

Minimizing Antimicrobial Resistance in Dogs and Cats: Do We Have a Problem?

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Introduction

“It is unwise to underestimate an adversary that has had a three billion year evolutionary head start” (Sayers, 2004). Attentiveness to emerging antimicrobial resistance is supported by the World Health Organization’s statistics indicating the sobering percent of resistance to first choice antimicrobials (generally fluoroquinolones, 3rd generation cephalosporins or carbapenems) currently present to over 50% of selected coliforms or gram positive organisms in partner hospitals. The American Veterinary Medical Association has promulgated through its member associations judicial antimicrobial use guidelines and the American Association of Veterinary Medical Colleges has partnered with the American Association of Public Land Grant Universities to promulgate Antimicrobial Drug Resistance learning outcomes targeting highschool, undergraduate and veterinary students. Most sobering: the Center for Disease Control indicates that the greatest risk factor for antimicrobial resistance is use.

Antimicrobial Resistance Patterns

Gram Negative: The advent of antimicrobial resistance is increasingly limiting therapeutic options in human and resistance to an antimicrobial varies with the species and strain. Among the most adaptable organisms is *E. coli*. Discovered in 1885 by the ediatrician Theodore Escherich, it was originally discovered in neonatal fecal samples. Dr. Erich recognized *E. coli* was acquired at birth and remained with us till death, with strains coming and going. It is the most thoroughly understood microbe, and is critically important as a research tool. Through *E. coli*, we have come to understand such diverse activities as intermediary metabolism, DNA replication and RNA transcription, protein synthesis and genetic recombination. Indeed, recombinant products would not be possible without *E. coli*. *Escherichia coli*, a member of the family Enterobacteriaceae, is a lactose fermenter, causing a distinct color on diagnostic agar. It is the predominant facultative anaerobe (in the normal intestine of both humans and many warm-blooded animals), playing a major role as normal microflora.¹⁻² However, it also is ubiquitous in the environment, as is recognized by its appearance as contaminants in food stuffs. It has or acquires genes that encode for flagella, making it mobile. Its presence in the environment is used as a sentinel of environmental contamination. Referred to as the “cockroach” of microbes because of its adaptability, *E. coli* rapidly divides, potentially doubling its population every 20 minutes. Further, it is highly mutagenic, with spontaneous mutations occurring in of 1 per 100 thousand to 1 per billion new progeny (assume 1 gm of feces contains 100 million *E coli*) thus assuring opportunity for spontaneous mutation even in the absence of stimuli, such as drugs. Resistance. The gastrointestinal environment is conducive to development of resistance. Environmental microbes maintain an ecological niche by suppressing competition through secretion of antibiotics. As such, commensal organisms are constantly being exposed to antibiotics. However, the microbe producing the antibiotic, as well as surrounding normal flora, are resistant to the antibiotic. Thus, genes for resistance develop along with genes directing antibiotic production and organisms are “primed” to develop resistance. Microflora of the GI tract can serve as reservoir of resistance genes. Exposure to antimicrobials may facilitate survival of isolates that have either spontaneously mutated or acquired resistant through other means. Resistance may be easily conferred to other potentially more virulent organisms.

E. coli rapidly develops resistance, particularly that associated with multiple drug resistance (MDR) when exposed to selected antimicrobials. *E. coli* develops resistance both vertically and horizontally. Shared resistance reflects the ability of bacteria to incorporate extrachromosomal DNA carrying the information for

resistance from other (including non-self) organisms. Extrachromosomal DNA (including plasmids and bacteriophages) encode for resistance to multiple drugs and can be transmitted vertically (to progeny) or horizontally, across species and genera. In general, resistance carried by plasmids “comes and goes”, meaning the presence of the drug may increase the likelihood of the plasmid being present, in large copy numbers; removal of the drug may be associated with resolution of the resistance. More disconcerting, resistance is easily conferred to more pathogenic organisms. In human medicine, *E. coli* has developed resistance to the fluorinated quinolones, beta-lactams, or both: it is among the Gram negative organisms that secrete extended

Table 1. The percent of *E. coli* feline and canine uropathogens from throughout the US (2009-2012) resistant to antimicrobial drugs.

