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Interpreting culture and susceptibility data in critical care: perks and pitfalls

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Abstract

Problem – The need for immediate, effective antimicrobial therapy in the critical care patient must be tempered by approaches which simultaneously minimize emergence of antimicrobial resistance. Ideally, therapy will successfully resolve clinical signs of infection, while eradicating infecting pathogens such that the risk of resistance is avoided. Increasing limitations associated with empirical antimicrobial choices direct the need for culture and susceptibility data as a basis of therapy. Even so, such in vitro data should be utilized within its limitations.

Objectives – To demonstrate the attributes and limitations of patient and population culture and susceptibility (pharmacodynamic) data in the selection of antimicrobial drugs and to demonstrate the design of individualized dosing regimens based on integration of pharmacodynamic (PD) and pharmacokinetic (PK) data.

Diagnosis – Limitations in culture and susceptibility testing begin with sample collection and continue through drug selection and dose design. Among the challenges in interpretation is discrimination between pathogens and commensals. Properly collected samples are critical for generation of data relevant to the patient's infection. Data are presented as minimum inhibitory concentrations (MICs). The MIC facilitate selection of the most appropriate drug, particularly when considered in the context of antimicrobial concentrations achieved in the patient at a chosen dose. Integration of MIC data with key PK data yields the C_{max}:MIC important to efficacy of concentration-dependent drugs and $T > \text{MIC}$, which guides use of time-dependent drugs. These indices are then used to design dosing regimens that are more likely to kill all infecting pathogens. In the absence of patient MIC data, population data (eg, MIC_{90}) may serve as a reasonable surrogate.

Conclusions – Properly collected, performed, and interpreted culture and susceptibility data are increasingly important in the selection of and design of dosing regimens for antimicrobial drugs. Integration of PK and PD data as modified by host and microbial factors supports a *hit hard, exit fast* approach to therapy that will facilitate efficacy while minimizing resistance.

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Introduction

Antimicrobials are among the most common and important drugs prescribed for the critical care patient (CCP).[1] Appropriate therapy is vital in the CCP; for human patients with sepsis, the risk of therapeutic failure increases up to 11% for every hour that appropriate antimicrobial therapy is delayed.[2,3] Yet, the implementation of therapy is a conundrum in that exposure to antimicrobials is a known risk factor for emerging antimicrobial resistance.[4,5] It is the sense of urgency associated with CCP care that generally swings the decision toward empirical antimicrobial use. This manuscript will address the role of culture and susceptibility (not *sensitivity*) testing (C&S) in the use of antimicrobial therapy. The discussion will include a focus on its attributes as well as limitations, and include the application of the population pharmacodynamic (PD) to the patient. Finally, the integration of PD and pharmacokinetic (PK) information in support of the dosing regimen will be addressed.

The goal of antimicrobial therapy is resolution of infection and specifically its clinical impact. However, the avoidance of emerging resistance is a second, often

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overlooked goal. Accepting that the 2 goals are not mutually exclusive is paramount to judicious and appropriate antimicrobial use in any patient. The risk of resistance is reduced if eradication of the infection – that is, killing of the infecting microbes – is achieved.

Several definitions are in order. An organism is represented by genus and species (eg, *Escherichia coli*, *Staphylococcus pseudointermedius*, and *Enterococcus feacalis*). The resident population, or inoculum, of the organism is comprised of thousands or hundreds of thousands of CFU or isolates, of which only 1 is isolated for culture reports. Whether or not the inoculum represents an infection rather than colonization reflects, in part, its pathogenicity, but also its size. Regardless of the size of the population, each CFU or isolate is characterized by its own minimum inhibitory concentration (MIC) for any drug. If each CFU associated with the infection were cultured, an MIC distribution could be generated. If the population distribution is normal, as might be expected in an antimicrobial naive patient (Figure 1), it is likely then that CFU isolated for MIC determination represents the most common MIC (mode), which should approximate the median MIC or MIC 50 (the 50th percentile). As such, a large percent of the inoculum infecting a patient is characterized by MIC that are lower and higher than that reported. The size of the infecting inoculum increases the risk of resistance. Any population that proliferates to an inoculum size of approximately $\geq 10^7$ increasingly is characterized by spontaneous genetic mutations such that at least one isolate in the inoculum will express resistance to any drug to which the population might be exposed. If the patient is treated with that drug, those isolates that are most susceptible to the drug will be inhibited. Reduced competition facilitates survival and growth of the remaining, more resistant isolates. Eventually, unless checked by the host immune response, or by appropriate drug therapy, a second, resistant population may emerge to cause infection (or reinfection) (Figure 1). Reculture will yield a new population whose representative MIC is likely to be higher than that of the first population. This newer population – comprised of first-step mutants – may not yet be resistant to the drug, but the increase in MIC is a prelude to resistance. As the

