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# Antimicrobial Resistance

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Development of antimicrobial resistance by microbial pathogens and commensals represents a major threat to animal and public health. In the United States, resistant bacterial infections are estimated to increase human health care costs by approximately $6000 to $30,000 per patient [1] and by at least $4 billion [2] annually. Although the economic impact of antimicrobial resistance in small companion animals is unknown, it is clear that the decreased efficacy of commonly used antibacterial agents and the need to use more expensive drugs not only limit therapeutic options but inflate the expense of treating infectious diseases. In several instances, the armamentarium of antibacterial drugs available to treat infections caused by certain resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), may be so restricted that the ability to cure an infection without producing toxicity is compromised.

Microbes are ubiquitous in the environment, including on the skin and mucous membranes as well as in the gastrointestinal tracts of animals. The ecologic success of microorganisms is largely attributable to their ability to survive hostile conditions and adapt to changes in the environment. Therefore, development of antimicrobial resistance is not a recent phenomenon but an inevitable consequence of microbial cell evolution. Indeed, the ability of bacteria to develop resistance was described soon after the introduction of the pioneer antibacterial agents [3,4]. Current concerns relating to antimicrobial resistance arise principally from the rapid rate of development of resistance relative to the slow rate at which new mechanistic groups of antibiotics are introduced and the conviction that development of resistance is accelerated by overuse of antibiotics. Furthermore, the ability of bacteria to acquire and transfer multiple resistance genes to other bacteria is alarming. The ease with which these genes are transferred between bacteria accelerates the emergence of antibacterial resistance in a particular animal species and increases the risk of spread of resistance to other species, including human beings. The latter is particularly relevant to concerns that use of antibacterial agents in food animals increases the occurrence of resistant infections in human consumers of food animal products. Recently, concerns have also been expressed that contact with small animal pets may serve as a source of infection for people, especially with regard to resistant

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*Staphylococcus* spp. Clearly, if the development and spread of resistance are to be retarded, it is necessary that veterinarians understand the mechanisms that bacteria use to resist antibacterial agents and the processes whereby this capacity is acquired and transferred.

## MECHANISMS OF ANTIMICROBIAL RESISTANCE

Microorganisms generally resist the actions of antimicrobial agents by (1) interfering with the stereospecific requirements necessary for binding of the drug to its target site, (2) destroying or altering the conformational integrity of the drug, or (3) preventing the drug from attaining an effective concentration at its site of action. The stereospecific requirements that must be met for antibacterial agents to interact with target receptors can be disrupted by mutations that produce structural changes to ribosomal binding sites (relevant to aminoglycosides, chloramphenicol, tetracyclines, macrolides, and lincosamides), enzymes responsible for nucleic acid synthesis and function (relevant to fluoroquinolones and rifampin), and enzymes responsible for synthesis of bacterial cell walls (relevant to β-lactams). This mechanism of resistance often results in large increases in minimal inhibitory concentration (MIC) values, as is encountered with the structural changes in DNA gyrase that cause substantial decreases in binding affinities to fluoroquinolones.

Examples of mechanisms involving destruction of antibacterial agents or changes in conformational integrity are hydrolysis of the β-lactam ring of penicillins and cephalosporins by β-lactamases and conjugation of aminoglycosides, thus preventing transport of drug into the bacterial cell. Initially identified as being produced by gram-positive staphylococci, β-lactamases are now recognized to be produced in multiple forms by many pathogenically important gram-positive and gram-negative bacterial genera, such as *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia*, *Salmonella*, *Shigella*, *Bacteroides*, and *Haemophilus*. The genes encoding production of β-lactamase enzymes may be located on the bacterial chromosome or extrachromosomally on plasmids. Gram-negative bacteria generally produce smaller amounts of β-lactamases than gram-positive bacteria, but this difference has little functional relevance, because gram-negative bacteria usually secrete these enzymes strategically in the periplasmic space, which is where the penicillin or cephalosporin binding proteins are located.

