotics were used extensively were resistant to tetracycline, neomycin, gentamicin, and apramycin, respectively, by the time pigs reached 63 days of age.

ANTIBIOTIC USE AND RESISTANCE ASSOCIATED WITH POULTRY

Antibiotics are used in the poultry industry therapeutically, nontherapeutically, and for growth promotion (CVP, 2006; Lu et al, 2006). Growth-promoting antibiotics used in poultry production include chlortetracycline, bacitracin, bambermycin, tylosin, and virginiamycin (CVP, 2006). Bacterial diseases—including colibacillosis, enteritis caused by *Clostridium* spp., mycoplasmosis, and several forms of salmonellosis—cause significant economic loss to the poultry industry (AHI, 2005; Barnes et al, 2003) and are a primary reason for treatment with antibiotics (Singer and Hofacre, 2006). Common antibiotics used for control these organisms include sulfonamides, amoxicillin, tetracyclines, tylosin, virginiamycin, neomycin, and penicillin. Until recently, enrofloxacin, a fluoroquinolone drug, was approved for control of colibacillosis; however, concerns that fluoroquinolone use in poultry may be linked to antibiotic-resistant *Campylobacter* infections in humans (Piddock, 1995; Murphy et al., 1996; Chu et al., 2004) caused the FDA to ban the use of that drug in poultry in 2005 (FDA, 2005).

NARMS data indicate that *Salmonella* from chickens have demonstrated increased resistance to amoxicillin/clavulanic acid, ceftiofur, cefoxitin, and tetracycline since the NARMS program was initiated (NARMS, 2005). In 1997, 0.5% of slaughter isolates were resistant to amoxicillin/clavulanic acid, 0.5% were resistant to ceftiofur, 0% were resistant to cefoxitin, and 20.6% were resistant to tetracycline. Preliminary data from 2005 indicate that 12.1%, 12.2%, 12.0%, and 28.3% of *Salmonella* isolates from poultry at slaughter were resistant to those same antibiotics, respectively. A slight increase in resistance to ampicillin was noted over that same time period, while little or no change was noted for resistance to chloramphenicol, ciprofloxacin, kanamycin, and streptomycin. Marked decreases have occurred in resistance to gentamicin and sulfa drugs over that same time period. In 1997, 17.8% and 24.8% of isolates were resistant to gentamicin and sulphamethoxazole; whereas in 2005, 4.3% and 8.5% were resistant to those same drugs, respectively.

In a study comparing the prevalence and resistance of bacteria from conventionally raised chickens and organically raised chickens in Maryland, all *Salmonella* isolates derived from conventionally raised birds were resistant to 5 or more antibiotics, whereas 79% of isolates from organically raised birds were sensitive to all 17 antibiotics tested (Cui et al., 2005). However, as isolates were derived from poultry products for retail markets, the effects, if any, of processing location or methods are un-

known. Harwood et al. (2001) studied fecal streptococci isolates in Florida and observed vancomycin-resistant *Enterococcus* (VRE) from chicken feces and from hospital waste water, but not from dogs, cattle, pigs, wild birds, raccoons, or surface water from rivers; however, the sources of the chickens and other animals were not described. In their study, *Enterococcus* spp. resistant to low concentrations of vancomycin (3 μg/mL) and harboring the *vanC* gene were isolated from chickens. By comparison, VRE (*E. faecium* and *E. avium*) resistant to high levels of vancomycin (10 μg/mL) and harboring the *vanA* gene were readily isolated from hospital waste water. Two *Enterococcus* isolates from chicken feces that were resistant to high levels of vancomycin were identified as *E. gallinarum*.

Fairchild et al. (2005) investigated the effects of tetracycline administration on cecal commensal bacteria, *Enterococcus* spp., *E. coli*, and *Campylobactor* spp. They observed that *Enterococcus* spp. and *E. coli* resistant to tetracycline and harboring a number of different tetracycline resistance genes were readily isolated from chickens, regardless of exposure or nonexposure to that drug. Tetracycline treatment in test birds did not produce tetracycline resistance in *Campylobactor* spp. in their study; however, tetracycline-resistant *Campylobactor* spp. were readily isolated from flocks that received and did not receive that antibiotic. The investigators concluded that complex population dynamics and genetics in enteric bacterial populations contribute to the antibiotic resistance observed in commercial flocks.

