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Antimicrobial Resistance and Its Epidemiology

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Introduction

Since the discovery of penicillin in the late 1920s, hundreds of antimicrobial agents have been developed for anti-infective therapy. Antimicrobials have become indispensable in decreasing morbidity and mortality associated with a host of infectious diseases and, since their introduction into veterinary medicine, animal health and productivity have improved significantly (National Research Council, Institute of Medicine, 1998). The emergence of antimicrobial resistance was not an unexpected phenomenon and was predicted by Alexander Fleming, who warned in his Nobel Prize lecture in 1945 against the misuse of penicillin. However, loss of efficacy through the emergence, dissemination, and persistence of bacterial antimicrobial resistance in many bacterial pathogens (defined as the ability of a microorganism to withstand the effect of a normally active concentration of an antimicrobial agent) has become a general problem and a serious threat to the treatment of infectious diseases in both human and veterinary medicine (Salyers and Amiable-Cuevas, 1997; Witte, 1998; Marshall and Levy, 2011).

Infections caused by resistant bacteria are more frequently associated with higher morbidity and mortality than those caused by susceptible pathogens (Helms et al., 2002; Travers and Barza, 2002; Varma et al., 2005). In areas of concentrated use, such as hospitals, this has led to lengthened hospital stays, increased health care costs, and, in extreme cases, to untreatable infections (Maragakis et al., 2008; Shorr, 2009). Contributing to this growing dilemma is the observation that the introduction of new classes or modifications of older classes of antimicrobials over the past 7 decades has been matched, slowly but surely, by the systematic emergence of new bacterial resistance mechanisms. Antimicrobial resistance mechanisms have been reported for all known antibiotics currently available for clinical use in human and veterinary medicine. Therefore, successful sustainable management of current antimicrobials (Prescott, 2008; Doron and Davidson, 2011; Ewers et al., 2011) and the continued development of new ones and of alternatives to antimicrobial drugs are vital to protecting animal and human health against infectious microbial pathogens.

Resistance Mechanisms

A large variety of antimicrobial resistance mechanisms have been identified in bacteria, and several different mechanisms can frequently be responsible for resistance to a single antimicrobial agent in a given bacterial species. The manually curated Antibiotic Resistance Genes Database (ARDB) lists the existence of more than 23,000 potential resistance genes from available bacterial genome sequences (Liu and Pop, 2009). Antimicrobial resistance mechanisms can be classified into four major categories (Figure 3.1): (1) the antimicrobial agent can be prevented from reaching its target by reducing its penetration into the bacterial cell; (2) the antimicrobial agent can be expelled out of the cell by general or specific efflux pumps; (3) the antimicrobial agent can be inactivated by modification or

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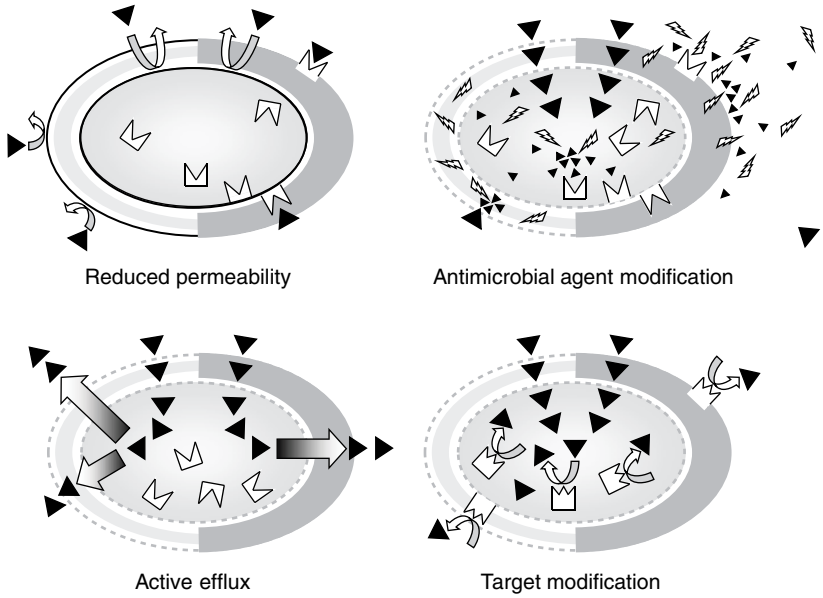

Figure 3.1. The four major mechanisms of antimicrobial resistance. Reduced permeability can be due to either lack of permeability of the outer membrane (e.g., down-regulation of porins in Gram-negatives) or of the cell membrane (e.g., lack of aminoglycoside active transport under anaerobic conditions). Active efflux can pump antimicrobial agents back into the periplasmic space (as with the TetA tetracyclines efflux pump in Enterobacteriaceae) or directly in the outer milieu (as for the RND multidrug efflux transporters). Antimicrobial agent modification by bacterial enzymes can take place either after the agent has penetrated into the cell (e.g., acetylation of chloramphenicol by CAT enzymes), in the periplasmic space (e.g., splitting of the beta-lactam ring by beta-lactamases in *Enterobacteriaceae*), or even outside of the bacterial cell (e.g., beta-lactamase produced by *Staphylococcus aureus*), before the agent has reached its target on the surface of the bacterium. Target modification has been described for both surface-exposed (e.g., peptidoglycan modification in vancomycin-resistant enterococci) and intracellular targets (e.g., macrolide resistance due to ribosomal methylation in Gram-positive bacteria).

degradation, either before or after penetrating the cell; and (4) the antimicrobial target can be modified or protected by another molecule preventing access of the antibiotic to its target, so that the antimicrobial cannot act on it anymore. Alternatively, the antimicrobial agent target can be rendered dispensable by the acquisition or activation of an alternate pathway by the microorganism. A few examples of each one of these resistance mechanisms are listed in Table 3.1 and more systematic information can be found in the following chapters of this book.

Types of Antimicrobial Resistance

In the context of antimicrobial resistance, bacteria display three fundamental phenotypes: susceptibility, intrinsic resistance, or acquired resistance.

Intrinsic resistance is natural to all the members of a specific bacterial taxonomic group, such as a bacterial genus, species, or subspecies. This type of resistance is most often through structural or biochemical characteristics inherent to the native microorganism. For example, many Gram-negative bacteria are naturally resistant to the activity of macrolides since these chemicals are too large to traverse the cell wall and to gain access to their cytoplasmic target. Other examples of innate resistance include the general reduced activity of aminoglycosides against anaerobes, because of the lack of aminoglycoside penetration into the cells under anaerobic conditions, and polymyxin resistance among Gram-positive bacteria because of the lack of phosphatidylethanolamine in their cytoplasmic membrane. A few examples of intrinsic resistance phenotypes for major bacterial taxa are presented in Table 3.2. These intrinsic

Table 3.1. Examples of resistance mechanisms (note that this is by far not a comprehensive list of all the resistance mechanisms known for each category of antimicrobials listed).

Antimicrobial Agent	Resistance Mechanism	Examples of Genetic Determinant
Tetracycline	2. Inducible efflux of tetracycline in *E. coli* and other *Enterobacteriaceae*	*tet*(A), *tet*(B), *tet*(C)
	4. Ribosomal protection in Gram-positive bacteria	*Tet*(O), *tet*(M)
Chloramphenicol	2. Efflux in *Enterobacteriaceae*	*cmlA*, *floR*
	3. Acetylation in *Enterobacteriaceae*	*catA*
Beta-lactams	3. Beta-lactamases in *Enterobacteriaceae*, and *Staphylococcus aureus*	bla_{TEM}, bla_{CTX-M}, bla_{CMY}, bla_{NDM}, bla_{Z}
Oxacillin, methicillin	4. Alternate penicillin-binding proteins in *Staphylococcus aureus*	*mecA*
Imipenem	1. Decreased porin formation in *Enterobacter aerogenes* and *Klebsiella* spp.	Mutations
Aminoglycosides	3. Phosphorylation, adenylation, and acetylation of aminoglycosides in Gram-negative and –positive bacteria	Numerous genes with a broad variety of specificities
Streptomycin	4. Modification of ribosomal proteins or of 16S rRNA in *Mycobacterium* spp.	Mutations
Macrolides, lincosamides, streptogramins	4. Methylation of ribosomal RNA in Gram-positive organisms	*ermA*, *ermB*, *ermC*
Macrolides, streptogramins	2. *Staphylococcus* spp.	*vga(A)*, *msr(A)*
Fluoroquinolones	2. Active efflux	*qepA*
	4. DNA topoisomeases with low affinity to quinolones	Mutations in *gyrA*, *gyrB*, *parC*, *parE*
	4. Target protection	Diverse *qnr* genes
Sulfonamides	4. Bypass of blocked pathway through additional resistant dihydropteroate synthase in Gram-negative bacteria	*sul1*, *sul2*, *sul3*
Trimethoprim	4. Bypass of blocked pathway through additional resistant dihydrofolate reductase	Diverse *dfr* genes

Table 3.2. Examples of intrinsic resistance phenotypes.

Organism	Intrinsic Resistance(s)
Most Gram-negative bacteria (*Enterobacteriaceae Pseudomonas* spp., or *Campylobacter* spp.)	Penicillin G, oxacillin, macrolides, lincosamides, streptogramins, glycopeptides, bacitracin
Klebsiella spp.	Ampicillin
Proteus vulgaris	Ampicillin, cephalosporins I, polymyxins
Proteus mirabilis	Tetracycline, polymyxins
Serratia marcescens	Ampicillin, amoxicillin-clavulanate, cephalosporins I, polymyxins
Enterobacter spp.	Ampicillin, amoxicillin-clavulanate, cephalosporins I, cefoxitin
Pseudomonas aeruginosa	Ampicillin, cephalosporins I and II, ceftriaxone, kanamycine, tetracycline, chloramphenicol, trimethoprim, quinolones
Haemophilus spp.	(Streptomycin, kanamycin), macrolides
Campylobacter jejuni and *Campylobacter coli*	Cephalosporins I, trimethoprim
Most Gram-positive bacteria	Polymyxins, quinolones
Streptococcus spp.	Aminoglycosides (low level)
Enterococcus spp.	Oxacillin, cephalosporins, aminoglycosides (low level), sulfonamides (*in vivo*), trimethoprim (*in vivo*)
Listeria monocytogenes	Oxacillin, cephalosporins, lincosamides
Bacillus anthracis	Cephalosporins, sulfonamides, trimethoprim
Anaerobes (including *Clostridium* spp.)	Aminoglycosides

Adapted from the Communiqué 2005 of the Comité de l'Antibiogramme de la Société Française de Microbiologie.

