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GENETICS OF ANTIMICROBIAL RESISTANCE

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Antimicrobial resistant strains of bacteria are an increasing threat to animal and human health. Resistance mechanisms to circumvent the toxic action of antimicrobials have been identified and described for all known antimicrobials currently available for clinical use in human and veterinary medicine. Acquired bacterial antibiotic resistance can result from the mutation of normal cellular genes, the acquisition of foreign resistance genes, or a combination of these two mechanisms. The most common resistance mechanisms employed by bacteria include enzymatic degradation or alteration of the antimicrobial, mutation in the antimicrobial target site, decreased cell wall permeability to antimicrobials, and active efflux of the antimicrobial across the cell membrane. The spread of mobile genetic elements such as plasmids, transposons, and integrons has greatly contributed to the rapid dissemination of antimicrobial resistance among several bacterial genera of human and veterinary importance. Antimicrobial resistance genes have been shown to accumulate on mobile elements, leading to a situation where multidrug resistance phenotypes can be transferred to a susceptible recipient via a single genetic event. The increasing prevalence of antimicrobial resistant bacterial pathogens has severe implications for the future treatment and prevention of infectious diseases in both animals and humans. The versatility with which bacteria adapt to their environment and exchange DNA between different genera highlights the need to implement effective antimicrobial stewardship and infection control programs in both human and veterinary medicine.

Keywords: Antimicrobial resistance genes; Antimicrobial resistance mechanisms; Conjugation; Integron; Plasmid; Transposon

INTRODUCTION

Since the introduction of penicillin during the 1940s, numerous antibacterial agents have been developed and marketed for clinical use and have ameliorated the massive human mortalities previously associated with bacterial infections before the ''antibiotic era.'' However, the initial optimism that all bacterial infections could be successfully treated with these new agents was quickly disabused when reports of resistance emerged soon after their introduction into clinical use (1–4). This was followed by studies in the 1950s and 60s showing that multiple drug resistance could be transferred to susceptible recipient cells (5). As of today, antimicrobial resistance mechanisms have been reported for all known antibiotics that are currently available for clinical use in human and veterinary medicine.

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In addition to the obvious benefits to humans animal health and productivity have improved significantly over the past several decades with the introduction of antimicrobials into veterinary medicine (6–9). However, their use in food animals, whether for growth promotion or prevention and treatment of infectious diseases, can select for resistance among both resident bacterial pathogens and commensal organisms. Even though some animal health pathogens remain generally susceptible to the majority of clinical antimicrobials, emerging resistance phenotypes have been documented among several important zoonotic Gram-negative bacterial pathogens (1,5,10–18). For example, multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 (DT 104) is typically characterized by resistance to more than 5 antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, also called R-type ACSSuT) and increased from 0.6% prevalence in human infections in the United States in 1979 to 34% in 1996 (19,20). Among human isolates obtained from the National Antimicrobial Resistance Monitoring System (NARMS), the multidrug resistant *Salmonella enterica* serotype Newport phenotype MDR-AmpC (resistant to over 9 antimicrobials and displays a decreased susceptibility to ceftriaxone) increased in prevalence from 0% in 1996 to 20.7% in 2003 (21). Regrettably, gaps still remain in our knowledge regarding the development and dissemination of bacterial resistance to antimicrobial agents in various animal production environments.

Despite the abundance of resistance phenotypes observed among bacteria, there are a limited number of mechanisms by which these resistance traits are acquired. The genes encoding antimicrobial resistance determinants may be located on the chromosome, where they are inherited by daughter cells, or they might be horizontally transmitted on mobile DNA elements, such as plasmids and transposons. The versatility of bacterial populations in adapting to toxic environments, along with their ability to exchange DNA, signifies that antibiotic resistance is an inevitable biological phenomenon that will likely continue to be a chronic public health problem. Therefore, successful management of current antimicrobials, and the continued development of new ones, is vital to protecting human and animal health against bacterial pathogens.