RISKS POSED TO HUMANS BY ANTIBIOTIC USE IN LIVESTOCK

It is well established that agricultural use of antibiotics results in increased prevalence of antibiotic-resistant bacteria in farm environments, thus contributing to the global pool of resistant organisms. However, what risk this poses to human health has not been clearly established. Foodborne transfer of bacteria carrying resistance genes is the most likely route through which agricultural use of antibiotics could affect human health. However, some evidence for direct animal-to-human transmission of resistant bacteria has been reported (Box et al., 2005; Hunter et al., 1994).

There has been some concern that farm use of antibiotics may also increase pathogen load in animals by selecting for pathogens that are known to possess resistant genes, integrons, or genetic islands containing resistance genes. These organisms may have an advantage under the selective pressure of antibiotic use, aiding in their colonization, which could then result in a greater pathogen load. A frequently cited study supporting this hypothesis (Williams et al., 1978) used swine infected with a chlortetracycline-resistant strain of *S*. Typhimurium and showed that subsequent treatment of infected pigs with chlortetracycline increased both the quantity and duration of shedding of that challenge organism. However, several subsequent studies failed to show that antibiotic use translated into increased pathogen loads, and following an extensive review of the literature (Goodman and Shum, 2000), the FDA determined that no evidence existed to support the need for pathogen load analysis as part of their Guidance 152 (FDA, 2003). In fact, some studies have shown that antibiotic use in livestock reduced shedding of foodborne pathogens (Ebner and Mathew, 2000; Kyriakis et al., 1996; Rattanatabtimtong et al., 2005).

There are several confounding factors that make the assessment of the risk posed by agricultural use of antibiotics difficult. A primary difficulty is that a large number of the antibiotics used in livestock production are also used in human and pet medicines, thus presenting difficulties in determining the initial sources or reservoirs for the resistant populations. For example, it would be difficult to assign blame for the increase in sulfonamide resistance to use of sulfa drugs in livestock, when sulfanamides have been used extensively in humans for prevention of acne, urinary tract infections, and diarrhea, among other common uses (Prado, 2004; van Boxtel and Britenhuis, 2001). Cross-resistance within and across families of antibiotics is also a confounding factor, as some antibiotics used solely in human medicine can select for resistance to other drugs which may be used primarily in livestock, and vice versa. Broad mechanisms of resistance, such as efflux

pumps, may be increased by use of a single antibiotic, but may subsequently confer resistance to unrelated antibiotics, making it difficult to determine the initial agent of selection. The fact that antibiotic resistance develops from both therapeutic and nontherapeutic use (Gellin et al., 1989; Kobland et al., 1987) presents additional difficulty in establishing a point from which to consider risk. Risk assessments focusing on nontherapeutic uses, the primary concern of agricultural use, would likely be confounded by resistance selection caused by therapeutic use commonly applied for chronic diseases. It follows that elimination of the veterinary use of some antimicrobial products may not translate into reductions of some resistance patterns in bacteria of concern.

It should be noted that the importance of resistance to the various antibiotics differs considerably, according to the role each antibiotic type may play in remediation of disease in humans. For those antibiotics that are critical for human use, resistance is of particular concern, whereas resistance to antibiotics of a lesser role in human medicine may not carry the same importance. For example, the importance of fluoroquinolones as one of the last lines of defense in treating MDR human pathogens dictates that resistance to that drug family carries a higher importance than resistance to a drug family, such as tetracyclines, long used in animal and human health, even though tetracycline resistance is more widespread in a number of significant pathogens. Guardabassi and Courvalin (2006) provide an excellent summary of the importance of the various antibiotic families used in animal agriculture with regard to human medicine.

Some efforts have been undertaken to model antimicrobial resistance and assess risk (quantitatively or semiquantitatively) associated with agricultural use of antibiotics (Barber et al., 2003; Vose et al., 2001). Such efforts have been conducted using defined risk assessment approaches, as opposed to precautionary principle approaches, for assessment and development of control strategies (National Research Council, 2003; Vose, 1998). The lack of numerical or empirical data in key areas has hampered those efforts and caused some to doubt the validity of such risk assessments. Still, these attempts have indicated that direct risks of on-farm antibiotic use may not be as significant as originally projected, primarily due to low risk elements in the steps between the movement of resistant organisms off farms and the projected failure of a human therapy as a result of agricultural use (Cox and Bufundo, 2002; Hurd et al., 2004).