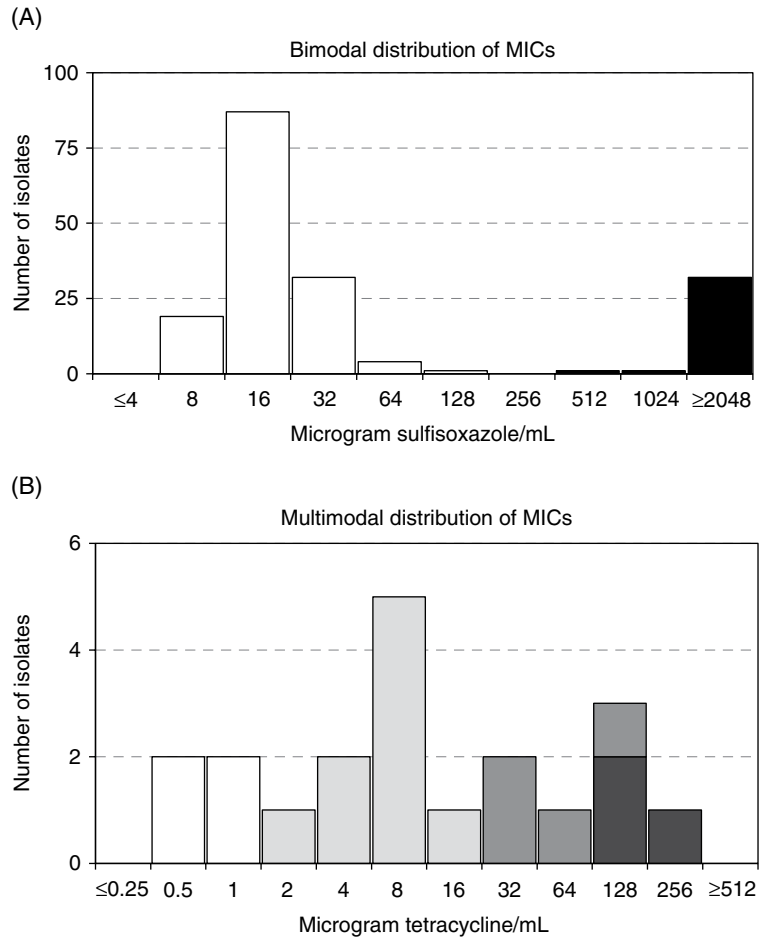

Figure 3.2. Examples of bimodal and multimodal distribution of minimal inhibitory concentrations. (A) Bimodal distribution of MICs for sulfonamides in a sample of commensal *Escherichia coli* isolates from swine and cattle. Susceptible isolates are in white and isolates with a resistance determinant are in black. Note the clear separation between the two groups. (B) Multimodal distribution of MICs for tetracycline in a sample of *E. coli* from a variety of origins. Fully susceptible isolates without any resistance determinant are in white. Isolates with a *tet*(C), *tet*(A), and *tet*(B) are in increasingly dark shades of gray. Note that depending on the respective frequency of each tetracycline resistance determinant, modes may or may not be clearly visible.

resistances should generally be known by clinicians and other users of antimicrobial agents so as to avoid inappropriate and ineffective therapeutic treatments. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides a very useful interactive list of antimicrobial susceptibility tables for a variety of organism/antimicrobial combinations on its website (http://mic.eucast.org/Eucast2/).

Antimicrobial resistance can also be acquired, such as when a normally susceptible organism develops resistance through some type of genetic modification. Acquisition of resistance usually leads to discrete jumps in the MIC of an organism and hence to clear bi- or polymodal distributions of MICs (Figure 3.2). However, in some instances such as for fluoroquinolone antimicrobials, acquisition of resistance (elevated MICs) may be a progressive phenomenon, through successive accumulation of multiple genetic modifications blurring the minimal changes in MIC provided by each modification into a smooth continuous MIC distribution curve, since mutations occur in particular topoisomerase genes in a step-wise manner (Hopkins et al., 2005; Table 3.3).

Table 3.3. Characterization of quinolone-resistant avian pathogenic *E. coli* ($n = 56$).[a]

No. of isolates	Mutation in[b]			MIC range (μg/ml)[c]			
	GyrA	GyrB	ParC	Nal	Orb	Enr	Cip
40	Ser83-Leu	None	None	64–>256	0.5–8	0.25–2	0.12–1
7	Asp87-Tyr	None	None	128	0.5–1	0.25–0.5	0.12–0.25
1	Asp87-Tyr	None	Ser80-Ile	>256	>16	16	8
1	Ser83-Leu; Asp87-Gly	None	None	128	1	0.5	0.25
1	Ser83-Leu; Asp87-Ala	None	None	>256	2	1	0.5
1	Ser83-Leu; Asp87-Gly	None	Ser80-Arg	>256	8	4	2
2	Ser83-Leu	Asp426-Thr	None	256	2	0.5	0.25–0.5
1	Ser83-Leu	Glu466-Asp	None	>256	8	2	1
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4

[a]Adapted from Zhao S, et al. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet Microbiol 107:215.
[b]Substituted amino acids, and the position number; e.g., Ser83-Leu indicates substitution of a leucine for a serine at position 83. Amino acids: Ser, serine; Asp, aspartic acid; Leu, leucine; Tyr, tyrosine; Glu, glutamic acid; Gly, glycine; I, isoleucine; Arg, arginine; Ala, alanine; Thr, threonine; None, wild-type. No mutations were identified in *parE* sequences.
[c]Nal, nalidixic acid; Orb, orbifloxacin; Enr, enrofloxacin; Cip, ciprofloxacin.

Acquired resistance can be manifested as resistance to a single agent, to some but not all agents within a class of antimicrobial agents, to an entire class of antimicrobial agents, or even to agents of several different classes. In the great majority of cases, a single resistance determinant encodes resistance to one or several antimicrobial agents of a single class of antimicrobials (such as aminoglycosides, beta-lactams, fluoroquinolones) or of a group of related classes of antimicrobials such as the macrolide-lincosamide-streptogramin group. However, some determinants encode resistance to multiple classes. This is, for example, the case for determinants identified in recent years such as the Cfr rRNA methyltransferase (Long et al., 2006) or the aminoglycoside acetyltransferase variant Aac(6′)-Ib-cr (Robiczek et al., 2006), or when multidrug efflux systems are upregulated, as is the case for the AcrAB-TolC efflux pump system (Randall and Woodward, 2002). The simultaneous acquisition of several unrelated genetic resistance determinants located on the same mobile genetic element is, however, more common as an explanation of multidrug resistance.

As should be clear from the discussion above, the acquisition of genetic determinants of resistance is associated with a variety of MICs and does not always lead to clinically relevant resistance levels. Therefore, the use of MIC data rather than categorical classification of bacteria into resistant and susceptible is encouraged. This would avoid many apparent contradictions and compromises between clinicians, microbiologists, and epidemiologists in setting appropriate susceptibility and resistance breakpoints. A clear distinction should be made between epidemiological cut-off values and clinical breakpoints, based on presence of acquired mechanisms causing decreased susceptibility to an antimicrobial or clinical responsiveness, respectively (Kahlmeter et al., 2003; Bywater et al., 2006).

Acquisition of Antimicrobial Resistance

Bacterial antibiotic resistance can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes, or from a combination of these mechanisms. Mutations occur continuously but at relatively low frequency in bacteria, thus leading to the occasional random emergence of resistant mutants. However, under conditions of stress (including those encountered

by pathogens when facing host defenses or in the presence of antimicrobials), bacterial populations with increased mutation frequencies can be encountered (Couce and Blázquez, 2009). This so-called mutator state has been suggested to be involved in the rapid development of resistance *in vivo* during treatment with certain antimicrobials such as fluoroquinolones (Komp Lindgren et al., 2003). However, for the majority of clinical isolates, antimicrobial resistance results from acquisition of extrachromosomal resistance genes.

Foreign DNA can be acquired by bacteria in three different ways (Figure 3.3): (1) uptake of naked DNA present in the environment by naturally competent bacteria (called transformation); (2) transfer of DNA from one bacterium to another by bacteriophages (transduction); and (3) transfer of plasmids between bacteria through a mating-like process called conjugation. Recently, the term *mobilome* was introduced to describe all mobile genetic elements that can move around within or between genomes in a cell. These have been divided into four classes: (1) plasmids; (2) transposons; (3) bacteriophage; and (4) self-splicing molecular parasites (Siefert, 2009). Although there are some examples of bacteriophage-mediated antimicrobial resistance transfer (Colomer-Lluch et al., 2011), the plethora of examples of transferable resistance plasmids found across a broad variety of bacterial hosts suggest that plasmids and conjugation are the major players in the global spread of antimicrobial resistance genes in bacterial populations.