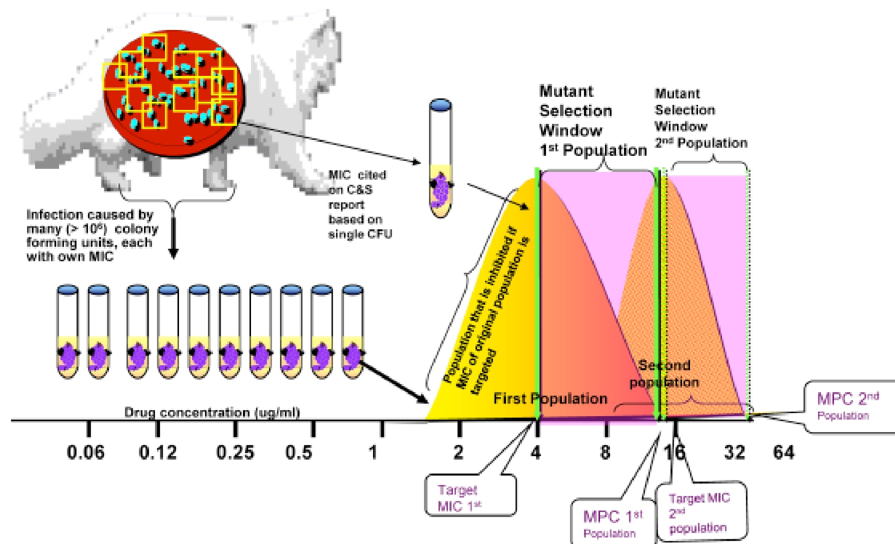

Figure 1: The inoculum infecting a patient is comprised of a population of isolates, each with its own minimum inhibitory concentration (MIC). The isolate most likely cultured probably will be among those with the most common MIC in the population. If the inoculum is large enough, spontaneous mutations will result in at least one isolate expressing resistance to any drug. If the patient is exposed to the drug, those isolates whose MIC is at or below the MIC will be inhibited. The mutant selection window can be used to describe the potential role of antimicrobial exposure in emergent resistance. Although most relevant to drugs for which resistance develops by point mutations, the concept is probably relevant to any population characterized by isolates with high MIC compared with the rest of the population. The window is comprised of the MIC reported on the culture report and the highest MICs of the infecting population (which generally is not known but might be approximated by the MIC 90 of the organism for the drug). The highest MIC is also referred to as the mutant prevention concentration (MPC) of the infecting inoculum. Should drug concentrations at the site of infection fall in this window, those isolates that have already mutated or have acquired some level of resistance are likely to repopulate more easily in the absence of competition, particularly in an at-risk patient. This new population may expand until a sufficient number of CFU occur, such that a second spontaneous mutation occurs, resulting in higher MIC. The initial goal of antimicrobial therapy ideally would target the MPC. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

mutant selection process repeats itself with the next generation of mutants, the risk of drug resistance increases. This step-wise development of resistance has been demonstrated with fluoroquinolones, for which resistance most commonly reflects a point mutation in one of 2 topoisomerase genes. Resistance is most likely to emerge if drug concentrations at the site of infection fall in a mutant selection window.[6] The lower threshold of the window is the MIC reported by susceptibility testing for the infecting isolate (Figure 1). The higher threshold is the 100th MIC percentile, that is, the highest MIC present for any isolate in the inoculum, also known as the mutant prevention concentration (MPC). If the drug concentration at the site of infection falls in the window, isolates with lower MIC will be inhibited, but those with higher MIC will survive to reinfect. The goal of therapy in this scenario would be to target the isolate with the MPC (Figure 1). Unfortunately, current C&S methods cannot predict the MPC.[7] Although this scenario most appropriately is applied to drugs for which resistance reflects point mutations in the gene, the concept is likely relevant for any population of isolates and any drug.

Healthy, immunocompetent patients may successfully suppress an emergent population, explaining why, perhaps, lower doses are often successful at resolving infection despite emergent resistance. However, it is the patients at risk, including CCP, for which reinfection with a resistant population is likely. For example, multidrug-resistant *E. coli* has emerged as a cause of nosocomial infections in dogs[8] and urinary tract infections (UTI) in canine CCP.[9,10] Bacterial translocation, invasive procedures, introduction of foreign surfaces conducive to bacterial colonization and biofilm formation (eg, catheters), immunocompromise, duration of hospitalization (increasing the likelihood of nosocomial infection), and changes in drug disposition that increase the risk of therapeutic failure or adverse drug reactions are among the factors that contribute to the risk.[1,11] As such, initial antimicrobial therapy should be directed to eradicate the entire infecting population such that mutants cannot survive to grow to a resistant population (ie, dead bugs do not mutate).

C&S testing is among the tools that might be used to slow the advent of antimicrobial resistance. Data generated from a properly collected culture can help determine the need for antimicrobial therapy. This includes both revealing the presence of bacteria at the sample site and identifying the infecting organism as to genus and species. Culture may help identify the isolate (and thus infecting organism) as a pathogen. Further, susceptibility data identify drugs to which the infecting organism is susceptible, and potentially the drug to which the representative isolate is *most* susceptible. Finally, because PD data (MIC) represent a therapeutic target (what is needed), C&S can support the design of a dosing regimen specifically targeting the infecting population in the individual patient.

Because a wrong decision can be lethal in the CCP, collection of a properly sampled culture (eg, blood cultures) is recommended before implementation of empirical antimicrobial therapy. Initial empirical choices for therapy in the CCP can be wrong, as has been demonstrated.[12,13] Culture is particularly important to confirm a nosocomial infection or in patients recently exposed to antimicrobial therapy. Pathogens isolated from an antimicrobial naïve patients are more likely to be characterized by susceptibility whereas resistance is more likely in patients exposed to antimicrobials.[14] The duration of time that can elapse between antimicrobial and return to a normal susceptibility pattern has yet to be determined. The author suggests that a 3-month antimicrobial-free period should lapse before the patient is considered to be antimicrobial naïve, a target that is not scientifically validated. An important consideration during decision making is the relevance of C&S data collected before therapy has begun. The results may not be returned for several days after therapy has begun. If the data indicate that the empirical choice of antimicrobials was wrong, the data may no longer be relevant to the initial infecting population in the face of the antimicrobial therapy. Even a single dose of antimicrobial can be associated with emergent resistance, as has been demonstrated for ciprofloxacin.[15] For this reason, and because the CCP is predisposed to develop resistance, empirical therapy should cover *any potential* pathogen in the CCP, including nosocomial organisms, thus lessening the likelihood that a wrong choice was made.[2,16,17] The risk of emerging environmental resistance associated with empirical therapy using the more potent (ie, ''big gun'') antimicrobials (eg, carbapenems)[18] might be reduced with a *hit hard, exit fast* approach: therapy can be de-escalated to a less powerful antimicrobial regimen as soon as possible, such as after resolution of clinical signs, targeting <5 days. This might occur, for example, after receipt of the culture data that have been collected before initiation of empirical therapy.[2,19] For the patient in which the initial choice was deemed incorrect upon receipt of susceptibility results, shifting to an alternative drug may not be necessary if the patient has responded to initial empirical therapy. For the nonresponsive patient, because of the impact that initial antimicrobial therapy may have had on the resident infecting populations, reassessment through culture is prudent. The addition of a second or third antimicrobial (and de-escalating as rapidly as possible) may be a reasonable approach.