ORIGINS OF ANTIMICROBIAL RESISTANCE

Resistance genes and transfer mechanisms most likely existed long before the discovery and use of modern therapeutic antimicrobials. For example, antimicrobial resistant bacteria estimated at being over 2000 years old have been recovered from glacial samples obtained from the Canadian Arctic Archipelago (22), while a more recent study detected TEM-type β-lactamases from a metagenomic library of cold-seep sediments of deep-sea Edison seamount (near Papua New Guinea) estimated to be about 10,000 years old (23). Resistant microorganisms have also been found among historic culture collections compiled before the advent of modern day antibiotics (24). Smith identified resistance to sulfadiazine, spectinomycin and tetracycline among *E. coli* isolates collected prior to 1950, whereas Barlow and Hall showed that *ampC* ß-lactamase genes recovered from *Citrobacter freundii* strains collected prior to the clinical use of antibiotics (the 1920s) were shown to be as effective at providing ß-lactam-resistance in *E. coli* as were the plasmid-borne alleles

from ß-lactam-resistant clinical isolates (24,25). Additionally, plasmids found in Gram-negative bacteria that were isolated before antibiotics were introduced into clinical practice were very similar to currently described plasmids, except that the early isolates did not possess any resistance genes (26,27).

Resistance to natural antimicrobial agents, synthetic derivatives, and completely synthetic antimicrobials has been recently observed in a collection of soil-dwelling actinomycetes, with some displaying resistance mechanisms not traditionally observed in clinical bacterial pathogens. Surprisingly, the investigators found that every isolate they tested displayed resistance to at least six to eight different antimicrobial agents and in some cases, as many as twenty (28). The use of novel resistance mechanisms by these organisms, coupled with the fact that these soil microbes are not as intensively exposed to antimicrobial selective pressures as are the clinical pathogens, emphasizes the fact that resistance is not a new phenomenon (28). The identification of this new reservoir of resistance genes highlights the possibility of future horizontal transfer of novel antimicrobial resistance determinants to bacteria of human and veterinary importance (28).

If bacterial antimicrobial resistance is not a new phenomenon, where did it originate? One popular belief is that antibiotic resistance mechanisms arose within antibiotic-producing microorganisms as a mechanism to protect them against the action of their own antibiotic (auto-toxicity) (28). This has been substantiated by the finding of aminoglycoside-modifying enzymes in aminoglycoside-producing organisms that display marked homology to modifying enzymes found in aminoglycoside resistant bacteria (13). Also, the essential genetic determinants associated with resistance to vancomycin; *vanA*, *vanH*, and *vanX*, appear to be very similar to the self protection mechanism employed in the vancomycin producing *Actinomyces* strains (29). In addition, some antibiotic preparations employed for human and animal use were found to be contaminated with chromosomal DNA of an antibiotic-producing organism, including antimicrobial resistance gene sequences (30). It has been postulated that this presence of DNA encoding antimicrobial resistance in antibiotic preparations has been a factor in the rapid development of multiple resistance by providing the resistance sequences that can then be taken up by the causative pathogen.

MECHANISMS OF RESISTANCE

In the context of antimicrobial resistance, bacteria can display one of three fundamental phenotypes: susceptibility, intrinsic resistance, or acquired resistance (31). Intrinsic resistance can be described as a natural phenomenon that is displayed by all members of a species and is a function of the physiological or biochemical makeup of that species. For example, enterococci are intrinsically resistant to cephalosporins due to a decreased binding affinity to the penicillin-binding proteins (32). Acquired resistance can result from the mutation of regulatory or structural genes, the acquisition of foreign resistance genes, or a combination of these two mechanisms and is present not in the entire species but within only a certain lineage of bacteria derived from a susceptible parent (13).