Plasmids are extrachromosomal self-replicating genetic elements that are not essential to survival but that typically carry genes that impart some selective advantage(s) to their host bacterium, such as antimicrobial resistance genes. Despite the apparent efficiency of these transfer mechanisms, bacteria possess a large variety of strategies to avoid being subverted by foreign

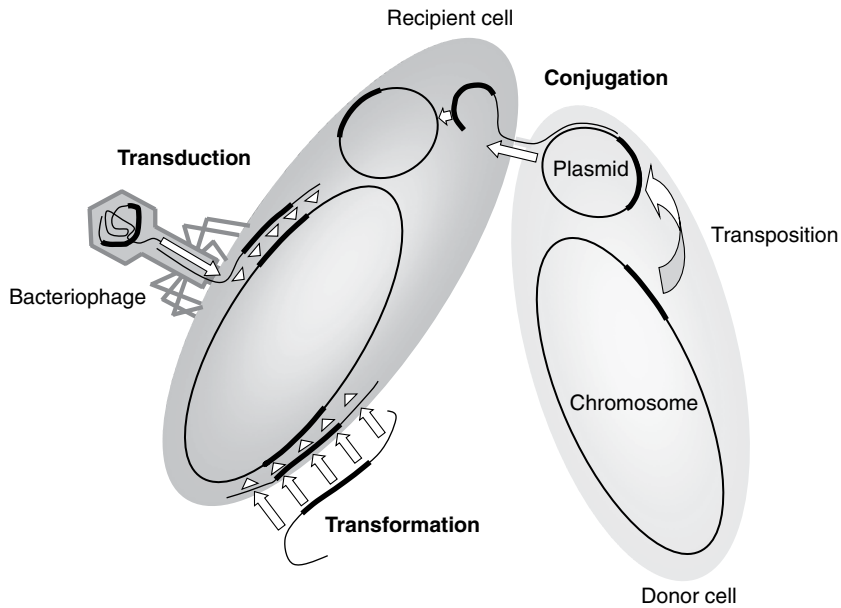

Figure 3.3. The three mechanisms of horizontal transfer of genetic material between bacteria. White arrows indicate the movement of genetic material and recombination events. The bold black line represents an antimicrobial resistance gene (or a cluster of resistance genes). In the case of transduction, a bacteriophage injects its DNA into a bacterial cell, and in the occurrence of a lysogenic phase, this DNA is integrated into the chromosome of the recipient cell. In the case of transformation, "naked" DNA is taken up by a competent cell and may recombine with homologous sequences in the recipient's genome. In the case of conjugation, a plasmid is transferred from a donor bacterium (transfer is coupled with replication and a copy of the plasmid remains in the donor) to recipient cell in which it can replicate. During its stay in various host bacteria, the plasmid may have acquired a transposon carrying antimicrobial resistance genes.

DNA, so that numerous obstacles have to be overcome to allow the stabilization and expression of genes in a new host (Thomas and Nielsen, 2005). In addition, plasmids compete for the replication and partition machinery within cells and plasmids that make use of similar systems and cannot survive for long together in the same cell. This "incompatibility" has led to the classification of plasmids into so-called incompatibility groups, a system widely used to categorize resistance plasmids into similarity groups and to study their epidemiology (Carattoli, 2011). Many studies have shown that antimicrobial resistance plasmids can be transferred between bacteria under a wide variety of conditions. This includes, for example, the relatively high temperature of the intestine of birds as well as other conditions and at the lower temperatures encountered in the environment. Some plasmids can be transferred easily between a variety of bacterial species, for instance between harmless commensal and pathogenic bacteria, thus leading in some cases to the emergence and massive establishment of newly resistant pathogen populations in individual animals within days (Poppe et al., 2005).

In addition to moving between bacteria, resistance genes can also move within the genome of a single bacterial cell and hop from the chromosome to a plasmid or between different plasmids or back to the chromosome, thus allowing development of a variety of resistance gene combinations and clusters over time. Transposons and integrons play a major role in this mobility within a genome. Transposons ("jumping genes") are genetic elements that can move from one location on the chromosome to another; the transposase genes required for such movement are located within the transposon itself. The simplest form of a transposon is an insertion sequence (IS) containing only those genes required for transposition. An advancement on the IS model is seen in the formation of composite transposons. These consist of a central region containing genes (passenger sequences) other than those required for transposition (e.g., antibiotic resistance) flanked on both sides by IS that are identical or very similar in sequence. A large number of resistance genes in many different bacterial species are known to occur as part of composite transposons (Salyers and Amiable-Cuevas, 1997).

Homologous recombination between similar transposons within a genome also play an important role in clustering passenger sequences such as antimicrobial resistance genes together on a single mobile element. Another group of mobile elements called IS*CR* that also help mobilize adjacent genetic material by mechanisms different from classical insertion sequences has been detected increasingly in relation with integrons (see below) and antimicrobial resistance genes (Toleman et al., 2006). Some bacteria (mainly anaerobes and Gram-positive bacteria) can also carry so-called conjugative transposons, which are usually integrated in the bacterial chromosome but can be excised, subsequently behaving like a transferable plasmid, and finally reintegrate in the chromosome of their next host. The magnitude of resistance development is also explained by the widespread presence of integrons, particularly class 1 integrons (Hall et al., 1999; Cambray et al., 2010). These DNA elements consist of two conserved segments flanking a central region in which antimicrobial resistance "gene cassettes" can be inserted. Multiple gene cassettes can be arranged in tandem, and more than 140 distinct cassettes have been identified to date conferring resistance to numerous classes of antimicrobial drugs as well as to quaternary ammonium compounds (Partridge et al., 2009). In addition, integrons are usually part of composite transposons, thus further increasing the mobility of resistance determinants.

The Origin of Resistance Genes and Their Movement across Bacterial Populations

Resistance genes and DNA transfer mechanisms have likely existed long before the introduction of therapeutic antimicrobials into medicine. For example, antimicrobial-resistant bacteria and resistance determinants have been found in Arctic ice beds estimated to be several thousand years old (D'Costa et al., 2011). More recently, molecular characterization of the culturable microbiome of Lechuguilla Cave, New Mexico (from a region of the cave estimated to be over 4 million years old) revealed the presence of bacteria displaying resistance to a wide range of structurally different antibiotics (Bhullar et al., 2012). Resistant microorganisms have also been found among historic culture collections compiled before the advent of antibiotic drugs as well as from humans or wild animals living in remote geographical settings (Smith, 1967; Bartoloni et al., 2004).

It is widely believed that antibiotic resistance mechanisms arose within antibiotic-producing microorganisms as a way of protecting themselves from the action

of their own antibiotic, and some resistance genes are thought to have originated from these organisms. 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. A number of antibiotic preparations employed for human and animal use have been shown to be contaminated with chromosomal DNA of the antibiotic-producing organism, including identifiable antimicrobial resistance gene sequences (Webb and Davies, 1993). However, as in the case of synthetic antimicrobials such as trimethoprim and sulfonamides, preexisting genes with other resistance-unrelated roles might have evolved through adaptive mutations and recombinations to function as resistance genes. Indeed, some have suggested that in their original host, antimicrobial resistance genes play a role in detoxification of components other than antimicrobials, and in a variety of unrelated metabolic functions (Martinez, 2008). A vast reservoir of such genes, now dubbed the *resistome*, is present in the microbiome of various natural environments (D'Costa et al., 2007; Bhullar et al., 2012), which can be transferred to medically relevant bacteria through genetic exchange (Wright, 2010).

Since resistance genes are frequently located on mobile genetic elements, they can move between pathogens, as well as between non-pathogenic commensal bacteria and pathogens. Thus, the issue of resistance has to be considered beyond the veterinary profession and specific pathogens. Indeed, there is growing evidence that resistance genes identified in human bacterial pathogens were originally acquired from environmental, non-pathogenic bacteria via horizontal gene exchange (Martinez et al., 2011; Davies and Davies, 2010). Resistance genes can spread quickly among bacteria, sometimes to unrelated genera. Even if an ingested bacterium resides in the intestine for only a short time, it has the ability to transfer its resistance genes to the resident microflora, which in turn may serve as reservoirs of resistance genes for pathogenic bacteria. The inclination to exchange genes raises the concern for the possible spread of antimicrobial resistance determinants from commensal organisms in animals and humans to human pathogens (Witte, 1998; Van den Bogaard and Stobberingh, 2000). Thus, the epidemiology of antimicrobial resistance goes beyond the boundaries of veterinary and human medicine. The complexity of movement of microorganisms and of horizontal gene transfer (HGT) involved in the epidemiology of global resistance is difficult to comprehend. The graphical depiction of this complex interaction in Figure 3.4 is the best attempt to date to capture this complexity.

On a long-term evolutionary scale, the epidemiology of antimicrobial resistance should be regarded as dominated by the stochastic or chaotic movement of resistance genes within a gigantic bacterial genetic pool. However, in the shorter term and on a local scale, this unrestricted approach may be too simple and of less practical relevance than considering only resistant pathogens. Because of the complexity of the resistance issue, numerous strategies to control the rise of antimicrobial resistance at every level have emerged in the scientific and medical communities. As with other complex issues that global society faces, no single intervention will be decisive alone, but numerous interventions are needed that cumulatively may preserve acceptable levels of efficacy for current and future antimicrobial drugs (Prescott et al., 2012).