Resistant bacteria can impede attainment of an effective concentration of an antibacterial agent at the site of action by preventing transport across the cell membranes or by active efflux of drug [5] from the bacterial cytoplasm. Impermeability of the cell wall or cell membranes of gram-negative bacteria frequently is a consequence of a reduction in the number of outer membrane porins [6]. Porins are transmembrane protein structures that provide access to relatively water-soluble antibacterial agents. Efflux pumps actively transport drugs from the inner phospholipid layer of the inner cytoplasmic membrane, a site that is sequestered from the aqueous cytoplasm, and therefore is accessible primarily to relatively lipid-soluble drugs. In contrast to mutational changes

in the structure of antibacterial target sites, which confer resistance to similar drugs that meet stringent stereospecific characteristics, changes in porin expression and the action of efflux pumps generally are less specific for individual antimicrobial agents. For example, multidrug-resistant efflux pumps exist that have wide substrate activities across a variety of different chemical groups of antibacterial agents.

Surveys of antimicrobial susceptibility trends indicate increasing resistance to multiple antibacterial agents, which greatly complicates selection of effective antimicrobial therapies. As described previously, a single gene operon encoding for a single mechanism, such as expression of outer membrane porins or an efflux pump, may confer multiple drug resistance because these mechanisms are not specific for particular classes of drugs but discriminate only on the basis of general physicochemical characteristics, such as lipid solubility. Multiple drug resistance may also arise from the accumulation of multiple gene operons, each encoding for a different mechanism of resistance. Accumulation of resistance-encoding genes is promoted by a variety of genetic processes that greatly facilitate transfer of nucleic acids between bacteria.

## ACQUISITION AND TRANSFER OF RESISTANCE GENES

De novo acquisition of resistance-encoding genes occurs by mutations arising from random errors in nucleic acid sequence resulting from polymerase-mediated replication of DNA. Mutation rates for antimicrobial-resistant phenotypes may range from 1 in $10^9$ to 1 in $10^6$ cell divisions. Although this rate may seem to be low, the likelihood of resistant mutants arising is actually quite high because of the high replication rate of bacteria. Indeed, resistant mutants generally can be produced after only a few sequential isolations and cultures on media containing a gradient of antibacterial agent concentration.

Once resistance has been acquired by mutation, it can be transferred by several processes, including conjugation, transduction, and transformation. Although transduction and transformation may be important in specific circumstances, such as transfer of resistance by transduction between staphylococci, conjugation presents the greatest risk for transfer of resistance genes among gram-negative bacteria. Conjugation involves transfer of a plasmid or circular extrachromosomal DNA via an intercellular bridge from a donor to a recipient bacterium [7]. The entire process, including formation of the intercellular bridge, known as a pilus, is encoded by genes on the plasmid (Fig. 1). Once the mating pair of bacteria is linked by the pilus, a single strand of the plasmid is cut and transferred to the recipient cell. Replication of the recipient and donor nucleic acid strands restores the resistance-encoding potential of the separated bacteria. In this manner, resistance genes can be transferred between bacteria of different strains, species, and possibly even genera.

Although resistance genes can be expressed from plasmids acquired by conjugation, the stability of such expression may be enhanced by insertion of these genes in the bacterial chromosome. This often involves a recombination event

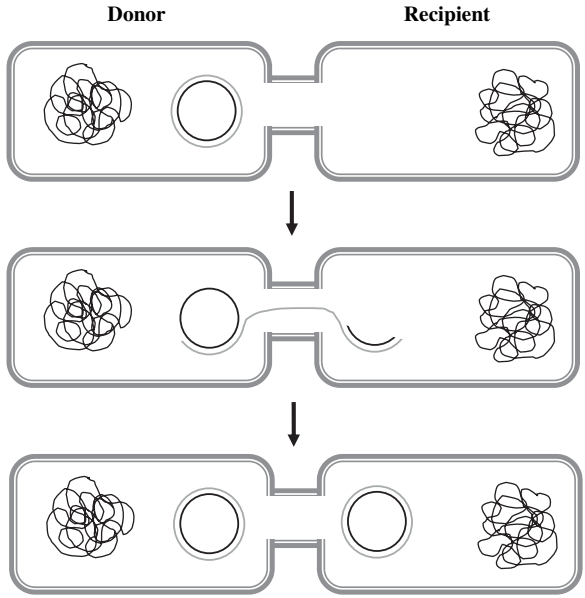


**Fig. 1.** Acquisition of antimicrobial resistance gene(s) by conjugation. After production of a pilus, a single strand of plasmid DNA is transferred to the recipient. During transfer, the remaining DNA strand in the donor and the newly acquired strand in the recipient undergo replication to produce functional double-stranded plasmids.

referred to as transposition [8]. Transposons are DNA elements that can move from one DNA location to another, including between plasmids and chromosomes. Transposases, encoded by the transposon, cut out donor DNA and insert this into the recipient, without regard to a stringent requirement that the DNA sequences have complementary base pairing. Such nonhomologous recombinations provide considerable mobility to antimicrobial resistance genes.