Drugs	Overall (n=1512)	95% CI	Species		Gender	
			Canine	Feline	Female	Male
AMX C	41.5	39.1-44.0	45.2	30.0	37.1	55.78
AMP	49.1	46.6-51.6	52.8	37.6	44.8	61.7
CPF	9.3	7.7-11.0	10.5	5.7	8.5	10.6
CFT	8.5	7.1-9.9	9.6	4.9	7.7	10.1
CFO	12.8	11.1-14.5	14.2	8.5	13.3	12.5
CFP	13.0	11.3-14.7	13.7	10.6	13.1	13.0
CFZ	8.9	7.5-10.5	10.4	4.4	8.3	10.6
CPL	98.9	98.2-99.3	99.0	98.6	99.0	99.3
CHP	12.6	11.0-14.3	13.7	9.3	12.2	13.8
DXY	100					
ENR	10.5	8.8-12.3	11.69	6.7	9.9	11.5
GEN	7.9	6.6-9.3	8.56	6.0	7.8	8.1
MRP	1.3	0.7-2.0	1.05	1.9	1.6	1.0
TCL	19.4	17.5-21.4	20.79	15.3	20.5	17.7
TMS	8.9	7.4-10.3	9.69	6.3	8.6	8.4

spectrum beta-lactamases (ESBL). Emergence of extended spectrum extended spectrum beta-lactamases (ESBL) is an example of the relentless adaptive nature of microbes toward designer drugs intended to preclude the advent of resistance. The ESBLs are encoded by large plasmids that can confer the information between strains as well as different species of organisms. The gene mutation confers resistance to newer cephalosporins including cefotaxime, ceftazidime and ceftriaxone, as well as cefpodoxime, or 4th generation including cefepime (no longer marketed in the USA); cefepime has been cited as possibly being effective against ESBL. The impact on clavulanic acid and sulbactam is not clear, although their use in place of

cephalosporins appears to reduce the emergence of ESBL and may reduce the emergence of other resistant pathogens such as *Clostridium difficile* and vancomycin-resistant enterococci. The ESBL are most commonly found in *Klebsiella* spp, *E. coli* or *Proteus mirabilis* (3.1-9.5%), but they also have been detected in other members of the Enterobacteriaceae and in *Pseudomonas aeruginosa*. Resistance to fluoroquinolones has also been well characterized. Normally associated with point mutations in topoisomerases (DNA Gyrase and Topoisomerase IV), such resistance is, like beta-lactamases, within class. However, in the presence of continued drug, efflux pumps appear to be induced. Such pumps serve to remove toxic compounds from the organism, including antimicrobials. At least 5 efflux pump systems have been characterized; they are associated with porins. They are characterized by broad substrate specificity, thus can impart multidrug resistance. The culture (above) and the antibiogram below typify the patterns of resistance that can emerge in animals which have received fluoroquinolones and in which resistance has emerged. *E. coli* resistance in isolates associated with urinary tract infections is particularly well described. One study demonstrated the gastrointestinal emergence of quinolone-resistant *E. coli* genetically distinct from infection-causing strains in patients treated with ciprofloxacin. Like virulence factors, transfer of resistance genes in isolates between animals in humans may present a public health risk, as was recognized by the FDA by 1999. We have demonstrated the impact of *E. coli* MDR through several of our studies. In healthy normal dogs, we have demonstrated the impact of 10 mg/kg amoxicillin bid and 5 mg/kg enrofloxacin once daily orally for 7 days on fecal *E. coli*. For either drug, 100% of *E. coli* became resistant to the drug, expressing high level (more than 8 times the MIC breakpoint). For amoxicillin, this resistance was limited to beta lactams and occasionally tetracyclines or sulfonamides;