The Effects of Antimicrobial Use on the Spread and Persistence of Resistance

The increased prevalence and dissemination of resistance is an outcome of natural selection, the Darwinian principal of "survival of the fittest." In any large population of bacteria, a few cells that possess traits that enable them to survive in the presence of a toxic substance will be present. Susceptible organisms (i.e., those lacking the advantageous trait) will be eliminated, leaving the remaining resistant populations behind. With long-term antimicrobial use in a given environment, the microbial ecology will change dramatically, with less susceptible organisms becoming the predominant population (Salyers and Amabile-Cuevas, 1997; Levy, 1998). When this occurs, resistant commensal and opportunistic bacteria can quickly become established as dominant components of the normal flora of various host species, displacing susceptible populations. Changes in antimicrobial resistance frequency when new antimicrobials appear on the market or when restrictions are implemented in the use of existing antimicrobials testify for the validity of these evolutionary rules. Several examples

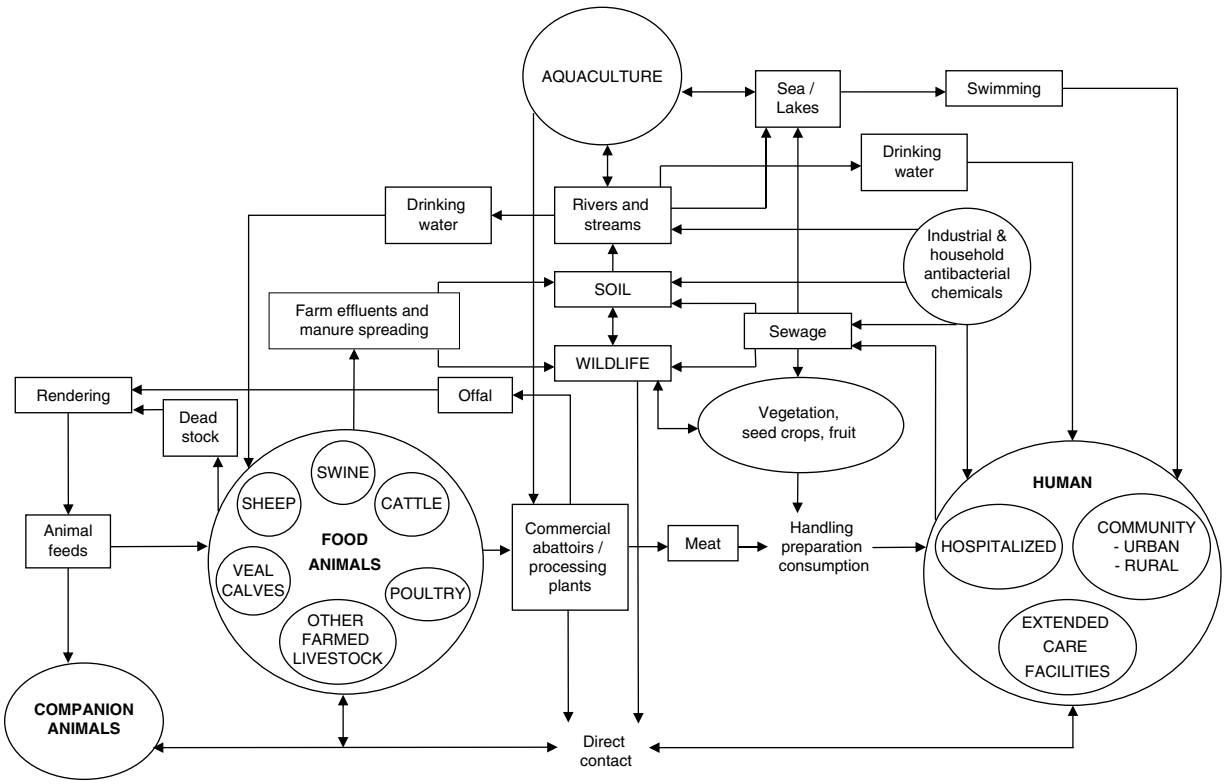

Figure 3.4. The ecology of the spread of antimicrobial resistance and of resistance genes. A schematic representation of resistant bacteria and antimicrobial resistance genes transmission routes across the multiple ecological compartments. This figure is a further development (Irwin et al., 2008) of an original one by Linton, 1977. Reproduced with permission.

of the rise and fall of antimicrobial resistance as selection pressures change are described later in this chapter.

The clustering of multiple resistance genes on plasmids, transposons, and integrons makes the problem of antimicrobial resistance challenging. Exposure to one antimicrobial may co-select for bacteria that are also resistant to several unrelated agents (Cantón and Ruiz-Garbajosa, 2011). There may also be non-antibiotic selection pressure for bacterial antibiotic resistance genes. Although much is only speculative on this subject (Meyer and Cookson, 2010), there is growing evidence showing that disinfectants and biocide may co-select for antimicrobial resistance (Yazdankhah et al., 2006; Hegstad et al., 2010). Not only can resistance determinants for antibiotics of a different class aggregate, but they may also form clusters with resistance genes for non-antibiotic substances such as heavy metals and disinfectants (Baker-Austin et al., 2006; Salyers and Amabile-Cuevas, 1997; Hall et al., 1999) or even with virulence genes (Boerlin et al., 2005; Da Silva and Mendonça, 2012; Johnson et al., 2010).

Carrying genetic material associated with resistance genes when they are not needed represents a burden for bacteria. Therefore, when a bacterial population is not under the selective pressure of antimicrobials, susceptible bacteria not carrying resistance genes may be at an advantage and the population as a whole is expected to slowly revert to a mainly susceptible state. A few examples of such a reversion have been described in the past (Aarestrup et al., 2001; Dutil et al., 2010). However, other studies have also shown that bacteria may exhibit resistance to antimicrobials despite a lack of specific selective pressures, as has been the case, for example, for chloramphenicol, glycopeptides, or streptothricin (Werner et al.,

2001; Bischoff et al., 2005; Johnsen et al., 2005). The mechanisms behind this persistence are unclear but likely to be multifactorial. They may include compensation for the metabolic load imposed by resistance genes by as yet not clearly understood mechanisms (Zhang et al., 2006), regulation of gene expression by the presence/absence of antimicrobials, and plasmid addiction systems. However, the real significance of each one of these mechanisms remains unclear. For instance, compensation for fitness loss has been shown to play a role in the case of resistance mechanisms associated with chromosomal mutations, but its role in the persistence of resistance associated with mobile genetic elements is much less evident. Although plasmid addiction systems may avoid reversion of plasmid carriers to a susceptible state, it is not clear if this is a real advantage for the affected bacteria (Mochizuki et al., 2006). When resistance genes are physically linked together or to other selectively advantageous genes, co-selection will lead to the persistence of all the resistance genes as part of the cluster. Several examples of co-selection are known, such as the maintenance of glycopeptide resistance in porcine enterococci by the use of macrolides, or the persistence and higher frequency of antimicrobial resistance in some pathogen populations due to linkage between virulence and resistance genes (Martinez and Baquero, 2002).

Finally, the effects of diverse drug administration protocols (administration route, timing, dosage) on the dynamics and persistence of susceptible and resistant bacteria and on the spread of resistance genes among bacterial populations at the global and individual level are complex and poorly understood (MacLean et al., 2010). Every effort should be made to define treatment protocols that avoid or minimize the windows for selection of resistant bacteria. This is of particular direct concern when low-level resistance mechanisms elevate the mutant selection window high enough to allow *in vivo* selection of fully resistant mutants, as can be the case for fluoroquinolones (Drlica and Zhao, 2007; Cantón and Morosini, 2011).

Antimicrobial Resistance and Public Health

Although most of the bacterial antimicrobial resistance observed in human medicine may be ascribed to use in human patients, it is being resolutely argued that antimicrobial use in veterinary medicine and food animal agriculture contributes to antimicrobial-resistant foodborne bacterial pathogens. These concerns are not new and in the 1960s led to the release in the United Kingdom of the Swann Report (Anonymous, 1969), which resulted in changes in antimicrobial use in agriculture. Despite the best efforts to date, there is no agreement regarding the scale of the impact of antimicrobial use in animals on human health. The fundamental and obvious concern over the agricultural use of antibiotics arises from the potential that antimicrobials used on the farm select for resistant bacterial strains that are transferred to humans via direct contact and ingestion of contaminated food and/or water (Figure 3.4). Numerous cases of transmission of resistant bacteria between animals and humans at risk, such as farmers, abattoir workers, and veterinarians, support these concerns (Hunter et al., 1994; van den Bogaard et al., 2002; Garcia-Graells et al., 2012). The parallel rise and decrease of resistance to glycopeptides in animal and human enterococci in some European countries after the introduction and subsequent ban of avoparcin (see below) and other antimicrobial growth promoters substantiate these fears. The identification of fluoroquinolone-resistant *Campylobacter* and quinupristin/dalfopristin-resistant enterococci from animal sources or their immediate environment has intensified this debate (Piddock, 1996; Witte, 1998). Food of animal origin has recently even been suggested to represent a potential reservoir of resistant extraintestinal pathogenic *E. coli* for humans, and uropathogenic *E. coli* in particular (Manges and Johnson, 2012). Methicillin-resistant *Staphylococcus aureus* (MRSA) seems to represent another resistant zoonotic agent (see below). This suggests that, because of their intimate contact with humans, pets and not just farm animals may represent another source of resistant bacteria and resistance genes of public health relevance (Ewers et al., 2010; Platell et al., 2011). A historical perspective on the issue of agricultural use of antimicrobial drugs and its impact on human health is available (Prescott, 2006).

Overall, there are clear and compelling data demonstrating that the use of antimicrobials in animals can have negative effects on antimicrobial resistance in bacteria and pathogens from humans. Although more research is needed to quantify the risk associated with this use in animals and the fraction of resistance in human pathogens attributable to it, this situation clearly warrants some caution and preventive measures.

Examples of Antimicrobial Resistance in Veterinary Medicine of Public Health Significance

Resistance in *Salmonella*

Although a large body of science is available on the prevalence of antimicrobial resistance and associated mechanisms in *Salmonella*, many aspects related to the emergence, persistence, and dissemination of antimicrobial resistance in these pathogens remain unclear.