Resistance phenotypes caused by point mutations often alter the binding targets of antimicrobials, resulting in lowered binding affinity. For example, point

mutations in the gene encoding DNA gyrase can alter the binding efficiency of quinolones, thereby reducing their efficacy. Multiple point mutations can occur leading to higher levels of resistance, as in the quinolone resistance-determining region (QRDR) of DNA topoisomerase genes, such as *gyrA*, *gyrB*, and *parC*, where multiple mutations cause higher minimum inhibitory concentrations (MICs) and therefore decreased susceptibility to quinolone antimicrobials (33). More striking are the effects with regards to β-lactam resistance, where point mutations in numerous β-lactamase genes have led to the identification and classification of over 300 enzymes associated with a range of β-lactam resistance phenotypes (34,35).

In addition to mutation, resistance also results from degradation or alteration of the antimicrobial, reduced uptake of an antimicrobial, and active efflux of an antimicrobial out of the cell. These mechanisms of resistance have been extensively reviewed elsewhere (36–40). Alteration or degradation of the antimicrobial is a common mechanism of resistance that reduces or eliminates antimicrobial activity. An example are the β-lactamases, which enzymatically cleave the β-lactam ring (40). A recently discovered example of enzymatic alteration of a synthetic antimicrobial involves a plasmid-borne variant of an aminoglycoside acetyltransferase (AAC(6′)-Ib)that acetylates fluoroquinolones and reduces their activity (41). Because fluoroquinolones are fully synthetic, naturally evolving antimicrobial resistance genes have not been considered a threat in the reduction of their activity (41). This variant enzyme acts against some fluoroquinolones and effects resistance to aminoglycosides, raising the implication that plasmids bearing this gene may co-select for multiple resistance. Some other examples of antimicrobials that are affected by enzymatic inactivation or modification include macrolides (hydroloysis), macrolides, and streptogramins, to name a few (40).

A number of different enzymatic modification mechanisms exist to inactivate antimicrobials, with acetylation being one of the most common. Aminoglycoside acetyltransferase, in addition to adding acetyl groups to antimicrobials effecting inactivation, also interferes with translation by binding to the A-site of the ribosome and interfering with the codon-anticodon translation mechanism (40). Macrolides also bind to the ribosome and interfere with protein synthesis, and bacterial genomic mutations that change the ribosomal 50S subunit binding site can decrease the efficiency of macrolide binding and effect resistance to this antimicrobial class (31).

Antimicrobial resistance can be associated with decreases in bacterial cell permeability, usually via porin alterations in Gram-negative bacteria. Outer membrane proteins (Omps) provide channels of entry for molecules to the cell membrane and internally into Gram negative bacteria, including antimicrobials, based on charge, shape, and size. Common Omps in *E. coli* are OmpF, OmpC, and OmpE, and the loss of function of one of these porins due to mutation can cause resistance to a wide variety of antibiotics (34,42,43). OmpF is the major channel in *E. coli* through which many molecules diffuse, and the alteration of this porin can result in decreased susceptibility to a number of different antimicrobial agents (34,42). Mutations in the genes that encode the outer membrane lipopolysaccharides (LPS) can contribute to resistance as well. It has been shown that changes in the O-antigen side chains of the LPS can change the shape and overall charge of the molecule, decreasing the binding efficiency of some cationic antibiotics. In fact, mutations in the LPS genes have been shown in conjunction with increasing antibiotic selective pressure (34,42,44,45).

Active efflux of an antimicrobial out of the bacterial cell is an energy-dependent mechanism employed by bacteria to decrease the cell's internal concentration of antimicrobials. Efflux pumps are naturally occurring and are present in both susceptible and resistant bacteria (34,39). In Gram-negative bacilli efflux pumps are often chromosomally encoded and most strains carry genetic determinants for several pumps, establishing a level of intrinsic resistance to a multitude of antimicrobials (46). Efflux mechanisms can confer resistance against a particular antimicrobial agent, class, or a number of antimicrobials resulting in multidrug resistance (47). Efflux mediated resistance is usually attributed to mutations in either the regulatory or effector genes of the efflux system. Mutations that effect resistance involve an increase in the expression of the efflux pump protein or in amino acid substitutions that makes the efflux protein more efficient in exporting antimicrobials out of the cell (39). Efflux pumps are often chromosomally encoded or transmissible on genetic elements, and can be constitutively expressed or triggered by various environmental stimuli (39,42,47).