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As helpful as C&S might be, basing antimicrobial therapy on the data does not guarantee therapeutic success, just as not doing so does not guarantee failure. As such, the advantages of C&S must be balanced by the pitfalls, several of which already have been discussed. The pitfalls begin with culture sampling, continue through identification and susceptibility testing, and conclude with interpretation and dose design. As with any tool, proper use depends on a recognition and respect of these limitations. Figure 1 delineates the approach to design of a dosing regimen for a patient based on either patient of population PD data.

Limits of Culture and Susceptibility Testing

Identifying the target organism

Sample collection: Culture data are only as good as the quality of the sample and the importance of proper culture techniques cannot be overemphasized (Table 1). The clinical veterinary microbiologist is a powerful ally in determining the significance of isolates yielded from a culture. However, optimal input depends, in part, upon the thoroughness of information regarding the patient provided with sample submission, including how the sample was collected. For the CCP patient, blood, urine, respiratory secretions (collected by bronchoscopy), and other pertinent body fluids (eg, pleural, peritoneal, or CSF) should be carefully sampled before antimicrobial therapy is begun. Swabs should be avoided when possible for a variety of reasons, the most compelling of which may be that only 3% of isolates collected with a swab will be successfully grown.[20] Whenever possible, fluids or tissues should be submitted. Results from samples collected from sites that contain commensal organisms or from environments that are easily contaminated must be interpreted in the context of the background contaminating noise that must be filtered out. Cleansing before sample collection is indicated, particularly for contaminated sites. For the same reason, cystocentesis is the most acceptable sample for interpretation of bacteriuria; catheterized samples often contain microbes colonizing the catheter and associated biofilm. Particular attention must be made to sampling for obligate anaerobes, for which even brief exposure to oxygen can be lethal.

Pathogen versus nonpathogen: Even the most properly collected culture may not confirm infection or identify the microbe as a pathogen. Cytology coupled with Gram staining should be considered when possible, with phagocytosis of the organism indicative of pathogenicity. Pathogenicity reflects virulence, which is often misconstrued as resistance. However, virulence

Table 1: Techniques in culture sampling.* (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

1. Commonalities regardless of site
   a. Site preparation: Don sterile gloves. Clean wound. Do not culture purulent or necrotic debris. Thoroughly cleanse wound by removing excessive debris, flushing with saline, blotting with sterile gauze. Change to sterile gloves prior to collection
   b. Tissue aspiration: Clean intact skin with antiseptic (eg, 70% alcohol and 10% povidone-iodine). Allow to air dry (do not fan). Expel air from an appropriately sized syringe to which is attached a 22-Ga needle. Insert needle into intact skin at the deepest portion of the lesion. Aspirate approximately 0.5-mL fluid. Needle can be moved back and forth at different angles in skin. Remove needle with hemostat. Discard needle and recap syringe with blood-gas cap or, particularly if anaerobes are suspected, transfer fluid to transport vial
   c. Swab techniques are acceptable only for eyes, ears, and uterine cultures. Note that only 3/100 CFU collected in a swab are likely to be successfully cultured. Use a swab in appropriate carrier media. The swab should be moistened with sterile preservative-free solution if wound is not moist. Sample should be collected without touching the edge of the wound or skin. Rotate swab over 1-cm area of open wound for 4 seconds. Place swab aseptically in transport sleeve making sure tip contacts liquid transport media (break ampule if present). One swab should be collected for each sample type (ie, aerobic, anaerobic, and a third if cytology is of interest)
2. Skin or wound biopsy: Swab strongly discouraged; aspirate acceptable (submit recapped needle if volume too small for syringe) macerated tissue preferred. Skin scraping of adequately cleaned skin may be preferred to swab for superficial skin infections. Clean intact skin with antiseptic and allow to air dry (do not fan). Collect biopsy aseptically. Place in transport tube containing liquid media. Clinical Microbiology Laboratory will macerate
3. Bone: Place in transport system. Moisten with sterile physiologic saline as necessary
4. Drain tube site: Treat as a contaminated wound. Care must be taken to not culture the biofilm associated with the foreign body. The tube should be removed, the site surgically cleaned and flushed, and the area cultured. Ideally, tissue will be collected at the presumed site of infection
5. Respiratory tract: Bronchoscopy specimens include bronchoalveolar lavage, bronchial washing, bronchial brushing, and transbronchial biopsy specimens. The bronchscope should be passed transorally in nonintubated patients or via the endotracheal tube in intubated patients. Bronchial wash or bronchoalveolar lavage specimens should be obtained before brushing or biopsy to minimize blood in the recovered fluid
   (i) For lavage, sterile nonbacteriostatic 0.85% NaCl should be injected from a syringe through a biopsy channel of the bronchoscope. Recovered fluid should be placed in the transport vial
   (ii) Bronchial brush specimens should be collected through a telescoping double catheter plugged with polyethylene glycol at the distal end (to prevent contamination of the bronchial brush) through the biopsy channel of the bronchoscope. The sample should be transported in a sterile container with a small amount of nonbacteriostatic sterile 0.85% NaCl
   (iii) Lung aspirations should be placed in transport vial for laboratory submission
   (iv) Pharyngeal samples are not acceptable
   (v) Nasal samples are of limited value. Culture requests should be limited to pathogen specific; *Bordetella bronchiseptica*