Salmonella can colonize and cause disease in a variety of food-producing and non-food-producing animals. Although all serotypes may be regarded as potential human pathogens, the great majority of infections are caused by only a limited number. Resistance in non-typhoidal *Salmonella* spp. has become an international problem (Threlfall, 2000; Poppe et al., 2001; Williams, 2001). The levels and extent of resistance vary and are influenced by antimicrobial use practices in humans and animals, as well as by geographical differences in the epidemiology of *Salmonella*. Drug resistance phenotypes have been associated with the use of antimicrobials in food-producing animals (Piddock, 1996; Wiuff et al., 2000; Molbak, 2004; Alcaine et al., 2005), in which resistance profiles generally reflect how long an agent has been in use. Thus, irrespective of source (food animals, food, humans), the most frequent resistances are usually to older antimicrobials such as ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Anderson, 1968; Chiappini et al., 2002; Molbak, 2004; Sun et al., 2005). However, there are increasing reports of *Salmonella* isolates worldwide displaying reduced susceptibility or resistance to extended-spectrum cephalosporins or fluoroquinolones (Threlfall et al., 2000; Zhao et al., 2001; Gupta et al., 2003; Alcaine et al., 2005; Johnson et al., 2005; Su et al., 2008; chapters 9 and 18). This is particularly troublesome since these antimicrobial classes are frequently used to treat *Salmonella* infections in children and adults, respectively (Angulo et al., 2004; Alcaine et al., 2005). Treatment will be more difficult with the recent emergence of carbapenemases in *Salmonella* (Savard et al., 2011).

Salmonella Typhimurium continues to be one of the serovars most frequently recovered from food animals worldwide (Zhao et al., 2005). In the United States, it is among the top four serovars most frequent in cattle, swine, chickens, and turkeys. Because of its broad host range, *S.* Typhimurium is also one of the most common serotypes isolated from human salmonellosis. Historically this serovar has often been associated with multiresistance, particularly in relation with phage type DT104, but this type may be decreasing in frequency, and a new multiresistant monophasic *S.* Typhimurium variant is now spreading globally (Butaye et al., 2006; Hauser et al., 2010).

An increase in *S.* Newport infections was reported by the CDC in 2000. Many of these strains exhibited a multidrug-resistant phenotype (commonly referred to as *S.* Newport MDR-AmpC) characterized by resistance to nine antimicrobials, including amoxicillin-clavulanic acid and ceftiofur. In addition to the characteristic resistance to nine specific antimicrobials, these strains also exhibited decreased susceptibility to ceftriaxone (MIC 16–32 μg/ml; Zhao et al., 2003). These strains are of particular clinical concern, as they possess plasmid- or chromosomally encoded AmpC beta-lactamases (e.g., bla_{CMY}) that confer decreased susceptibility to a wide range of beta-lactams, including ceftriaxone, the drug of choice for treating complicated salmonellosis in children (Gupta et al., 2003). Slightly later, a similar increase in third-generation cephalosporin resistance related to bla_{CMY} plasmids was observed in *S.* Heidelberg in Canada, which was attributed to the use of this class of antimicrobials in poultry (Dutil et al., 2010; chapter 9). In both cases, MDR-AmpC strains found their way into the food chain and were linked to human foodborne infection (Gupta et al., 2003; Zhao et al., 2003; Dutil et al., 2010). Multidrug-resistant *Salmonella* have also been associated with illness in animals and humans in equine and companion animal veterinary facilities (Wright et al., 2005). These latter reports frequently describe poor hand-washing practices by employees, eating in work areas, and previous antimicrobial drug therapy in affected humans or animals.

Methicillin-Resistant *Staphylococcus aureus*

MRSA has emerged as a major nosocomial pathogen in human hospitals. This problem had remained limited to hospital settings, but MRSA is now present in the human community too. However, MRSA has been emerging rapidly in animals in recent years, for reasons that are not clear (chapter 8), and represents an important example of both the spread of resistance and the links between resistance in human and animal medicine.

There are an increasing number of reports on MRSA colonization and infections in animals (Weese, 2010), demonstrating spread into animal populations (chapter 8). Most early reports of MRSA in animals were from horses and from dogs and cats; MRSA have remained a rarity in cattle despite extensive use of cloxacillin in mastitis treatment. A recent report from Belgium (Vanderhaegen et al., 2010) suggests that this situation may be changing. MRSA isolates were originally recovered more frequently from horses in relation with nosocomial surgical wound infections possibly originating from humans (Seguin et al., 1999). Equine MRSA usually belong to a specific clone that seems to be maintained within equine populations (Weese et al., 2005a,b). This clone is also occasionally found in humans, particularly in horse personnel, but is not one of the most prevalent human MRSA clones. Investigations suggest that transmission of MRSA goes in both directions between humans and horses and may be associated with clinical disease in both groups.

The epidemiology of MRSA in dogs and cats may be different since the clones found in dogs and cats, and occasionally transmitted between animals, are the same as those frequently found in nosocomial and community infections in humans. In addition, many reports show that the same MRSA strain from clinical infections or from healthy carriage can be found in pets and humans with close contact (van Duijkeren et al., 2004a,b; Rankin et al., 2005). In recent years, the MRSA ST398 clone has emerged massively in livestock (Smith and Pearson, 2011). This clone seems to be particularly frequent in pigs and veal calves (Voss et al., 2005) but has also been described in poultry, dairy cattle, and other species, as well as in meat products. The reasons for the emergence of this clone in livestock are not completely understood. Although people working with livestock (farm workers, veterinarians) are at higher risk of carrying MRSA ST398, its transmission between humans seems not to be as active as for other MRSA.

Antimicrobials in Animal Feeds and Association with Resistance in Bacteria of Human Health Significance

It has been known for decades that continuous oral administration of low concentrations of antimicrobials increases feed conversion and weight gain and reduces shipping stress-associated diseases in food animals (Butaye et al., 2003; Dibner and Richards, 2005). Past studies have shown that this practice is also a potentially significant driving force in accelerating the emergence of resistant bacteria that could infect humans (Wegener, 2003; Kelly et al., 2004; Dibner and Richards, 2005). The use of antimicrobial agents for growth promotion is discussed in chapter 22.

Most classes of antimicrobials used in animals have analogues used in humans and are therefore capable of selecting for resistance to human medical antibiotics. The important exceptions are the ionophores (e.g., lasalocid, monensin, narasin, salinomycin), the quinoxalines (e.g., olaquindox), bambermycins (flavophospholipol), and avilamycin (Turnidge, 2004). Among the former group, two classes of antimicrobials that have received particular attention in the scientific community are the streptogramins (quinupristin/dalfopristin, virginiamycin) and glycopeptides (avoparcin, vancomycin).

Virginiamycin in feed has been approved since 1975 for food-producing animals for growth promotion and prevention or control of certain diseases in turkeys, swine, cattle, and chickens (Kelly et al., 2004). The human analogue, Synercid, a mixture of the two streptogramin antibiotics quinupristin and dalfopristin (QD), was approved in September 1999 by the U.S. FDA for treatment of bacteremias in humans, particularly against vancomycin-resistant *Enterococcus faecium* (VREF) and for the treatment of skin and soft tissue infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Synercid was considered then to be a last resort of therapy for potentially life-threatening bloodstream infections caused by VREF. The approval of Synercid focused increased attention on the use of virginiamycin in animal husbandry; specifically, whether farm use of virginiamycin resulted in streptogramin resistance in bacteria that could result in impaired Synercid therapy in humans (Wegener 2003; Kelly et al., 2004). Synercid-resistant *E. faecium* (SREF) are common in the poultry production environment, including samples from litter and transport containers (McDermott et al., 2005). SREF is also common on poultry meat products at retail, suggesting that such meats serve as a continual source of resistant strains and/or their resistance genes (McDermott et al., 2005). Foodborne strains might transfer plasmidborne resistance determinants to human native enterococci *in vivo* (Jacobsen et al., 1999), which in turn might donate

these genes to other strains causing human infections. The food safety implications prompted the FDA (http://www.fda.gov/downloads/AnimalVeterinary/NewsEvents/CVMUpdates/UCM054722.pdf) and others (Cox and Popken, 2004; Kelly et al., 2004) to propose risk assessment models examining the potential public health consequences of virginiamycin use. The potential for streptogramin resistance genes to transfer from foodborne enterococcal isolates to those causing disease in humans remains difficult to assess, because of complex interplays between bacterial specificity for hosts and gene transfer (Hammerum et al., 2010). In addition, while new resistance genes and new variants thereof keep emerging and spreading in Gram-positive organisms (Witte and Cuny, 2011), a significant proportion of the streptogramin-resistance determinants from enterococci remain unknown in many recent studies. Therefore, estimations of the potential health risks to humans resulting from virginiamycin use in animal husbandry require further study.

Early studies in the 1990s provided evidence in favor of a causal association between the use of avoparcin and the occurrence of VREF on farms in Europe (Bager, 1999; Aarestrup et al., 2000). This suggested that food animals constitute a potential reservoir of infection for VREF in humans (Wegener, 2003). In response to continued pressure from the "major harm" position, the European Union took the "precautionary principle" and followed the earlier move of Scandinavian countries by suspending the use of the "growth promoter" in feed antibiotics: avoparcin, bacitracin, virginiamycin, spiramycin, and tylosin because of their ability to select for resistance to antimicrobials of human importance (Turnidge, 2004; chapter 26). The frequency of resistance to vancomycin and to growth promoters in enterococci from animal origin generally declined after the ban of antimicrobial growth promoters (Aarestrup et al., 2001; Sorum et al., 2004). Interestingly, because of the plasmid-based linkage of glycopeptide and macrolide resistance genes in swine VREF, the decrease of VREF frequency in swine isolates after the ban on avoparcin was slow until tylosin was also banned as a growth promoter (Aarestrup et al., 2001). Some studies have also demonstrated a parallel declining trend in VREF isolated from food and humans after the ban, thus supporting the effectiveness of the ban (Klare et al., 1999; Pantosti et al., 1999). However, VREF are still persisting in animals (Heuer et al., 2002) and isolates similar to those from animals could be recovered from humans several years after the ban of avoparcin (Hammerum et al., 2004; Hammerum, 2012). Thus, antimicrobial resistance associated with the use of antimicrobial growth promoters will not vanish as quickly as early studies had led us to hope (Johnsen et al., 2011). In addition, the global ban of antimicrobial growth promoters might have undesirable consequences on animal health, consequences that remain to be assessed precisely (Casewell et al., 2003). It also increases, at least initially, the use of therapeutic antimicrobials (Grave et al., 2006). As part of the federal strategy for controlling antimicrobial resistance in the United States, the Food and Drug Administration (FDA) in 2012 released Guidance for Industry #209 "The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals," which focuses on two primary principles: (1) limiting medically important antimicrobial drugs to uses in food-producing animals that are considered necessary for assuring animal health; and (2) limiting such drugs to uses in food-producing animals that include veterinary oversight or consultation (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf). This guidance, which represents FDA's current thinking on this topic, is a very important development in the field (chapter 26).