Of more recent concern is the identification and description of bacteria that employ multiple resistance mechanisms against a single antimicrobial class. For example, resistance to β-lactam antimicrobials can be mediated by all of the above described mechanisms. The most common mechanism of β-lactam resistance is production of β-lactamase enzymes, which physically destroy the antimicrobial. Additionally, alteration of the antimicrobial drug's binding to a target effects β-lactam resistance, where mutations in the penicillin binding proteins (PBPs) of the bacterial cell can result in decreased affinity for the β-lactam to the PBP. Resistance to β-lactams has also been associated with the accumulation of point mutations in certain porin genes, restricting the permeability of the bacterial cell to the antimicrobial, as well as by energy-dependent efflux systems (31,36).

TRANSFER OF ANTIMICROBIAL RESISTANCE

The acquisition of mobile DNA elements, in addition to single or accumulative mutations in physiological genes, has accelerated the development and exchange of resistance in bacteria. The spread of mobile genetic elements, in particular plasmids, transposons, and integrons, has greatly contributed to the rapid dissemination of antimicrobial resistance among several bacterial genera of human and veterinary importance (48,49). Plasmids are extra-chromosomal segments of DNA that replicate independently of the chromosome and can be exchanged among various bacteria via a hair-like transfer appendage called a pilus (50). Plasmids are categorized into incompatibility (Inc) groups according to their mode of replication and maintenance in a bacteria cell (51). Incompatibility is a manifestation of plasmid relatedness based on the commonality of replication machinery, whereby plasmids of different modes of replication are able to reside in the same bacterium while two different plasmids exploiting the same replication mechanism are mutually incompatible and are unable to persist in the same bacterium for prolonged periods (51,52).

Plasmids are not essential to survival, but typically carry genes that impart some selective advantage to the host bacterium, such as virulence determinants, adhesions, and antimicrobial resistance genes. Plasmids that carry resistance genes are called R plasmids or R factors. Since their discovery in the 1950s (53), antimicrobial

resistance plasmids have been increasingly associated with both Gram-positive and Gram-negative bacterial pathogens and commensal organisms. Plasmid-associated resistance genes have been characterized for the majority of clinically available antimicrobials, including most recently the quinolones (54–56), and it is not uncommon for a single plasmid to simultaneously mediate resistance to multiple antimicrobials and be shared among different bacterial genera (57). For example, interserovar exchange of plasmids between *Salmonella* Serovar Muenchen and S. typhimurium has been shown in animals where both of the serovar strains shared similar plasmid profiles and carried similar antimicrobial resistance genes on their plasmids (58). Also, the spread of multiple extended spectrum β-lactamases has been partly attributed to their presence on multidrug resistance plasmids (33,34).

Until recently, quinolone resistance was believed to arise solely from chromosomal mutations in genes encoding target enzymes or via active efflux. However, in 1998, a novel mechanism of plasmid-mediated quinolone resistance (termed *qnr*) was discovered in a *Klebsiella pneumoniae* isolate from Birmingham, Alabama, collected in 1994 (59). *qnr* was found in an integron-like structure near Orf513 on the multidrug resistance plasmid, pMG252 (60). Qnr, the gene product, is a member of the pentapeptide repeat family of proteins and has been shown to block the action of ciprofloxacin on purified DNA gyrase and topoisomerase IV (55). Subsequently, *qnr* plasmids have been reported from clinical isolates of *E. coli*, *Citrobacter fruendii, Enterobacter* species, *K. pneumoniae, Providencia stuartii*, and *Salmonella* species from across the globe (54,61). The different *qnr* genes reported to date include *qnrA, qnrB*, and *qnrS* (55). The plasmid-encoded Qnr proteins derived from *E. coli*, *Klebsiella oxytoca* and *K. pneumoniae* isolates recovered from different geographic sources (China, Europe and USA) show almost identical residues, indicating these proteins most likely have similar origins (56). There is also evidence supporting the role played by these plasmids by providing genetic linkage between resistance to quinolones and extended-spectrum β-lactamase production (62).