Table 1: Continued

6. Urine: Samples should be collected by cystocentesis only. Catheterized samples generally not preferred. Sample can be submitted in a red-top serum collection tube. Samples should be kept cold, submitted on ice such that samples are received by the laboratory within 24 hours of submission
7. Blood culture: Liquid media is indicated. Volume is critical to maximize recovery. Bacteremia may consist of less than 1 CFU/mL of whole blood. Sterile prep the collection site, and for blood cultures, at least 3 collections at 3 different time points are indicated. Collection during a fever spike is recommended. For blood the volume should be 1 part blood to 10 parts broth
8. CSF or joint fluids: Use blood culture bottle and add entire sample aseptically in to broth
9. Other body fluids: see tissue aspirate
10. Ocular: 1 or 2 drops of topical anesthetic are generally instilled. Organisms are more readily detected in scrapings than from a swab
 (i) For conjunctival scrapings, scrape the lower tarsal conjunctiva with a sterilized spatula and place material directly into medial. Alternatively, use a calcium alginate swab or a cotton-tipped applicator to swab the inferior tarsal conjunctiva (inside surface of eyelid) and the fornix of the eye
 (ii) Consider collecting a conjunctival sample first such that it might help assess the possibility of contaminations. Using short, firm strokes in 1 direction, scrape multiple areas of ulceration and suppuration with a sterilized spatula. The eyelid should remain open and care should be taken to avoid eyelashes. Multiple scrapings are recommended because the depth and extent of viable organisms may vary. Inoculate each scraping directly to appropriate media
 (iii) Intraocular fluid should be collected via needle aspiration. Aspirate should be used to directly inoculate appropriate media with immediate transport to the laboratory in an anaerobic transport system
11. Gastrointestinal tract: Fecal specimens are submitted primarily for the detection of *Campylobacter*, *Shigella*, and *Salmonella* species, *Clostridium difficile* and in certain cases to detect *Yersinia*, *Vibrio*, and *Aeromonas* species and enterohemorrhagic *Escherichia coli*. Care should be taken to make sure sample is not contaminated with urine. Fecal WBC should be ordered on liquid stools to indicate degree of inflammation. Stool specimens should be mixed with transport medium to maintain viability of pathogens that may be present. Sample should be collected digitally wearing a sterile glove or using a sterile fecal loop

*The laboratory to which the sample will be submitted should be consulted before collection such that their recommendations can be followed. These general guidelines are offered in the absence of specific guidance.

indicates the pathogen's ability to infect and cause disease.[21,22] Virulence is dependent upon virulence factors such as adherence molecules and cytotoxins that, respectively, facilitate microbial infection and induction of disease. Rapid detection of virulence factors using molecular diagnostic tools is particularly helpful in the identification of target pathogens.[23] In contrast, resistance describes the ability of the isolate to avoid antimicrobial-induced harm. In general, emerging resistance is believed to be associated with *decreased* rather than increased virulence, although not all experts support this assumption. Community-acquired infections may be associated with increased virulence, but less resistance. However, community-acquired methicillin-resistant *Staphylococcus aureus* increasingly is associated with increasing virulence, increasing the risk of spreading infections.

The inability of C&S to discriminate colonization from infection is particularly problematic for tissues characterized by normal flora. Most normal flora are commensals that neither harm nor help the host. However, some commensals are opportunistic, able to cause disease without the support of virulence factors. A population shift from colonization to infection is more likely to occur in at-risk patients, such as the CCP. Infection generally reflects, for example, normal flora, such as *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *S. pseudointermedius*. However, opportunistic organisms also may be acquired from the environment.

Growth characteristics of the culture may provide some guidance in the differentiation of a pathogen from a commensal organism. For example, *Streptococcus spp.* all species abbreviations in this form should have a period after, as indicated here, please review and correct throughout. pathogenicity can be associated with its ability to hemolyze hemoglobin, with α designation (hemoglobin is simply reduced) being the least and β (RBCs disrupted) potentially the most hemolytic and pathogenic. γ-Hemolysis is actually the absence of hemolysis, and is demonstrated by *Enterococcus spp.* (previously a subset of *Streptococcus spp.*). However, again, exceptions occur. For example, *Enterococcus* has expressed β-hemolysis and α-hemolytic *Streptococcus* can be pathogenic under the right circumstances. An example might be the CCP that has undergone invasive procedures such as intubation. The laboratory may choose to not implement susceptibility testing for those isolates considered nonpathogenic, with the interpretation of pathogenicity by the microbiologist depending on the host circumstances, including sampling site. The number of isolates or the extent of growth might indicate pathogenicity, although this also must be interpreted in the context of patient factors. If the site is one that is easily contaminated, cultures yielding only light growth or growth that requires incubation in enriched nutrient broth might be indicative of colonization rather than infection. In contrast, for tissues that are normally sterile, such as blood cultures and CSF, any bacterial growth (not considered to be a contaminant by a microbiologist) may be considered indicative of infection; specialized procedures may be necessary to identify growth in these tissues. Thus, as few as 2 colonies of *Pseudomonas sp.* cultured from a properly collected bronchial alveolar lavage might be considered significant, whereas growth of $<10^5$ CFU from a site that is