As an example, Hurd et al. (2004) conducted a semiquantitative risk assessment of the potential impact of using the macrolide antibiotics tylosin and tilmicosin in various livestock. The analysis was conducted based on guidelines outlined in Guidance 152 (FDA, 2003). In that analysis, it was conservatively assumed that all occasions of tylosin and tilmicosin use in pigs or poultry would lead to macrolide-resistant bacteria, including *S. enterica* serovars, *Campylobacter* spp., and *E. faecium*; that those bacteria would contaminate meat and poultry products, and cause foodborne illness at rates cited by FoodNet data. However, as human foodborne illnesses caused by those organisms are seldom, if ever, treated with macrolide antibiotics, the risk of failure of a macrolide-mediated antibiotic therapy was negligible. In the analysis, the overall national risk for poultry, swine, and beef was estimated to be 1 in 14,000,000, 1 in 53,000,000, and 1 in 236,000,000 cases per year for *Campylobacter* (combined risk *C. coli* and *C. jejuni*), respectively, and 1 in 3,000,000,000, 1 in 21,000,000,000, and 1 in 29,000,000,000 cases per year for *E. faecium*, respectively. As a comparison, the FDA, in their risk assessment of fluoroquinolone-resistant *Campylobacter* attributed to the consumption of chicken, estimated the risk at 1 in 32,900 cases, and determined this level of risk to be "low" (FDA-CVM, 2001). Using similar techniques, Cox and Ricci (2005) estimated that a ban on enrofloxacin use in poultry in the United States would prevent less than 1 severe incident per year, while causing approximately 6,600 additional cases of campylobacteriosis and more than 40,000 excess illness days.

BENEFITS OF ANTIBIOTIC USE IN AGRICULTURE

Scientists also note the potential benefits associated with using antibiotics in food animals,

including the treatment of disease, improvement of carcass quality, and improvement of feed efficiency (Andreasen et al., 2005). The use of feed-based antimicrobials has consistently been shown to benefit livestock production, increasing the ability of farms to maintain profitable margins (Cromwell et al., 1996; NRC, 1999; Phillips et al., 2004), lowering manure output and thus the effects of animal wastes on the environment (Roth and Kirchgessner, 1993), and lowering animal pathogen loads and carriage of foodborne pathogens in livestock (Kyriakis et al., 1996; Ebner and Mathew, 2000; Rattanatabtimtong et al., 2005). Many foodborne pathogens are not easily controlled in livestock by vaccines; as these organisms have a commensal association with their food animal hosts, making eradication difficult, if not impossible, limiting their numbers in the gut with antibiotic-based feed or water additives may be a practical approach to limiting foodborne transfer of these organisms (Phillips et al., 2004).

It appears prudent that such benefits be included in risk assessment models evaluating antibiotic use in food animals, so that more realistic evaluations result and a balance is achieved that provides the greatest protection from inherent risk while maximizing the overall benefits to society. It should be noted that risk assessments may be strictly defined by FDA or other agency standards that may not allow inclusion of benefits in the assessment formula.

CONCLUSION

The issues surrounding antibiotic resistance in bacteria of food animal origin are complex and of high relevance to agricultural industries, consumers, and health care providers. While use of antibiotics in livestock for disease therapy, prophylaxis, and growth enhancement is known to select for resistant bacteria, the impact of these organisms and the resistance genes they carry on human health are not clearly known. In contrast to Europe, regulatory agencies in the United States, other countries in the Americas, Asia, and elsewhere, have not yet imposed total bans on the nontherapeutic growth-promoting use of antibiotics. Newer restrictions in the United States, such as the recent withdrawal of approval of the use of enrofloxacin in poultry, have focused on antibiotics deemed critical for use in humans. Judicious use guidelines (Vogel, 2003), along with educational programs and surveillance aimed at detection and intervention in the case of high priority resistance issues, remain the primary strategies to limit the spread of antibiotic resistance in agricultural production. Production economics and increasing restrictions imposed by retail food and restaurant chains (McDonald's, 2003; KFC, 2006) may have a greater impact on the future use of some food animal antibiotics than the current debate among the scientific community.

It is likely that both risks and benefits are realized in the use of antibiotics for food animal production. As consumers, the animal industries, and regulating agencies consider strategies to limit risks associated with antibiotic use, it is imperative that science-based information provide the foundation for such considerations and decisions.

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