In addition to carrying resistance genes, plasmids can serve as vehicles for other resistance elements, such as transposons and integrons. Transposons are gene sequences that can move from one location on the chromosome to another or from the chromosome to a transmissible plasmid. Transposons are made up of insertion sequences (IS), intervening DNA, and the enzyme responsible for the transposition, called a transposase. This "jumping" set of genes carries the enzymes and genetic sequences required for movement located within the transposon to randomly "jump" from one genetic location to another (50). It has been suggested that intracellular DNA movement via transposons and other mobile DNA elements is mediated by enzymes that are similar to enzymes used for viral insertion into chromosomes (50).

Transposons can be very simple, composed of the IS elements and the transposase, or much more complicated as in composite transposons. Composite transposons consist of a central region containing genes other than those required for transposition, such as antibiotic resistance genes, flanked on both sides by IS that are very similar in sequence and usually in an inverted orientation. A large number of resistance determinants in many different bacterial species are transmitted via composite transposons (5). Transmission of a transposon from one bacterial species to another can be accomplished by insertion into a conjugative plasmid or via a

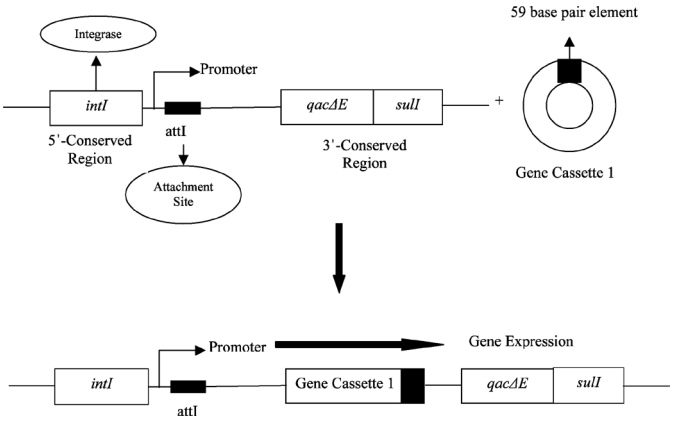

Figure 1 Schematic representation of gene cassette capture and expression mechanism by a Class I integron.

conjugative transposon (50). Conjugative transposons appear to be a hybrid between transposons and plasmids. Conjugative transposons promote their excision from the donor cell's genome and form a covalently closed single-stranded DNA circular structure which, unlike plasmids, does not replicate except to synthesize a duplicate strand to become double-stranded. These transposons then promote their conjugation to a neighbouring bacterium and, following conjugation, integrate into the recipient chromosome or recipient host's plasmid (63). Conjugative transposons have been identified in Gram negative as well as Gram positive bacteria (63).

The magnitude of resistance development and dissemination is further explained by the discovery of a novel genetic system for the movement of antibiotic resistance genes called the integron (64). Integrons are mobile DNA elements with a specific structure consisting of two conserved segments flanking a central region in which antimicrobial resistance "gene cassettes" can be inserted (64,65). Gene cassettes exists as free circular DNA structures around 500–1000 bp that are not expressed on their own due to the lack of the promoter region. A 59 bp element is located downstream of the promoter-less resistance gene and serves as the recombination site. Insertion of the cassette into the integron structure via the recombination process at the *attI* recombination site downstream from a promoter helps in the expression of the gene encoded by the cassette (Figure 1). Multiple gene cassettes can be arranged in tandem, and more than 60 distinct cassettes have been identified (64). Cassette-associated genes have been shown to confer resistance to beta-lactams, aminoglycosides, phenicols, trimethoprim, streptothricin, sulfonamides, and quaternary ammonium compounds (64–66). At least four classes of integrons have been discovered based on the integrase gene sequence, and among enteric isolates and a number of different genera, class I integrons are the most frequently identified (67). Integrons are usually identified in conjunction with streptomycin and trimethoprim-sulfamethoxazole resistant bacteria isolated from food animals and human infections (68,69). The inclination to exchange genes increases concern about the possible spread of antibiotic resistance determinants from commensal or nonclinical organisms in animals and man to human pathogens (5).