resistance tended to resolve by the 3 week study end period. Enrofloxacin resistance however, not only was multidrug resistance, but tended to persist. The relationship between enrofloxacin resistance and multidrug resistance was also demonstrated in a pilot surveillance study of approximately 400 *E. coli* pathogens collected from dogs or cats. The pattern of resistance varied regionally, being as much as 50% to amoxicillin or amoxicillin clavulanic acid and in the south, approximately 30% to enrofloxacin. Although the number of isolates resistant to beta lactams only (expressing single drug resistance) was high, single drug resistance to enrofloxacin was rare. If resistance was expressed to enrofloxacin, it was multidrug in nature. We have an ongoing study involving 3000 isolates throughout the United States sponsored by the Morris Animal Foundation and Idexx laboratories. Currently, regional differences in resistance continue to persist. Overall resistance is greatest to cephalexin (9%) as is demonstrated in Table 1; 100% of isolates are also resistant to doxycycline using the new interpretive criteria established by CLSI. Further, 40% are resistant to amoxi-clavulanic acid and 50% to ampicillin (amoxicillin). This latter statistic suggests that for treatment of *E. coli*, Clavamox® may not have that much advantage over amoxicillin alone and this would also be true if treating *Enterococcus*. However, if treating other organisms, protection against betalactamases may be helpful.

Gram Positive Organisms: Methicillin resistance (MRSA; *S. aureus*; MRSP; *S. intermedius* [pseudintermedius]) Multidrug resistance is now considered the normal response to antibiotics for Gram positive cocci pneumococci, enterococci and staphylococci. Methicillin resistance (MRSA; *S. aureus*; MRSP; *S. intermedius* [pseudintermedius]) is indicated by the presence of the *mecA* gene, which encodes a mutation in penicillin binding protein (PBP) resulting in formation of PBP2a rather than PBP2. As such, affinity is reduced for the beta-lactam ring, rendering the organism resistant to all beta-lactams. Protectors such as clavulanic acid are ineffective. Detection of MRSA or MRSP on C&S generally is based on resistance to oxacillin, which is more stable than methicillin in disks used for testing. However, increasingly, laboratories are indicating MRS based on absence of susceptibility to any beta-lactam. In our hospital, approximately 25 to 30% of *Staphylococcus pseudintermedius* express methicillin resistance. Antibiotics and especially cephalosporins are associated with induction, selection, and propagation of MRSA. MRSA in human patients has evolved from a hospital-acquired (HA-MRSA; nosocomial), in which occurs most commonly in patients immunocompromised by disease, drugs, procedures and duration of hospitalization, to a community acquired infection (CA-MRSA), in which otherwise healthy persons are infected, usually in the skin or soft tissue. Crowded conditions, shared items and poor hygiene increase the risk of community acquired infection. Although it is community acquired MRSA strain USA300 that appears to be most commonly associated with increased colonization in dogs and cats, it is USA100, most commonly associated with hospital acquired-MRSA infections in humans, that most commonly is associated with infections in dogs and cats animals. The impact of MRSA in veterinary medicine is increasingly problematic, not only because of its impact on the patient, but the public health considerations. The *mec* gene has been detected in methicillin-resistant *Staphylococcus aureus* organisms infecting dogs and MRSA has been associated with infection in dogs. However, MRSA also has been found in up to 4% of healthy dogs, with identification complicated by the need for multiple sampling sites (nasal and rectal or perineal). Infections have been isolated in family members and pets in the same household, but this is likely to reflect transmission from humans to the pet. It is likely that colonization is transient in animals. However, healthy pets have been demonstrated to be potential reservoirs for transmission of MRSA to healthy handlers and a potential health risk to immune-compromised patients (human and presumably other animals in the household). Human colonization with MRSP is unusual. However, MRSP has been reported as a cause of infection in human patients and transmission from pets with pyoderma has been confirmed. It is the very immunocompromised patient that is at risk for MRS infection acquired from an animal. In such cases, the carrier or infected animal should be removed from the environment until successfully treated for methicillin-resistant *Staphylococcus*.