Conjugation and transposition may collaborate with integrases to facilitate transfer and accumulation of multiple genes encoding for antimicrobial resistance (Fig. 2). Integrases are site-specific recombinases encoded by integrons, which are genetic units that provide for assembly of multiple resistance gene cassettes in a single DNA location, including within a transposon or plasmid. Each gene cassette may contain an antimicrobial resistance gene and an integrase-specific recombination site. Under the influence of integrases, multiple gene cassettes may be sequentially inserted in the integron at a cassette receptor site. Expression of these genes can be regulated collectively by a single promoter, thus providing for coordinated defense against many different antimicrobial agents. Therefore, once acquired by random mutation, the collaborative function of conjugation, transposons, and integrons can greatly facilitate transfer and expression of multiple drug resistance, thus demonstrating that bacteria have a phenomenal adaptive ability to survive antibacterial therapy.

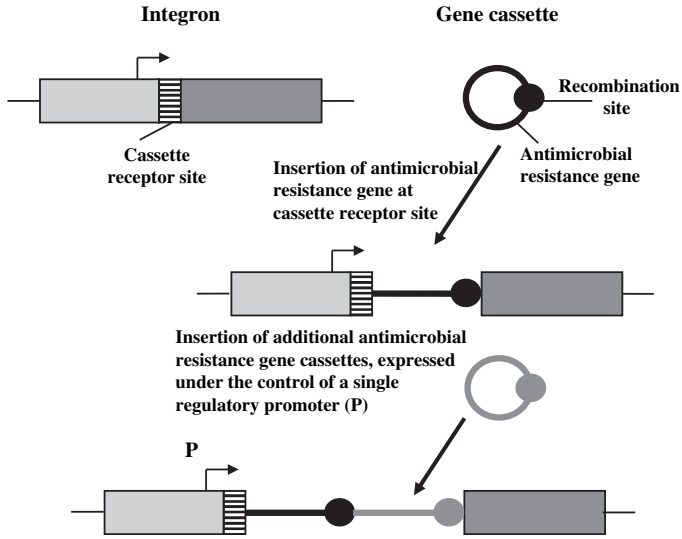


**Fig. 2.** Accumulation of mobile antimicrobial resistance gene cassettes in an integron.

## SELECTION AND EXPRESSION OF RESISTANCE RESULTING FROM EXPOSURE TO ANTIBACTERIAL AGENTS

Generally, antibiotic exposure does not cause a susceptible strain to mutate to a resistant one. Nevertheless, exposure to antimicrobial agents promotes emergence of resistance by facilitating the survival of resistant strains or inducing the expression of existing antimicrobial resistance genes. Classically, resistance in a bacterial population can be identified by the existence of at least two distinct subpopulations separated on the basis of MIC values. Survival of the relatively resistant subpopulation is promoted by exposure to concentrations of antibiotics that inhibit only the susceptible subpopulation. As a result of this differential effect, resistant strains increase in number until they represent a larger proportion of the population as a whole, thus increasing the likelihood that they cause infectious diseases. Within environments that are subject to frequent and consistent antibacterial use patterns, such as intensive care units, the emergence of predominant populations of resistant strains is accelerated, particularly when little care is taken to prevent transfer of resistant strains between patients. Antibiotic exposure not only promotes the survival of drug-resistant pathogenic bacteria but increases the population of drug-resistant nonpathogenic bystanders, many of which are commensals in the upper respiratory and gastrointestinal tracts, thus increasing the reservoir of resistance in the bacterial population as a whole and increasing the opportunity for resistance to be transferred to pathogenic bacteria by processes like conjugation and transposition.