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easily contaminated (eg, wounds, clean catch, or catheterized urine) might mitigate the need for antimicrobial therapy.[24] For cystocentesis, up to 10^3 CFU/mL may not be significant in normal dogs but may be indicative of infection in a patient that is not concentrating urine (eg, due to renal disease, diuretic, or fluid therapy, etc.).[10]

The number of different organisms isolates might be indicative of pathogenicity. Vibrant growth of a single organism generally is indicative of infection by a pathogen, even in an environment that is easily contaminated. In contrast, isolation of multiple organisms (ie, >3) may be indicative of normal flora colonization. In the event that multiple pathogens are isolated from a well collected sample, those isolates characterized by lighter growth might be de-emphasized in favor of organisms with significant growth. Controlling the heavier growth may facilitate the patient's capacity to eradicate the lighter growth isolates. For example, *E. coli*, *Staphylococcus intermedius* group (SIG) or α-hemolytic *Streptococcus* are rapid growers and if present together, the organisms with the greater growth might be targeted. However, *P. aeurginosa* is an example of a slow grower that is easily overwhelmed by other organisms (hence the need to refrigerate). The presence of such organisms in a properly collected sample generally indicates the need for treatment. In contrast, other pseudomonads may reflect contamination, depending on the patient circumstances.

Among the contributions of the veterinary microbiologist is an awareness of organisms that are more likely to be contaminants rather than pathogens. For example, *Bacillus spp.*, *Corynebacterium spp.*, and nonhemolytic *Streptococcus spp.* are common wound contaminants and may be indicative of a poorly representative sample. Some organisms are considered contaminants at some sites, but pathogenic at others. For example, β-hemolytic *Streptococcus spp.* are likely to be significant if collected from a wound but not the ear. If C&S data indicate contamination, the site should be resampled (tissue collection rather than swab) after proper cleansing. Tissues generally can be submitted without maceration, allowing the microbiologist to properly prepare the sample.

Although the susceptibility patterns of an isolate may offer clues as to pathogenicity, care must also be taken with this approach. Contaminants are often characterized by patterns of susceptibility rather than resistance. However, such an isolate may yet be a pathogen, particularly in a patient with no previous history of antimicrobial exposure. Complex patterns of resistance may suggest the isolates is an infecting pathogen rather than a colonizing commensal. This is exemplified by nosocomial organisms associated with medical treatments (arising within 48 h of hospital admission). However, *Stenotrophormona* or *Serratia* are common contaminants of antiseptics or disinfectants that are characterized by complex patterns of resistance. Multidrug resistance must also be considered in the context of the inherent susceptibility of the organism, being relevant only if expressed toward drugs to which the organism should be susceptible. For example, *P. aeruginosa* may be tested toward drugs to which it is inherently resistant, yielding results that look MDR. However, MDR should not be considered unless expressed toward ticarcillin, carbapenems, or aminoglycosides.

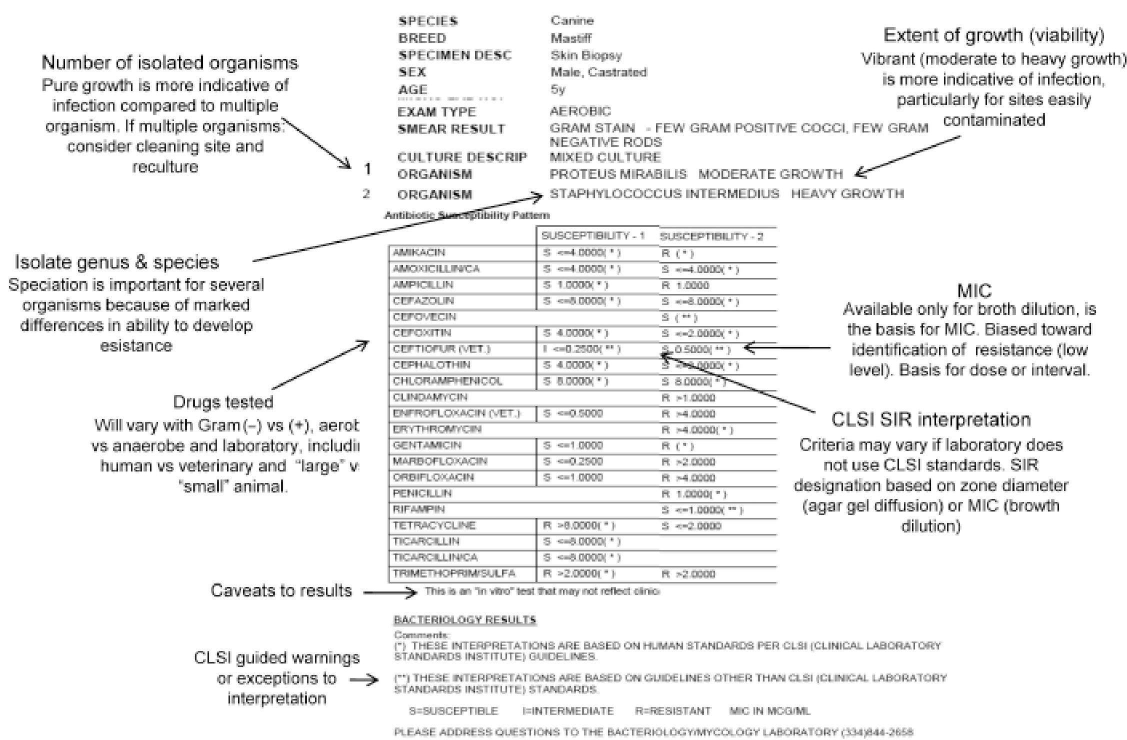
Thus far, the discussion has focused on identification of a cultured isolate as a pathogen. However, another potential limitation of C&S testing is failure to speciate the isolate. Some isolates are identified to the level of genus but not species, with the extent varying among laboratories. This may be more common in laboratories that also support human medicine. Speciating genera such as *Enterococcus* and *Staphylococcus* are especially important. Whereas *Enterococcus faecalis* tends to be susceptible to a variety of drugs, *Enterococcus faecium* is often characterized by multidrug resistance. *Staphylococcus* may be identified only as coagulase negative (*Staphylococcus schleiferi* subsp. *schleiferi*) or positive (*S. aureus*, *S. intermedius* group, and *S. schleiferi* subsp. *coagulans*). However, staphylococcal virulence factors occur in both categories, with the types of infection varying. For example, the 'slime' produced by coagulase negative organisms facilitates infection associated with foreign bodies.[25] As such, even a coagulase negative *Staphylococcus sp.* may be significant if cultured from an otherwise sterile site. Speciating *Streptococcus* is particularly important in identifying the source of infection.