Glycopeptides such as vancomycin are the initial drugs used to treat MRSA in humans, although increasingly vancomycin resistant staph infections (VRSA) have emerged.

Reducing Resistance: The Three “D”s.

Regardless of the organism, the most significant mechanism by which bacterial resistance is likely to be reduced is implementation of behaviors that are designed to reduce patient risk such as length of hospital stay, and design, implementation of and adherence to infection control practices. Consider the three DE-s:

(1) **DESCALATE.** Because previous antimicrobial therapy is one of the most important factors associated with resistance, approaches which minimize indiscriminant antimicrobial use will be important. Examples of human strategies include improving appropriate antimicrobial use (eg, including less ideal strategies such as strict adherence to prescribed formularies, setting limits on the duration of antimicrobial therapy); potentially reasonable strategies such as requiring prior approval for use of certain antibiotics such that proper use can be verified; and more rationale strategies such as narrowing the spectrum of empiric antibiotics, and rotating the use of antimicrobial drugs on a regular schedule); primary prevention by decreasing length of hospital stay, decreased use of invasive devices, and newer approaches such as selective digestive decontamination and vaccine development. Probably the single most important first step in judicious antimicrobial use and avoiding resistance is questioning/confirming the need for therapy. This is no small task, being fraught with the lack of effective diagnostic aids. Probably the most common– and least correct mindset is failure to recognize that we are in conflict with our directive of “above all else do no harm” if we use antimicrobials in the absence of infection. De-escalation includes taking actions that stay the hand in reaching for drugs if they are not really necessary. For urinary tract infections, increasingly the need for treating asymptomatic bacteria is questioned. What constitutes an infection may not depend only on the inoculum size (e.g, 1000 CFU/ml) but the presence of clinical signs.

(2) **DESIGN.** Dosing regimens should be designed to assure that adequate drug concentrations are reached at the site of infection to kill, not simply inhibit, microbial growth. **DEAD BUGS DON’T MUTATE!** Once the decision to use the antimicrobial is made, efforts should focus selecting a drug to which the bug is most susceptible. A practice-based antibiogram (see above) may be helpful. If an animal has not been exposed to antimicrobials, the chances are improved that any infecting pathogens are among the susceptible isolates. A narrow spectrum is preferred to a broad spectrum drug. Once the drug is chosen, design focuses on the dosing regimen to assure that concentrations adequate to kill the infecting microbe are achieved at the site of infection. This involves not only selecting the drug to which the isolate is most susceptible (and the drug most likely to reach the target site), but also designing the dosing regimen based on the MIC, potentially the MPC and time or concentration dependency of the drug (see parts II and III if relevant). An antibiogram (see figure; top number is % susceptible, bottom number of each cell is number tested) generated for each practice can support empirical selection of antimicrobial drugs although increasingly, culture is indicated in all but the antimicrobial naïve patient (this includes direct - or indirect through a household member - exposure). (Note that squares without information are drugs which should not be tested toward that bug).

(3) **DECONTAMINATE:** Hospital strategies include: improving infection control (eg, selective decontamination procedures, prevention of horizontal transmission via handwashing, use of disinfectants, glove and gown use, alternatives to soap, and improving the workload and facilities for health care workers), and identification of specific areas for treatment of potentially infectious agents (ie, bandaging areas that can be easily cleaned). Increasingly “detergent” should be applied to the patient and its home. For example, recurrent infections might be reduced if successful initial therapy is coupled with cleansing of the environment in which the pet is located such that it is not continued to be exposed to the infecting bug. For UTI infections, this may become particularly important in that urine contaminates the environment.