Aside from the effect of antimicrobial exposure on survival of resistant mutants, antimicrobial agents may also induce the expression of existing resistance

genes. For example, antimicrobial agents can induce the expression of the multiple antibiotic resistance (*mar*) locus in *Salmonella enterica* serovar Typhimurium, which regulates the expression of the AcrAB multidrug efflux pump and OmpF porin, both of which contribute to multiple drug resistance by affecting the permeation of antibacterial agents across bacterial cell membranes [9,10]. The Mar phenotype possesses increased resistance to a wide range of organic solvents, disinfectants, and antibiotics, including fluoroquinolones. Although the *marRAB* system confers relatively low-level resistance (MIC values are usually only two to four times higher) [9], it is considered to be clinically important because expression of this system serves as a stepping stone to higher levels of resistance, particularly when bacteria are exposed to marginal and/or subtherapeutic concentrations of antibiotics. The Mar phenotype has been implicated in antibiotic-resistant *Salmonella* infections in human beings and a variety of domestic animals [10], and there is evidence that active efflux may be the primary mechanism of resistance to ciprofloxacin used by the *Salmonella enterica* serovar Typhimurium [11]. Furthermore high-level resistance to fluoroquinolones, mediated primarily by DNA gyrase mutations, may depend on concurrent expression of the AcrAB multidrug efflux pump [12].

Another example of an inducible mechanism of resistance is the protection conferred by the *erm* genes of staphylococci. Under the influence of macrolides and lincosamides, expression of *erm* genes encodes for a change in the bacterial ribosomal binding site targeted by these antibacterial agents. A recent study conducted in the United Kingdom indicated a high incidence of inducible *erm*-mediated resistance to clindamycin in MRSA and recommended routine screening of these isolates for inducible resistance [13].

## EMERGENCE OF RESISTANCE IN SMALL COMPANION ANIMALS

In comparison with people and food animals, relatively few studies have addressed the emergence of resistance in small companion animals and the relationships between the use of antibacterial agents and development of resistance. The small number of investigations that have tracked changes in resistance over time generally indicate that resistance of notable bacteria, such as *Escherichia coli* and *Staphylococcus* spp, to newer antibacterial therapies is increasing. For example, a review of antimicrobial drug use and resistance in dogs based on 15 years of records (1984–1998) from a teaching hospital in Canada concluded that the incidence of resistant coagulase-positive *Staphylococcus* spp varied according to concurrent use patterns for individual antibacterial agents [14]. There was a significant decrease in resistance to penicillin G and ampicillin, which correlated with a decline in the use of these antibacterial agents over a similar period, whereas resistance to cephalothin and enrofloxacin increased concurrently with increased use of these newer antibacterial agents. During the same period, isolation of multidrug-resistant *Enterococcus* spp and *Pseudomonas aeruginosa* from urinary tract infections increased relative to other causes of infection, again suggesting that exposure to antibacterial agents promoted

emergence of resistant bacterial genera and strains. A more recent study conducted in the United States indicated that ciprofloxacin and enrofloxacin resistance of bacteria isolated from the urinary tracts of dogs had increased, although more than 80% of isolates were still susceptible to these fluoroquinolones [15]. The bacteria most commonly isolated were *E coli*, *Proteus mirabilis*, and *Staphylococcus intermedius*. In the United Kingdom, a retrospective study of resistance of *E coli* and *Staphylococcus* spp to antimicrobials used in a small animal referral hospital revealed significantly increasing trends only with respect to resistance of *E coli* to amoxicillin, enrofloxacin, and streptomycin [16]. No changes in resistance were identified for most bacterial-drug interactions; neither were there any significant differences in prevalences of multiple drug-resistant *E coli*, *Proteus* spp, *Pseudomonas* spp, staphylococci, or streptococci. Analysis of resistance of *E coli* to antimicrobials used in a companion animal community practice revealed significant rising trends of resistance to clavulanate-amoxicillin and streptomycin and in multiple drug resistance of *E coli*, *Proteus* spp, and *Pseudomonas* spp, whereas resistance of *Staphylococcus* spp to ampicillin and penicillin G was observed to decrease [17]. In contrast to the results of these studies, the introduction of marbofloxacin in Europe was not associated with any significant increase in resistance over a 7-year period (1994–2001) [18].