Susceptibility testing

Standards setting: The PD data generated by susceptibility testing serve as the target concentration that ideally will be achieved at the site of infection and is a basis for the design of the dosing regimen. The sophisticated nature of in vitro susceptibility testing offers many opportunities for mistakes and misinterpretations. Aspects subject to variability, in the absence of standards, include pH, media volume, cation content and osmolality, inoculum size, temperature, humidity, and duration of incubation. Variability in each will potentially alter the results. The method by which growth, or absence thereof, is detected (eg, computer-enhanced images or spectrophotometry versus human observation) also contributes to variability.[26] Quality assurance protocols are necessary to assure proper implementation of C&S procedures. Variability also should cause clinicians to cautiously interpret preliminary data, quick *snap* tests, or other methods intended to generate rapid results.

Without standardization, susceptibility data cannot be interpreted. The Clinical and Laboratory Standards Institute (CLSI; formally the National Committee for Laboratory Standards) provides protocols, guidelines, and interpretive standards necessary to validate C&S testing. Guidelines are based on a review of PD data collected on target organisms and PK data generated in the target species. Once established, in order to be accepted, the guidelines must be demonstrated to have clinical relevancy. A subcommittee of CLSI promulgates interpretive criteria specific for animal-approved drugs, and when appropriate, will make recommendations regarding the applicability of human drug guidelines. New PD and PK data are incorporated into CLSI considerations as they become available. Because CLSI criteria are a continuum of a work in progress, criteria are intermittently adjusted in the face of changes that might accompany, for example, emerging resistance. Standards provided by CLSI represent consensus decision making among committee members comprised of practitioners and members of government, industry, and academia. As such, CLSI standards tend to be unbiased and peer reviewed, a desirable basis for guidelines in clinical practice. It is important to note that veterinary microbiology laboratories are not compelled to follow CLSI standards, and the prudent clinician might assure that susceptibility data are submitted to laboratories that do so. However, despite its important role in quality assurance, CLSI itself faces limitations.

Limitations despite standards: For veterinary medicine, efforts of CLSI are limited by the lack of properly generated PD data for many potential pathogens in dogs and cats. This reflects, in part, the lack of a robust antimicrobial surveillance system for these species. Other limitations are presented by technology and constraints of commercial testing systems. For example, in the interest of space and cost, susceptibility of several drug classes may be represented by model drugs, which ideally behave similarly to those the model represents. Exceptions to extrapolation are indicated by CLSI in its interpretive guidelines and these exceptions should be noted by the testing laboratory on the report (Figure 2). For example, in vitro, ampicillin represents amoxicillin, amoxicillin-clavulanic acid represents ampicillin-sulbactam and sulfamethoxazole/trimethoprim represents other potentiated sulfonamides. In contrast, cephalothin, a first-generation cephalosporin (which is no longer used therapeutically in the United States) is more stable in vitro and thus represents cephalexin and other first-generation cephalosporins;



SPECIES Canine
BREED Mastiff
SPECIMEN DESC Skin Biopsy
SEX Male, Castrated
AGE 5y
EXAM TYPE AEROBIC
SMEAR RESULT GRAM STAIN - FEW GRAM POSITIVE COCCI, FEW GRAM NEGATIVE RODS
CULTURE DESCRIP MIXED CULTURE
1 ORGANISM PROTEUS MIRABILIS MODERATE GROWTH
2 ORGANISM STAPHYLOCOCCUS INTERMEDIUS HEAVY GROWTH