Surveillance Programs and the Role of Diagnostic Laboratories

The seriousness of the antimicrobial resistance threat has prompted many governments to initiate surveillance programs, which include bacteria of animal origin. These programs provide a tool to globally assess the extent of the problem, to follow its evolution over time, and to evaluate the effectiveness of control measures. Such systems include, among others, the National Antimicrobial Resistance Monitoring System (NARMS) in the United States, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in Canada, and the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) in Denmark. On the veterinary side, most of the national surveillance programs only include bacteria considered as indicators of the general resistance situation (i.e., *Escherichia coli* and *Enterococcus* spp.)

and zoonotic bacterial agents (*Salmonella enterica* and *Campylobacter* spp.). Only a few surveillance programs obtain antimicrobial susceptibility data from bacterial pathogens of animals, the most visible being the BfT-GermVet Monitoring Program in Germany (Schwarz et al., 2007). Surveillance programs are of particular interest when, like DANMAP, they include the collection of data on antimicrobial use and try to link the latter with the evolution of resistance. Because of the past problems in lack of standardization of antimicrobial susceptibility testing, it is encouraging that these national surveillance programs use similar (if not identical) methodologies and provide increasingly comparable data.

There is a wealth of information on the prevalence of antimicrobial resistance in animal pathogens (Aarestrup, 2006). However, because of the geographically local and temporarily limited nature of these studies and their different sampling and susceptibility testing methodologies, it is difficult to draw reliable conclusions on the global antimicrobial resistance situation in veterinary medicine. Constant efforts are made by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) to develop agreed veterinary standards for susceptibility testing methodologies (chapter 2). However, investigation shows that many veterinary laboratories do not strictly follow these standards. There is a great need for diagnostic laboratories to adhere to standards so as to provide reliable and reproducible susceptibility data for clinicians and other users. It should be recognized, however, that most studies of antimicrobial resistance in veterinary pathogens are not based on a representative sample of pathogen populations but rather on diagnostic laboratory submissions, so that these reports may overestimate the prevalence of resistance in target pathogen populations. Consequently, better-designed studies are needed for the assessment of the real antimicrobial resistance situation in veterinary pathogens at every level, starting from the farm and all the way up to the global national and international level.

Susceptibility testing of clinical isolates is a cornerstone for prudent use of antimicrobials and for an adequate management of single clinical cases (chapters 2 and 7). Unfortunately, microbiological analysis and susceptibility testing are still frequently performed only when a problem has not been resolved by empirical antimicrobial therapy.

Nosocomial Infection and Antimicrobial Resistance in Veterinary Hospitals

Because of the high selection pressure exerted by the heavy use of antimicrobial agents in human hospitals, resistance first emerged as a significant problem in bacteria associated with nosocomial infections. Veterinary hospitals and practices, and their intensive care units, keep increasing in size. In parallel, companion animal medicine is increasingly more sophisticated and intensive. Consequently, antimicrobial resistance problems similar to those from human hospitals have appeared in companion animal practice. Compared, however, to human medicine, few publications are available on nosocomial infections with multiresistant pathogens in animals. Nevertheless, what there is shows that the similarities between veterinary and human hospitals are striking. The heavy use of antimicrobial agents in intensive care units is associated with increased antimicrobial resistance (Ogeer-Gyles et al., 2006a), multidrug resistant organisms are widespread in veterinary clinics and hospital environments (Murphy et al., 2010), and indwelling devices as well as surgical procedures are "hot spots" for nosocomial infections (Ogeer et al., 2006b; Bubenik et al., 2007; Marsh-Ng et al., 2007; Jones et al., 2009).

Besides the problem with MRSA in horses (Anderson et al., 2009) and companion animals (Wieler et al., 2011) mentioned above, and increasingly frequent outbreaks in veterinary clinics (van Duijkeren et al., 2010), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is now emerging as a major problem organism in the veterinary world, including in hospital settings (van Duijkeren et al., 2011; chapter 8). These organisms seem to be resistant to a large number of other antimicrobials of a variety of classes, making treatment of MRSP infections even more challenging than treatment of MRSA (Steen, 2011). Interestingly, the emergence of MRSP is related to the spread of a very few major clonal lineages (Perreten et al., 2010), suggesting the importance of infection control as one approach to improving antimicrobial stewardship (chapter 7).

Other multiresistant nosocomial pathogens have been reported in veterinary hospital and intensive care units, including *Salmonella enterica*, *E. coli*, *Acinetobacter baumannii*, and enterococci, but other resistant pathogens common in human hospitals are also reported sporadically.

Multiresistant *Salmonella* is one of the most regularly encountered causes of nosocomial infections in veterinary hospitals. Equine clinics seem to be particularly prone to such problems (Dargatz and Traub-Dargatz, 2004), and resistance profiles are increasingly problematic (Dallap Schaer et al., 2010). However, multiresistant *Salmonella* outbreaks also happen in companion animal clinics (Wright et al., 2005). As in human hospitals, multidrug-resistant *Enterobacteriaceae* resistant to extended-spectrum cephalosporins are increasingly being reported in veterinary nosocomial infections. Both AmpC- and ESBL-type beta-lactamases have been described in *Salmonella*, *E. coli* (Sanchez et al., 2002), and *Klebsiella* (Haenni et al., 2011). This may also be a precursor trend toward the emergence of carbapenemases in these organisms (chapter 10).

Acinetobacter baumannii is another often multiresistant Gram-negative organism of environmental origin causing major nosocomial human hospital infection problems. Recent reports suggest that this may also occur in veterinary clinics (Endimiani et al., 2011; Zordan et al., 2011). Multiresistant *A. baumannii* strains seem to persist better in hospitals under antimicrobial pressure than susceptible organisms. This was the case in a series of *A. baumannii* infections in a veterinary hospital, in which persistent strains were multiresistant, whereas sporadic ones all presented only few resistances. After eradication of a first multiresistant strain through hygienic measures, another persistent multiresistant strain readily replaced the first (Boerlin et al., 2001).

Antimicrobial stewardship and clinical use guidelines are discussed in chapter 7.

Accumulation and Persistence of Antimicrobial Resistance in Pathogens

Resistance gene linkage and co-selection are one of the reasons for the accumulation and persistence of resistance in bacterial populations (Bischoff et al., 2005; Johnsen et al., 2005). However, this does not in itself explain why pathogens are more frequently resistant to antimicrobials than the normal flora. The most frequently cited explanation for this difference is the higher selection pressure exerted on pathogens by repeated treatments. Linkage of resistance and virulence genes on plasmids is likely to be an additional factor explaining the higher prevalence of resistance among many pathogens. Such linkages have already been described sporadically in the past (Martinez and Baquero, 2002), but evidence gathered in molecular epidemiology studies is accumulating to show that it may be a relatively widespread phenomenon, at least in organisms such as *E. coli*. For instance, tetracycline resistance genes are frequently linked to enterotoxin genes in enterotoxigenic *E. coli*, which may explain why tetracycline resistance is more frequent in ETEC than in commensal *E. coli* populations (Boerlin et al., 2005). Similarly, the linkage of chloramphenicol resistance genes to enterotoxins genes may partially explain why, despite the ban of chloramphenicol approximately 2 decades ago, chloramphenicol resistance is still widespread in porcine ETEC but less frequent in commensal *E. coli*.

Recent research aimed at characterizing broad host range plasmids recovered from numerous bacterial species has shed additional light on potential gene linkage associations. For example, DNA sequencing of multidrug resistant plasmids from *Salmonella* Kentucky revealed highly conserved backbones shared with avian pathogenic *E. coli* (APEC) virulence plasmids (Fricke et al., 2009). Specifically, the largest plasmid identified carried resistance determinants for streptomycin and tetracycline as well as important virulence genes found in APEC strains. Given the shared intestinal habitat, it is likely that *S.* Kentucky acquired APEC-like plasmids from commensal and/or pathogenic *E. coli* strains in the chicken intestine. These results show that antimicrobial resistance determinants and APEC virulence factors important in avian and possibly human *E. coli* pathogenesis can be encoded by the same plasmid. Under antimicrobial selection, the propagation of these virulence factors within bacterial communities could potentially lead to the emergence of new virulent strains from the commensal microflora of both animals and humans.

Do virulence genes accumulate in bacterial populations because of their genetic linkage with resistance genes and because of the selection exerted by antimicrobial use? The extent of genetic linkage and the degree to which co-resistance and virulence are related is an important consideration in assessing risks associated with antimicrobial use.