Irrespective of whether trends have been identified whereby increases in resistance are correlated with the use of specific antibacterial agents, it is clear that resistance of certain small animal pathogens is high enough to justify concern. This is particularly true of *Staphylococcus* spp, which is the subject of much attention in the human health domain, especially with regard to methicillin and vancomycin resistance. An analysis of canine *S intermedius* isolated in France in 2002 revealed that more than 60% of strains produced β-lactamases against penicillin G [19]. Lower levels of resistance were measured for alternative non-β-lactam antibacterial agents, such as oxytetracycline (46%), chloramphenicol (30%), erythromycin (28%), clindamycin (22%), doxycycline (6%), gentamicin (2%), and fluoroquinolones (2%). No resistance was observed to the combination of clavulanate and amoxicillin, cephalosporins, the combination of sulfonamide-trimethoprim, and oxacillin, which serves as a reliable indicator of methicillin resistance. These observations are consistent with those of studies conducted in the United States, United Kingdom, Denmark, and Germany between 1986 and 1995, in which resistance of *S intermedius* to penicillin and tetracycline was relatively high but resistance to the sulfonamide-trimethoprim combination and enrofloxacin was either low or nonexistent [20].

More recent studies published in 2005 indicate that higher levels of methicillin resistance exist among small animal isolates than were described in earlier reports, however. A survey of seven veterinary teaching hospitals in the United States revealed that 11% of *S aureus* isolated from dogs was methicillin resistant [21]. Studies conducted in the United Kingdom and Ireland also confirm the recent emergence of MRSA, often associated with postoperative and wound infections [22,23]. In addition to methicillin resistance, these isolates may be

resistant to other antibacterial agents, such as macrolides, lincosamides, and fluoroquinolones, thus presenting few alternatives for successful therapy [22].

Although resistance of gram-negative bacteria seems to vary according to geographic origin, there is evidence that resistance to newer antibacterial agents, including those with important human therapeutic value, may be emerging. Results of a study conducted in Sweden during 2002 and 2003 indicate that resistance of *E coli* derived from urogenital infections was generally low and no different from levels of resistance reported approximately 10 years earlier [24]. The highest proportion of resistance observed (26%) in this study was for strains isolated from urine of dogs in hospitals during 1991 through 1993. Similarly, resistance of *E coli* isolated from dogs in Finland, irrespective of prior antimicrobial therapy, was reported to be low [25]. Nevertheless, a study conducted in Italy over the period 2001 through 2003 reported that 45% of *E coli* strains were resistant to tetracycline, 34% to the combination of sulfamethoxazole and trimethoprim, 15% to enrofloxacin, and 7% to cefotaxime [26]. Likewise, in Trinidad, more than one third of all *Salmonella* spp isolated from dogs were resistant to cephalothin [27], thus suggesting that resistance of Enterobacteriaceae to cephalosporins may present a therapeutic concern, especially considering the importance of this group of antibacterial agents in the treatment of gastrointestinal infections in human patients.

In summary, antimicrobial resistance of small animal pathogens varies considerably depending on geographic location, the history of exposure to antibacterial agents, and the specific microorganism of interest. Although resistance of microorganisms affecting small animals may be less widespread than in human beings and food animals, probably because of differences in antibacterial exposure, there are nevertheless sufficient data to conclude that the prevalence of resistance in dogs and cats is high enough to pose therapeutic challenges and to justify development and implementation of strategies to retard further development of resistance.

## HUMAN HEALTH IMPLICATIONS OF ANTIBACTERIAL RESISTANCE IN SMALL ANIMALS

Emergence of antibiotic-resistant bacteria in food animals and the risk posed to human consumers when these resistant bacteria contaminate food products have been subjects of considerable concern in the veterinary and human health communities. Indeed, the use of newer drugs, such as fluoroquinolones, to control animal diseases has been the target of much criticism by the human health community because of the possibility that selection of resistant bacteria and subsequent bacterial contamination of food products may lead to an increase in resistant infections in people [28,29]. Until recently, less attention has been directed at the possibility of direct transfer of resistant microorganisms between small animal pets and people, despite their close physical contact in home environments and use of the same antibacterial agents in human and veterinary practices. In their excellent review of the role of pet animals as reservoirs of antimicrobial-resistant bacteria, Guardabassi and colleagues [30] noted that

veterinarians frequently use first-line antibacterials, such as amoxicillin-clavulanate, cephalosporins, and fluoroquinolones, in pet animals and that the ensuing resistance in pathogenic bacteria as well as commensals presents a significant risk for zoonotic transfer between pets and people. Resistance phenotypes of particular clinical interest include MRSA (including strains producing the highly pathogenic Panton-Valentine leukocidin toxin [31]), vancomycin-resistant enterococci (VRE), and multidrug-resistant *Salmonella* spp.