Antibiotic Susceptibility Pattern

	SUSCEPTIBILITY - 1	SUSCEPTIBILITY - 2
AMIKACIN	S <=4.0000(*)	R (*)
AMOXICILLIN/CA	S <=4.0000(*)	S <=4.0000(*)
AMPICILLIN	S 1.0000(*)	R 1.0000
CEFAZOLIN	S <=8.0000(*)	S <=8.0000(*)
CEFOVECIN		S (**)
CEFOXITIN	S 4.0000(*)	S <=2.0000(*)
CEFTIOFUR (VET.)	I <=0.2500(**)	S 0.5000(**)
CEPHALOTHIN	S 4.0000(*)	S [illegible](*)
CHLORAMPHENICOL	S 8.0000(*)	S 8.0000(*)
CLINDAMYCIN		R >1.0000
ENFROFLOXACIN (VET.)	S <=0.5000	R >4.0000
ERYTHROMYCIN		R >4.0000(*)
GENTAMICIN	S <=1.0000	R (*)
MARBOFLOXACIN	S <=0.2500	R >2.0000
ORBIFLOXACIN	S <=1.0000	R >4.0000
PENICILLIN		R 1.0000(*)
RIFAMPIN		S <=1.0000(**)
TETRACYCLINE	R >8.0000(*)	S <=2.0000
TICARCILLIN	S <=8.0000(*)	
TICARCILLIN/CA	S <=8.0000(*)	
TRIMETHOPRIM/SULFA	R >2.0000(*)	R >2.0000

This is an "in vitro" test that may not reflect clinic

BACTERIOLOGY RESULTS

Comments:
(*) THESE INTERPRETATIONS ARE BASED ON HUMAN STANDARDS PER CLSI (CLINICAL LABORATORY STANDARDS INSTITUTE) GUIDELINES.

(**) THESE INTERPRETATIONS ARE BASED ON GUIDELINES OTHER THAN CLSI (CLINICAL LABORATORY STANDARDS INSTITUTE) STANDARDS.

S=SUSCEPTIBLE I=INTERMEDIATE R=RESISTANT MIC IN MCG/ML

PLEASE ADDRESS QUESTIONS TO THE BACTERIOLOGY/MYCOLOGY LABORATORY (334)844-2658

Figure 2: An example C&S report from a dog with a skin infection. A gram-negative and -positive isolate were cultured from the submitted sample. The key components to a report are identified. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

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for similar reasons, oxacillin represents methicillin. However, cefazolin, another first-generation drug, generally is not well represented by cephalothin because it is less effective toward *S. aureus* and more effective against *E. coli*.[27] The spectrum of third- and fourth-generation cephalosporins markedly differs, limiting extrapolations of a model drug. As such, multiple third-generation cephalosporins might be present on a susceptibility report. Extrapolations among aminoglycosides are also limited. Gentamicin is generally more effective toward *S. aureus* whereas amikacin generally is more effective toward *Pseudomonas spp*.

Limitations in extrapolations are not limited to spectrum, but also may reflect a mismatch between in vitro and in vivo response. This may include differences in PK that cannot be adequately represented by the in vivo testing system. For example, despite in vitro evidence of susceptibility, aminoglycosides should not be used to treat *Enterococcus spp*. or as sole agent to treat *Staphylococcus spp*. despite an 'S' designation on a susceptibility report. Potentiated sulfonamides are not considered by CLSI to be clinically effective toward enterococci despite in vitro susceptibility. However, recent reports in the literature challenge this assessment, underscoring the importance of continued surveillance of the data by CLSI. Generally, laboratories will not test drugs against organisms for which clinical efficacy has not been demonstrated. This is most obviously exemplified by gram-negative versus gram-positive susceptibility panels, with the drugs tested against the isolate grouped according to anticipated efficacy for the type of organism (eg, gram-negative isolates will not be tested toward clindamycin or erythromycin, gram-positive isolates generally are not be tested toward ticarcillin, which was developed for gram-negative infections, and anaerobes will not be tested toward aminoglycosides, etc.).

A more recently recognized limitation of susceptibility testing is detection of acquired resistance that is rapidly induced by the presence of the drug. This might be best exemplified by gram-negative organisms that produce extended spectrum β-lactamases (ESBL). These enzymes destroy selected third- and fourth-generation cephalosporins but are induced at the site of infection by the presence of the drug.[28] As such, ESBL generally are not expressed by the isolate culture in vitro. Rather, their detection requires additional testing of the isolate in the presence of cefpodoxime or ceftazidime alone and again for each of these drugs in the presence of clavulanic acid. Because the latter is not susceptible to ESBL, a 4-fold or more reduction in the respective cephalosporin MIC associated with clavulanic acid indicates the presence of ESBL. Not all laboratories have incorporated special testing for ESBL; for those who do not, caution is recommended when interpreting isolate susceptibility to later generation cephalosporins. Newer ESBL are constantly emerging as resistance evolves. For example an ESBL produced by *K. pneumoniae*, which targets carbapenems, was recently identified, thus highlighting the need for rapid incorporation appropriate testing procedures into microbiology testing labs.[29]

Methods of in vitro testing

Guidelines have been promulgated by CLSI for a variety of microbial testing methods.[30–33] The PD information varies with the testing procedures, with the disk diffusion and broth dilution methods representing the most common methods upon which clinical C&S is based (Figure 3).[25,26] The newer E-test represents a compromise between the two (Figure 4). The broth dilution procedure yields MIC and generally is considered the gold standard method to which all others are compared.[34,35] Each method, but particularly the broth dilution, requires rapid growth of organisms, thus potentially limiting the application to aerobes that are not fastidious in nature.

For all procedures, the isolate must be grown to a standard inoculum (usually based on a turbidity test). Once achieved, a known amount of the inoculum is used to inoculate the testing media. The commonly used agar gel diffusion procedure involves placement of disks containing the drug of interest on the inoculated plate. Drug diffuses from the disk into surrounding media at a known rate (Figure 3), and with incubation at standard conditions, a zone of no growth (inhibition) emerges whose size reflects susceptibility of the isolate to the drug. The size of the inhibitory zone around each disk roughly correlates to the MIC, but the limitation in zone size precludes establishing a predictable relationship between zone and MIC, even with mathematical modeling. As such, agar gel diffusion data are semiquantitative data, being limited to susceptible ('S'), resistant ('R'), or intermediate ('I') interpretations. An advantage of the disk diffusion method is that poorly or slowly growing organisms that are not testable with tube dilution (eg, many streptococci and enterococci or anaerobes) may be more amenable to disk diffusion. Further, multiple drugs can be simultaneously tested on one plate.