The Control of Antimicrobial Resistance

It is doubtful whether new classes of antimicrobial agents will be available for veterinary use in the coming years. Novel antimicrobials are likely to be restricted to

human medicine and economic considerations will limit development of new antimicrobials only for animal use. Thus, the antimicrobials available to veterinary medicine will probably remain the same as today. Therefore, continued efforts should be made to preserve their efficacy. Many professional associations, governmental agencies worldwide, and international committees are developing or have provided guidelines for responsible and prudent use of antimicrobial agents in veterinary medicine and agriculture (chapter 7). Additionally, economic incentives and the development of new market segments, such as the production of food from organic farms and "antibiotic-free" animals may reduce the use of antimicrobial agents in animals. The role of alternatives to antimicrobials such as vaccines, as well as pre- and probiotics, also remains to be thoroughly assessed and defined. Finally, maintenance and improvement of good management practices in companion animal medicine as well as in food animal husbandry represent cornerstones in the reduction of antimicrobial use and in the control of antimicrobial resistance.

In conclusion, the optimism of the early antimicrobial discovery era has been tempered by the emergence of bacterial strains displaying resistance to almost every antimicrobial therapeutic in use. Today, many clinically important bacteria are characterized by multiple antibiotic resistance phenotypes, the legacy of past decades of antimicrobial use and misuse. This modern predicament of widespread antimicrobial resistance has led recognition internationally that the benefits of these agents may be lost, unless there is comprehensive and concerted action to combat the present problem and to reverse anticipated developments. Resistance is an inevitable biological phenomenon: the challenge is to prevent it from continuing to be a persistent and serious obstacle to modern medicine.

Bibliography

Aarestrup FM (ed). 2006. Antimicrobial Resistance in Bacteria of Animal Origin. Washington, DC: ASM Press.

Aarestrup FM, et al. 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb Drug Resist 6:63.

Aarestrup FM, et al. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother 45:2054.

Alcaine SD, et al. 2005. Ceftiofur-resistant *Salmonella* strains isolated from dairy farms represent multiple widely distributed subtypes that evolved by independent horizontal gene transfer. Antimicrob Agents Chemother 49:4061.

Anderson ES. 1968. Drug resistance in *Salmonella typhimurium* and its implications. Brit Med J 3:333.

Anderson ME, et al. 2009. Retrospective multicentre study of methicillin-resistant *Staphylococcus aureus* infections in 115 horses. Equine Vet J 41:401.

Angulo FJ, et al. 2004. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med B Infect Dis Vet Pub Health 51:374.

Anonymous. 1969. Joint Committee on the Use of Antimicrobial Drugs in Animal Husbandry and Veterinary Medicine. London: HMSO.

Bager F. 1999. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. Microb Drug Res 5:53.

Baker-Austin C, et al. 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol 14:176.

Bartoloni A, et al. 2004. High prevalence of acquired resistance unrelated to heavy antimicrobial consumption. J Infect Dis 189:1291.

Bhullar K, et al. 2012. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One 7:e34953.

Bischoff KM, et al. 2005. The chloramphenicol resistance gene *cmlA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. FEMS Microbiol Lett 243:285.

Boerlin P, et al. 2001. Transmission of opportunistic pathogens in a veterinary teaching hospital. Vet Microbiol 82:347.

Boerlin P, et al. 2005. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. Appl Environ Microbiol 71:6753.

Bubenik LJ, et al. 2007. Frequency of urinary tract infection in catheterized dogs and comparison of bacterial culture and susceptibility testing results for catheterized and noncatheterized dogs with urinary tract infections. J Am Vet Med Assoc 231:893.

Butaye P, et al. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clin Microbiol Rev 16:175.

Butaye P, et al. 2006. The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. Microbes Infect 8:1891.

Bywater R, et al. 2006. Antimicrobial breakpoints—definitions and conflicting requirements. Vet Microbiol 118:158.

Cambray G, et al. 2010. Integrons. Annu Rev Genet 44:141.

Cantón R, Morosini MI. 2011. Emergence and spread of

antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev 35:977.
Cantón R, Ruiz-Garbajosa P. 2011. Co-resistance: an opportunity for the bacteria and resistance genes. Curr Opin Pharmacol 11:477.
Carattoli A. 2011. Plasmids in Gram-negatives: molecular typing of resistance plasmids. Int J Med Microbiol 301:654.
Casewell M, et al. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52:159.
Chiappini E, et al. 2002. Results of a 5-year prospective surveillance study of antibiotic resistance among *Salmonella enterica* isolates and ceftriaxone therapy among children hospitalized for acute diarrhea. Clin Ther 24:1585.
Colomer-Lluch M, et al. 2011. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs and poultry. Antimicrob Agents Chemother 55:4908.
Couce A, Blázquez J. 2009. Side effects of antibiotics on genetic variability. FEMS Microbiol Rev 33:531.
Cox LA, Popken DA. 2004. Quantifying human health risks from virginiamycin used in chickens. Risk Anal 24:271.
Da Silva GJ, Mendonça N. 2012. Association between antimicrobial resistance and virulence in *Escherichia coli*. Virulence 3:18.
Dallap Schaer BL, et al. 2010. Outbreak of salmonellosis caused by *Salmonella enterica* serovar Newport MDR-AmpC in a large animal veterinary teaching hospital. J Vet Intern Med 24:1138.
Dargatz DA, Traub-Dargatz JL. 2004. Multidrug-resistant *Salmonella* and nosocomial infections . Veterinary Clinics of North America. Equine Pract 20:587.
Davies J, Davies D. 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol 74:417.
D'Costa VM, et al. 2007. Expanding the soil antibiotic resistome: exploring environmental diversity. Curr Opin Microbiol 10:481.
D'Costa VM, et al. 2011. Antibiotic resistance is ancient. Nature 477:457.
Dibner JJ, Richards JD. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84:634.
Doron S, Davidson LE. 2011. Antimicrobial stewardship. Mayo Clin Proc 86:1113.
Drlica K, Zhao X. 2007. Mutant selection window hypothesis updated. Clin Infect Dis 44:681.
Dutil L, et al. 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis 16:48.
Endimiani A, et al. 2011. *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. J Antimicrob Chemother 66:2248.
Ewers C, et al. 2010. Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing *Escherichia coli* among companion animals. J Antimicrob Chemother 65:651.
Ewers C, et al. 2011. Extended-spectrum beta-lactamases-producing Gram-negative bacteria in companion animals: action is clearly warranted! Berl Munch Tierarztl Wochenschr 124:94.
Fricke WF, et al. 2009. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic Escherichia coli strains in *Salmonella enterica* serovar Kentucky isolates from poultry. Appl Environ Microbiol 75:5963.
Garcia-Graells C, et al. 2012. Livestock veterinarians at high risk of acquiring methicillin-resistant *Staphylococcus aureus* ST398. Epidemiol Infect 140:383.
Grave K, et al. 2006. Usage of veterinary therapeutic antimicrobials in Denmark, Norway and Sweden following termination of antimicrobial growth promoter use. Prev Vet Med 75:123.
Gupta A, et al. 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J Infect Dis 188:1707.
Haenni M, et al. 2012. Veterinary hospital-acquired infections in pets with a ciprofloxacin-resistant CTX-M-15-producing *Klebsiella pneumoniae* ST15 clone. J Antimicrob Chemother 67:770.
Hall RM, et al. 1999. Mobile gene cassettes and integrons in evolution. Ann NY Acad Sci 870:68.
Hammerum AM. 2012. Enterococci of animal origin and their significance for public health. Clin Microbiol Infect 18:619.
Hammerum AM, et al. 2004. A vancomycin-resistant *Enterococcus faecium* isolate from a Danish healthy volunteer, detected 7 years after the ban of avoparcin, is possibly related to pig isolates. J Antimicrob Chemother 53:547.
Hammerum AM, et al. 2010. Antimicrobial-resistant enterococci in animals and meat: a human health hazard? Foodborne Pathog Dis 7:1137.
Hauser E, et al. 2010. Pork contaminated with *Salmonella enterica* serovar 4,[5],12:i:-, an emerging health risk for humans. Appl Environ Microbiol 76:4601.
Hegstad K, et al. 2010. Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? Microb Drug Resist 16:91.
Helms M, et al. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. Emerg Infect Dis 8:490.
Heuer OE, et al. 2002. Vancomycin-resistant enterococci (VRE) in broiler flocks 5 years after the avoparcin ban. Microb Drug Res 8:133.
Hopkins KL, et al. 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. Int J Antimicrob Agents 25:358.
Hunter JE, et al. 1994. Apramycin-resistant Escherichia coli isolated from pigs and a stockman. Epidem Infect 112:473.
Irwin R, et al. 2008. *Salmonella* Heidelberg: ceftiofur-related resistance in human and retail chicken isolates in Canada. Proc ASM Conference: Antimicrobial Resistance in