Antimicrobial-resistant strains isolated from small animals often are indistinguishable from strains isolated from people caring for these animals [23]. For example, using pulsed-field gel electrophoresis, investigators in Ireland observed that the most frequently occurring pattern of MRSA from veterinary sources was the same as the most prevalent strain found in the human population [22]. Based on the epidemiologic analyses of disease outbreaks, transfer of a variety of resistant infections between pets and people has been reported, including multiple drug-resistant *S typhimurium* [30]. In most instances, however, transfer seems to be from people to their pets [32], as is probably true for the transfer of most MRSA strains [33,34] and VRE. Nevertheless, such transfer still poses a risk to human health, because pets can then serve as reservoirs of these microorganisms and as a source of infection for other susceptible people and animals living in the same household.

## NEW ANTIMICROBIAL DRUGS ON THE HORIZON

Historically, the principal strategy used against antimicrobial resistance has been the introduction of new antibacterial agents with novel mechanisms of action to which previously resistant microorganisms are susceptible. For example, initial β-lactamase–mediated resistance to natural penicillins was addressed by the development of the antistaphylococcal penicillins, such as methicillin and cloxacillin. When multidrug-resistant bacteria subsequently developed resistance to methicillin, veterinarians and physicians turned to vancomycin as a drug of last resort, thus severely limiting therapeutic options. Although newer products, such as teicoplanin and linezolid, may be used systemically to treat vancomycin-resistant staphylococcus, the pace at which new drugs are being introduced is clearly insufficient to keep up with the rate at which resistance currently develops. Indeed, the number of new antibacterial agents approved by the US Food and Drug Administration in recent years has declined by more than 50% relative to the approval rate 20 years ago [35]. Challenges involved in antimicrobial drug development as well as economic incentives in favor of developing other drugs used to treat chronic medical conditions in elderly people, such as hypercholesterolemia, hypertension, psychologic and/or psychiatric disorders, and arthritis, have discouraged pharmaceutical companies from developing new molecular entities with activity against microorganisms. During the period 1998 through 2002, nine new antibacterial agents were approved for use in human beings, and only two of these (linezolid and daptomycin) exert their antibacterial activity via novel mechanisms [35]. Based on

public disclosures of the world's 15 largest pharmaceutical companies, five new antibacterial molecular entities were estimated to be under development in 2004, whereas the numbers of new molecular entities being developed to treat erectile dysfunction, bladder hyperactivity, and anxiety were four, eight, and nine, respectively [35]. None of the antibacterial agents currently under development seem to have novel mechanisms of action.

Clearly, concerted efforts need to be directed at improving the prospects for development of new antibacterial agents, including modification of existing drugs and discovery of novel bacterial target mechanisms [36]. Irrespective of any successes in this regard, however, it is imperative that currently available agents be used in such a manner that the inevitable development of resistance is retarded and their efficacy is prolonged as much as possible.

## STRATEGIES TO AVOID DEVELOPMENT OF ANTIMICROBIAL RESISTANCE

Strategies considered effective in delaying development of resistance involve minimizing the use of antimicrobial agents and using dosage regimens to achieve drug concentrations at the site of infection that eliminate pathogenic organisms without promoting survival of more resistant microbial subpopulations. In support of these strategies, a plethora of antimicrobial prudent use guidelines have been published by a variety of agencies, including the American Veterinary Medical Association [37], the American Animal Hospital Association [38], and the American Association of Feline Practitioners [39]. Generally, these guidelines adhere to the following principles:

1. Antibacterial agents should only be used when bacterial infection is confirmed or strongly suspected to exist. Obviously, patients that have viral, mycotic, neoplastic, or parasitic diseases do not respond to antibacterial therapy. Furthermore, increased body temperature does not necessarily indicate the presence of a bacterial infection but can also be caused by immune-mediated diseases, neoplasia, drug reactions, exercise, excitement, and increased environmental temperature. In particular, antibacterial agents should be avoided in the treatment of viral upper respiratory tract infections unless secondary bacterial infection is confirmed. Studies conducted in children have demonstrated that antibacterial treatment of these uncomplicated infections does not enhance resolution of the disease, even in the presence of purulent exudates from the nares or throat [40]. Similarly, antibacterial agents are seldom indicated in the treatment of feline lower urinary tract infections, which generally do not have a bacterial cause.

Use of antimicrobial agents should also be minimized by using sound patient care management practices, such as vaccination and isolation of infected animals. The effectiveness of strategies designed to prevent the spread of resistant microorganisms between infected individuals is best illustrated by the success of containing the spread of MRSA in human hospitals by diagnostic screening of patients, followed by isolation, use of disposable gloves and gowns by medical personnel, and washing of hands with antiseptic soaps [41,42].

2. When possible, in vitro sensitivity testing and pharmacokinetic data should be used to ensure attainment of an efficacious concentration of antibacterial drug at the site of infection. It is essential that an effective concentration of antimicrobial agent be attained at the site of infection for a duration sufficient to achieve elimination of the infection and minimize development of resistance. Therefore, pharmacodynamic as well as pharmacokinetic factors need to be considered in the selection of specific antibacterial agents and appropriate dosage regimens. The former involves use of in vitro sensitivity testing, especially for microbial isolates that have an unpredictable response to antibacterial therapy, such as Enterobacteriaceae, *Staphylococcus* spp, and *Pseudomonas* spp. The latter involves consideration of such factors as lipid solubility of the drug and whether there are diffusional barriers that impede distribution of the drug between the vascular system and the site of infection, such as the blood-brain barrier and infectious lesions that become sequestered by accumulation of exudates and fibrous tissue. Doses used should always be high enough to inhibit growth and survival of more resistant subpopulations of microorganisms, irrespective of whether the antibacterial agent is used prophylactically or to treat existing infection.

An approach developed by Drlica and his coworkers [43,44] to promote administration of doses that are high enough to minimize survival of relatively resistant strains of bacteria involves the use of MIC and mutant prevention concentration (MPC) values. The latter is defined as the lowest drug concentration at which growth of the least susceptible single-step mutant strain is inhibited (Fig. 3). Drug doses that achieve concentrations at the site of infection that are higher than the $MIC_{90}$ but lower than the MPC can be expected to promote emergence of resistant subpopulations of bacteria because of the competitive advantage resulting from the inhibitory effects of the antibacterial agent on more susceptible strains. Doses that result in concentrations that exceed the MPC limit the selection of resistant mutants, however. When the difference between the $MIC_{90}$ and MPC (termed the *mutant selection window*) is wide and no single drug can be identified that can be used safely to produce concentrations higher that the MPC for a particular pathogen, consideration should be given to combining antibacterial agents with different mechanisms of action. Estimates of MPC values for particular drug-pathogen combinations can be generated by culturing large numbers of bacteria on media containing a range of antibacterial drug concentrations. Considering that the frequency of single-step mutations conferring resistant phenotypes can be lower than $10^{-7}$, at least $10^{10}$ bacteria are needed to ensure the presence of resistant subpopulations in the test culture. Using this approach, mutant selection windows have been estimated for a variety of drug-pathogen combinations [45]. Although there clearly are several challenges in using these data to predict in vivo activity, such as differences in efficacy requirements for concentration-dependent (eg, aminoglycosides, fluoroquinolones) versus time-dependent (eg, β-lactams, macrolides) antibacterial agents, using mutant selection windows offers a quantitative approach to designing drug doses that are less likely to promote emergence of antibacterial resistance.