One example of rapid dissemination of integron-associated antimicrobial resistance genes is *Salmonella* Typhimurium phage type DT104, commonly known as R-type ACSSuT. This phage type has had a significant public health impact and is a global concern. It was first documented in cattle from UK in 1984 and was soon reported in animals and humans the world over (20,70–72). The genetic determinants for this R-type are contained in a 43 kb island (Salmonella Genomic Island [SGI]), comprised of integrons containing respectively the ASu (bla_{CARB-2} and *sul1*) and SSp (*aadA2*) genes with intervening plasmid-derived genes coding for resistance to chloramphenicol/florfenicol (*flo*) and tetracyclines (*tet*G) (73). All isolates of multidrug-resistant (MDR) DT104 with the ACSSuT phenotype have contained the same gene cassettes irrespective of source (food animal or human), or country of origin. Of note in recent years has been the identification of SGI1 in several different *Salmonella* serovars, including *S*. Agona, *S*. Albany, and *S*. Paratyphi B variant Java (74). Furthermore, it has been shown experimentally that the DT104 MDR cluster can be efficiently transduced by P22-like phages (75). Upstream of the first integron in the MDR locus is a gene encoding a putative resolvase enzyme, which demonstrates greater than 50% identity with the Tn*3* resolvase family (76). These findings support the potential for horizontal spread of the MDR gene cluster among *Salmonella* and other bacteria.

The term ''superintegron'' was coined by Mazel *et al.* in 1998 (77,78) for integrons that have incorporated hundreds of gene cassettes. One such superintegron (SI) has been identified in the *Vibrio cholerae* chromosome that is 179 kb long and harbours 179 gene cassettes (79). Another SI has been identified in *Vibrio metschnikovii* that consists of approximately 26 gene cassettes (79). The role played by the SIs has not been investigated in detail but SIs are likely to play an important role in bacterial evolution. Their complex structure, presence of multiple gene cassettes, chromosomal integration, and possible co-integration of virulence factors can lead to a potential selection of SI among other bacterial pathogens.

More recently, a new element called orf*513* has been increasingly identified in association with multiple antimicrobial resistance genes and with class 1 integrons, spawning the idea of ''complex class 1 integrons'' (80). This complex integron, termed insertion sequence common regions (ISCR1), can mediate resistance to chloramphenicol, trimethoprim, aminoglycosides, tetracyclines, and an array of β-lactams (80). The orf*513* region is thought to be similar to a common region element (CR), which is a group of potentially mobile DNA elements found in the *Salmonella* pathogenicity islands and on the SXT conjugative element in *Vibrio cholerae* (81). Some studies suggest that CR elements replicate by rolling circle replication, and may be a subset of a family of unusual IS elements, IS*91*. Replication using the rolling circle mechanism allows for genetic rearrangements that may not be possible by traditional rearrangement mechanisms, and therefore may present a new evolutionary advancement in the class I integron, and new clinical concerns (80).

Mobile DNA elements can transport multiple antimicrobial resistance genes in tandem and are more than likely responsible for the rapid dissemination of these genes among different bacteria (5,13,16,82). A confounding factor is the fact that there may be non-antibiotic selection pressure for bacterial antibiotic resistance genes. Recent data indicates that different resistance determinants can aggregate in linked clusters on a single mobile element, such that antibiotics of a different class

or even non-antibiotic substances such as heavy metals or disinfectants might select for antimicrobial resistant bacteria (64,65,82–84).