Zoonotic Bacteria and Foodborne Pathogens, 15–18 June 2008, Copenhagen, Denmark.
Jacobsen BL, et al. 1999. Horizontal transfer of the *satA* gene encoding streptogramin A resistance between isogenic *Enterococcus faecium* strains in the gastrointestinal tract of gnotobiotic rats. Microb Ecol Health Dis 11:241.
Johnsen PJ, et al. 2005. Persistence of animal and human glycopeptide-resistant enterococci on two Norwegian poultry farms formerly exposed to avoparcin is associated with a widespread plasmid-mediated *vanA* element within a polyclonal enterococcus faecium population. Appl Environ Microbiol 71:159.
Johnsen PJ, et al. 2011. Retrospective evidence for a biological cost of vancomycin resistance determinants in the absence of glycopeptide selective pressures. J Antimicrob Chemother 66:608.
Johnson JM, et al. 2005. Antimicrobial resistance of selected *Salmonella* isolates from food animals and food in Alberta. Can Vet J 46:141.
Johnson TJ, et al. 2010. Sequence analysis and characterization of a transferable hybrid plasmid encoding multidrug resistance and enabling zoonotic potential for extraintestinal *Escherichia coli*. Infect Immun 78:1931.
Jones ID, et al. 2009. Factors contributing to the contamination of peripheral intravenous catheters in dogs and cats. Vet Rec 164:616.
Kahlmeter G, et al. 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52:145.
Kelly L, et al. 2004. Animal growth promoters: to ban or not to ban? A risk assessment approach. Int J Antimicrob Agents 24:7.
Klare I et al. 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Res 5:45.
Komp Lindgren P, et al. 2003. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. Antimicrob Agents Chemother 47:3222.
Levy SB. 1998. Multidrug resistance, a sign of the times. New Engl J Med 338:1376.
Linton AH. 1977. Antibiotic resistance: the present situation reviewed. Veterinary Record 100:354.
Liu B, Pop M. 2009. ARDB-Antibiotic resistance genes database. Nucleic Acids Res 37:D443.
Long KS, et al. 2006. The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. Antimicrob Agents Chemother 50:2500.
MacLean RC, et al. 2010. The population genetics of antibiotic resistance: integrating molecular mechanisms and treatment contexts. Nat Rev Genet 11:405.
Manges AR, Johnson JR. 2012. Food-borne origins of *Escherichia coli* causing extraintestinal infections. Clin Infect Dis 55:712.
Maragakis LL, et al. 2008. Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther 6:751
Marshall BM, Levy SB. 2011. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24:718.
Marsh-Ng ML, et al. 2007. Surveillance of infections associated with intravenous catheters in dogs and cats in an intensive care unit. J Am Anim Hosp Assoc 43:13.
Martinez JL. 2008. Antibiotics and antibiotic resistance genes in natural environments. Science 321:365.
Martinez JL. 2010. Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. Front Microbiol 2:265.
Martinez JL, Baquero F. 2002. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev 15:647.
McDermott PF, et al. 2005. Changes in antimicrobial susceptibility of native *Enterococcus faecium* in chickens fed virginiamycin. Appl Environ Microbiol 71:4986.
Meyer B, Cookson B. 2010. Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control? J Hosp Infect 76:200.
Mochizuki A, et al. 2006. Genetic addiction: selfish gene's strategy for symbiosis in the genome. Genetics 172:1309.
Molbak K. 2004. Spread of resistant bacteria and resistance genes from animals to humans—the public health consequences. J Vet Med B Infect Dis Vet Pub Health 51:364.
Murphy CP, et al. 2010. *Escherichia coli* and selected veterinary and zoonotic pathogens isolated from environmental sites in companion animal veterinary hospitals in southern Ontario. Can Vet J 51:963.
National Research Council, Institute of Medicine. 1998. The Use of Drugs in Food Animals: Benefits and Risks. Washington, DC: National Academy Press.
Ogeer-Gyles J, et al. 2006a. Evaluation of catheter-associated urinary tract infections and multi-drug-resistant *Escherichia coli* isolates from the urine of dogs with indwelling urinary catheters. J Am Vet Med Assoc 229:1584.
Ogeer-Gyles J, et al. 2006b. Development of antimicrobial drug resistance in rectal *Escherichia coli* isolates from dogs hospitalized in an intensive care unit. J Am Vet Med Assoc 229:694.
Pallechi L, et al. 2008. Antibiotic resistance in the absence of antimicrobial use: mechanisms and implications. Expert Rev Anti Infect Ther 6:725.
Pantosti A, et al. 1999. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet 354:741.
Partridge SR, et al. 2009. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev 33:757.
Perreten V, et al. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother 65:1145.

Piddock LJ. 1996. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? J Antimicrob Chemother 38:1.

Platell JL, et al. 2011. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. Antimicrob Agents Chemother 55:3782.

Poppe C, et al. 2001. Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin, and the environment of animal production in Canada, 1994–1997. Microb Drug Res 7:197.

Poppe C, et al. 2005. Acquisition of resistance to extended-spectrum cephalosporins by *Salmonella enterica* subsp. *enterica* serovar Newport and *Escherichia coli* in the turkey poult intestinal tract. Appl Environ Microbiol 71:1184.

Prescott JF. 2006. History of antimicrobial usage in agriculture. In: Aarestrup FM (ed). Antimicrobial Resistance in Bacteria of Animal Origin. Washington, DC: ASM Press, pp. 19–28.

Prescott JF. 2008. Antimicrobial use in food and companion animals. Anim Health Res Rev 9:127.

Prescott JF, et al. 2012. Conference report: antimicrobial stewardship in Canadian agriculture and veterinary medicine. How is Canada doing and what still needs to be done? Can Vet J 53:402.

Randall LP, Woodward MJ. 2002. The multiple antibiotic resistance (*mar*) locus and its significance. Res Vet Sci 72:87.

Rankin S, et al. 2005. Panton-Valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 108:145.

Robicsek A, et al. 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 12:83.

Salyers AA, Amiable-Cuevas CF. 1997. Why are antibiotic resistance genes so resistant to elimination? Antimicrob Agents Chemother 41:2321.

Sanchez S, et al. 2002. Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. J Clin Microbiol 40:3586.

Savard P, et al. 2011. First NDM-positive *Salmonella* spp. strain identified in the United States. Antimicrob Agents Chemother 55:5957

Schwarz S, et al. 2007. The BfT-GermVet monitoring program-aims and basics. Berl Munch Tieraztl Wochenschr 120:357.

Seguin JC, et al. 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J Clin Microbiol 37:1459.

Shorr AF. 2009. Review of studies of the impact on Gram-negative bacterial resistance on outcomes in the intensive care unit. Crit Care Med 37:1463.

Siefert JL. 2009. Defining the mobilome. Methods Mol Biol 532:13.

Smith DH. 1967. R factor infection of *Escherichia coli* lyophylized in 1946. J Bacteriol 94:2071.

Smith TC, Pearson N. 2011. The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis 11:327.

Sorum M, et al. 2004. Prevalence of vancomycin resistant enterococci on poultry farms established after the ban of avoparcin. Avian Dis 48:823.

Steen S. 2011. Methicillin-resistant strains of *Staphylococcus pseudintermedius* in companion animals. Vet Rec 169:53.

Su LH, et al. 2008. An epidemic of plasmids? Dissemination of extended-spectrum cephalosporinases among *Salmonella* and other Enterobacteriaceae. FEMS Immunol Med Microbiol 52:155.

Sun HY, et al. 2005. Occurrence of ceftriaxone resistance in ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis isolates causing recurrent infection. Clin Infect Dis 40:208.

Thomas CM, Nielsen KM. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Rev Microbiol 3:711.

Threlfall EJ. 2000. Epidemic *Salmonella typhimurium* DT 104-a truly international multiresistant clone. J Antimicrob Chemother 46:7.

Threlfall EJ, et al. (2000). Spread of resistance from food animals to man—the UK experience. Acta Vet Scand S93:63.

Toleman MA, et al. 2006. ISCR elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev 70:296.

Travers K, Barza M. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin Infect Dis 34 S3:S131.

Turnidge J. 2004. Antibiotic use in animals—predjudices, perceptions and realities. J Antimicrob Chemother 53:26.

Van den Bogaard AE, Stobberingh EE. 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents 14:327.

Van den Bogaard AE, et al. 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 49:497.

Van Duijkeren E, et al. 2004a. Methicillin-resistant staphylococci isolated from animals. Vet Microbiol 103:91.

Van Duijkeren E, et al. 2004b. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 10:2235.

Van Duijkeren E, et al. 2010. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. Vet Microbiol 141:96.

Van Duijkeren E, et al. 2011. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 66:2705.

Vanderhaeghen W, et al. 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. Vet Microbiol 144:166.

Varma JK, et al. 2005. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. J Infect Dis 191:554.

Voss A, et al. 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965.

Webb V, Davies J. 1993. Antibiotic preparations contain DNA: a source of drug resistance genes? Antimicrob Agents Chemother 37:2379.

Weese J, et al. 2005a. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. Emerg Infect Dis 11:430.

Weese J, et al. 2005b. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. J Am Vet Med Assoc 226:580.

Weese JS. 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 51:233.

Wegener HC. 2003. Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol 6:439.

Werner G, et al. 2001. Aminoglycoside-streptothricin resistance gene cluster aadE-sat4-aphA-3 disseminated among multiresistant isolates of *Enterococcus faecium*. Antimicrob Agents Chemother 45:3267.

Wieler LH, et al. 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol 301:635.

Williams RJ. 2001. Globalization of antimicrobial resistance: epidemiological challenges. Clin Infect Dis 33 S3:S116.

Witte W. 1998. Medical consequences of antibiotic use in agriculture. Science 279:996.

Witte W, Cuny C. 2011. Emergence and spread of *cfr*-mediated multiresistance in staphylococci: an interdisciplinary challenge. Future Microbiol 6:925.

Wiuff C, et al. 2000. Quinolone resistance among *Salmonella enterica* from cattle, broilers, and swine in Denmark. Microbial Drug Res 6:11.

Wright GD. 2010. Antibiotic resistance in the environment: a link to the clinic? Curr Opin Microbiol 13:589.

Wright JG, et al. 2005. Multidrug-resistant *Salmonella* Typhimurium outbreak. Emerg Infect Dis 11:1235.

Yazdankhah SP, et al. 2006. Triclosan and antimicrobial resistance in bacteria: an overview. Microb Drug Resist 12:83.

Zhang Q, et al. 2006. Fitness of antimicrobial-resistant *Campylobacter* and *Salmonella*. Microbes Infect 8:1972.

Zhao S, et al. 2001. Identification and expression of cephamycinase *bla*(CMY) genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrob Agents Chemother 45:3647.

Zhao S, et al. 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J Clin Microbiol 41:5366.

Zhao S, et al. 2005. Characterization of *Salmonella Typhimurium* of animal origin obtained from the National Antimicrobial Resistance Monitoring System (NARMS). Foodborne Path Dis 2:169.

Zordan S, et al. 2011. Multidrug-resistant *Acinetobacter baumannii* in veterinary clinics, Germany. Emerg Infect Dis 17:1751.