3. Avoid prolonged use of antibacterial agents. Although it is important that the use of antibacterial agents be minimized, treatment should not be discontinued before the infection is eliminated. Premature termination of therapy can result in the persistence of relatively resistant strains of microorganisms that

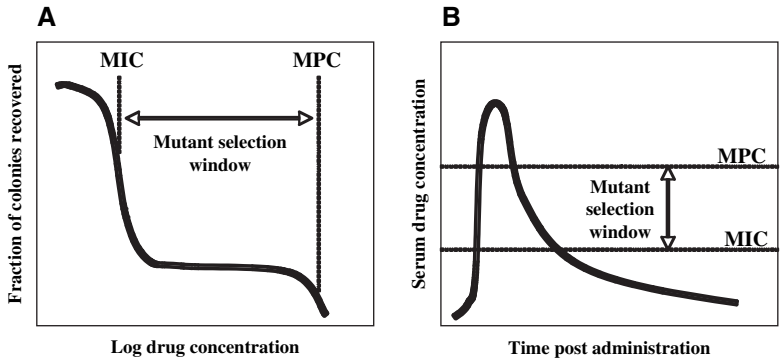


**Fig. 3.** Mutant selection window (MPC – MIC), expressed in terms of the fraction of microbial colonies that survive exposure to a particular drug concentration (*A*) and the time after drug administration (*B*). (*Adapted from* Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin Infect Dis 2001;33:S149, S151; with permission.)

require longer exposure periods for successful treatment. The resolution of clinical signs often is not a reliable indicator of elimination of the infection. A meta-analysis of 3-day versus longer antibacterial therapy of cystitis in women confirmed that there was no difference in symptomatic cure rates between the two durations of therapy but that the prolonged treatment was more successful in achieving bacteriologic cure and reducing recurrence of the infection [46]. Collection of clinical samples for culture and sensitivity testing at the conclusion of treatment may be used to determine whether therapy needs to be prolonged or if an alternative drug should be used.

Generally, antimicrobial therapy should be continued for 7 to 10 days or for 4 to 5 days after resolution of fever [38]. Patients that have compromised host defenses, such as those that are leukopenic, should be treated for 10 to 14 days. Chronic infections may require 4 to 6 weeks of treatment.

4. When possible, select narrow-spectrum agents, based on definitive identification of the infectious agent, rather than broad-spectrum drugs. Efficacious antimicrobial therapy is accomplished and emergence of resistance is minimized when the concentration of antibacterial agent achieved at the site of infection is high enough to eliminate susceptible and relatively resistant strains. This objective is most likely to be achieved when attention is focused on the specific relationships between drug dose, tissue concentration of drug, and susceptibility of the target pathogen. Broad-spectrum antimicrobials affect a wide variety of microorganisms (but with varying sensitivity) and may thus select relatively resistant strains of nontarget microorganisms. Even when these collateral effects involve nonpathogenic microorganisms, emergence of resistance is promoted because the propagation of these resistant bystanders increases the reservoir of resistance in the population.
5. Monitor development of resistance by means of well-designed surveillance schemes. Long-term preservation of the effectiveness of antimicrobial agents

relies on the implementation of strategies to monitor emergence of resistance associated with specific practices (eg, use of fluoroquinolones in food animals, treatment of staphylococcal infections using glycopeptides). Although such surveillance usually is performed by governmental regulatory agencies [47], such as the National Antimicrobial Resistance Monitoring System (NARMS) [48], veterinary clinical service facilities also have a responsibility for monitoring antimicrobial agent use and susceptibility profiles of prevailing pathogens. Unfortunately, based on the paucity of reports pertaining to studies in the scientific literature involving small companion animals, little attention is devoted to this responsibility including in veterinary teaching hospitals.

## SUMMARY

Development of antimicrobial resistance is an inevitable consequence of exposure of microorganisms to antimicrobial agents. It represents an evolutionary consequence of multiple genetic strategies designed to ensure survival in a hostile and changing environment. Although emergence of resistance cannot be prevented, it can be retarded by minimizing use of antimicrobial agents and avoiding selection of relatively resistant pathogenic and nonpathogenic strains caused by exposure to tissue concentrations that confer a competitive advantage. Most attention in veterinary medicine has focused on the emergence of resistance in food-borne pathogens, with relatively little attention being devoted to small companion animals, despite the frequent use of antimicrobial agents in these animals, evidence that resistance is emerging, and potential for transfer of resistance between companion animals and people. To retard further emergence of resistance in small companion animals, it is imperative that surveillance programs be instituted to monitor development of resistance.

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