In addition to self-transmissible mobile DNA elements, antimicrobial resistance determinants can be spread among bacteria via uptake of naked DNA from the surrounding environment (transformation) or upon infection with a bacteriophage carrying resistance genes (transduction). Transformation was the first mechanism of DNA transfer to be discovered among prokaryotes and involves DNA scavenging by a bacterium after the death and deterioration of a nearby bacterium (50). The DNA in a dead bacterium degrades and is broken into fragments released into the surrounding milieu, which can be taken up by transformation competent recipients. If antibiotic resistance genes are included in the degraded DNA, they can be taken up by a nearby bacterium and incorporated into the bacterial genome. Genetic exchange via transduction involves bacteriophage infection of a bacterium, phage replication, and packaging of some of the bacterial DNA with the phage DNA (which may include resistance determinants), and lysis of that bacterium and infection of subsequent bacteria. Upon subsequent infection, those resistance determinants may be transferred to the infected bacterium (50). The contribution of transformation and transduction in the evolution of multidrug resistance is difficult to assess but laboratory demonstrations indicate that it theoretically plays a role in antimicrobial resistance development (85).

ARE WE FIGHTING AN UPHILL BATTLE?

The observation that the introduction of new classes or modifications of older classes of antimicrobials over the past six decades has been matched, slowly but surely, by the systematic development of new bacterial resistance mechanisms reminds us of the Greek mythology story of Sisyphus, who was condemned to eternal punishment by pushing a heavy rock to the top of a steep hill, where it would always roll down again. One must remember that the development of bacterial antimicrobial resistance is an expected phenomenon based on the Darwinian principle of survival of the fittest. However, since resistance theoretically is expected to emerge in a bacterial population under selective pressure, what happens when that selective pressure is no longer present? Does the population return to a predominantly susceptible phenotype or will resistance persist to some degree, only to return to high levels present prior to antimicrobial removal when the antimicrobial is reintroduced into the environment? Further investigation is necessary to address these questions.

The question of what may occur once antimicrobial selective pressure is removed in the form of growth promoters has begun to be addressed in Sweden and Denmark (86,87). In Denmark, the use of avoparcin was banned in 1995 and the use of virginiamycin was banned in 1998. A survey was then conducted to assess the resultant prevalence of resistant bacteria. From 1995 to 2005 glycopeptide-resistant enterococci prevalence in broilers decreased from 72.7% to 5.8%, illustrating the return to a more susceptible bacterial population once the selective pressure was removed (87). However, in pigs the occurrence of glycopeptide-resistant enterococci remained stable at 20% from 1995 to 1997. It was discovered that a linkage existed between macrolide resistance genes and glycopeptide resistant genes, and only when use of both classes of antimicrobials were significantly decreased did the prevalence

of resistance decrease from 20% to 6% in 2000 (87). The ''Danish experiment'' highlights an important point in the debate about antimicrobials. Is the removal of the selective pressure enough to drive the prevalence of resistance down? Or will the ever increasing genetic linkage of resistance genes preclude the ''easy fix'' of banning only certain antimicrobials or certain classes of antimicrobials? An increase in the scientific knowledge on this subject is needed to adequately begin to fully understand the risks in agricultural antimicrobial use.

Furthermore, the human health implications of agricultural antimicrobial use are not fully understood. A serious health crisis is posed by the emergence of human and veterinary resistant pathogens and risk assessment strategies are being developed in an attempt to address the situation. Prevention of infection should be the ultimate goal, aided by the development of efficacious vaccines and competitive exclusion products, which have been suggested as alternatives to our reliance on antimicrobials (10,18,88,89). A further understanding of the complex ecological, biochemical, and molecular aspects of antimicrobial resistance is also needed, both to develop new antimicrobial agents and to protect the potency of the currently available agents. Further insights into these areas should lead to better strategies for managing antimicrobial resistance development and limiting resistance dissemination.

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