In contrast, for broth dilution procedures, several tubes (macrodilution) – or more commonly, wells (microdilution; Figure 4) – are dedicated to the drug of interest. Each well contains the drug in concentrations that increase logarithmically (Figure 3). The low and high range of concentrations tested for each drug vary, based on the PK of the drug in the target species, that is, the concentration likely to be achieved in plasma at the recommended dose. Again, following standardized

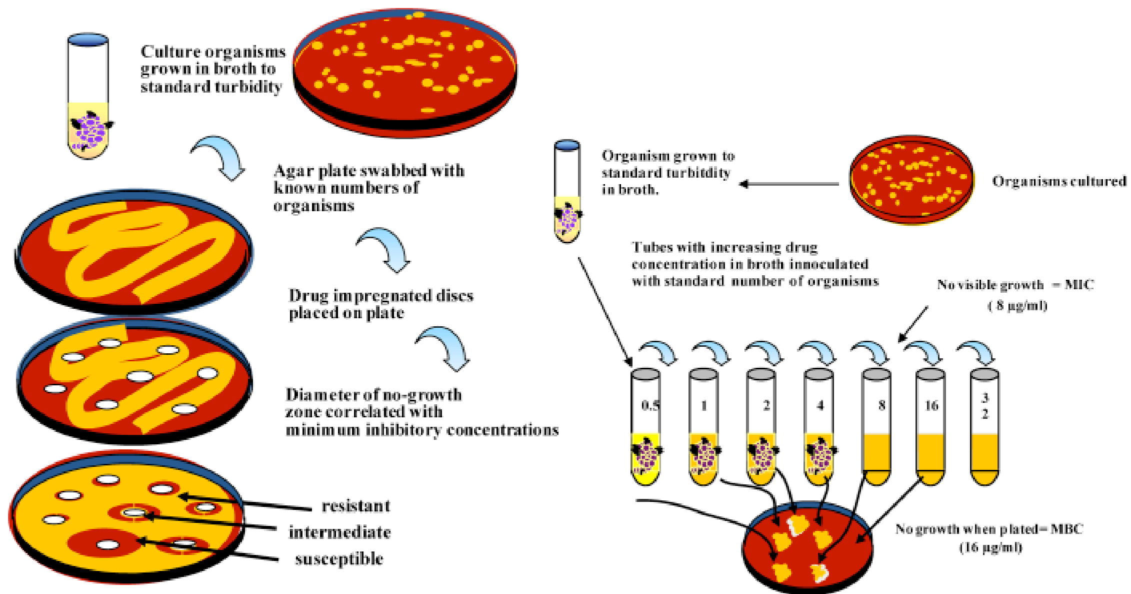

Figure 3: The most common methods of culture and susceptibility testing are disk diffusion (eg, Kirby Bauer) and broth dilution. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

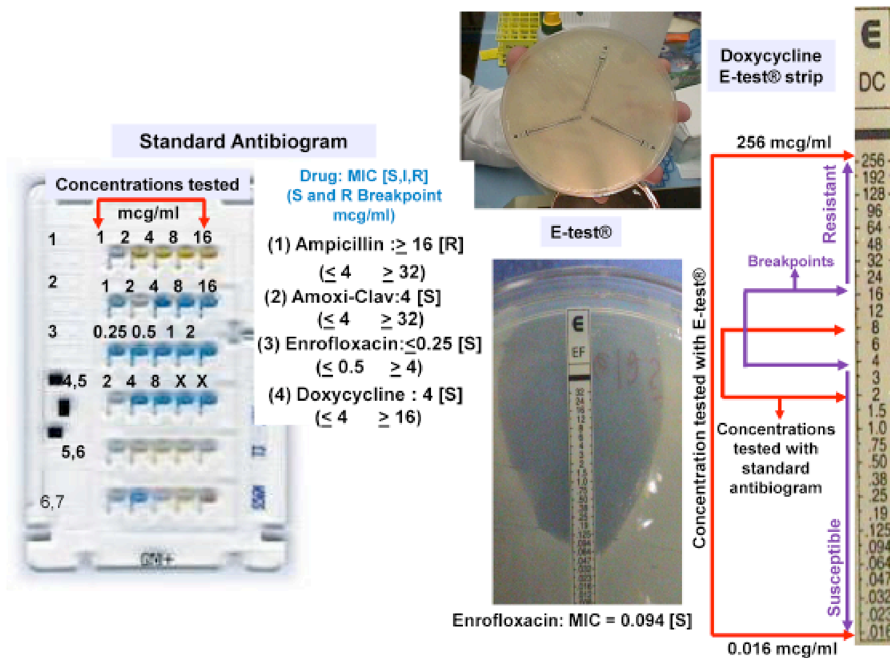

Figure 4: An example of a commercial antibiogram card based on (micro) broth dilutions (left) and an E-test (right). Both generate minimum inhibitory concentrations (MICs). For the card, the MIC for each drug has been indicated to the right, with the number in parentheses indicating the susceptible and resistant breakpoints for that drug as determined by Clinical and Laboratory Standards Institute (CLSI). The range of concentrations for the E-test is much wider than the card, including concentrations well above and below the breakpoints set by CLSI (indicated by inside arrows), as is demonstrated for enrofloxacin, whose MIC for this isolate is $\leq$ 0.25 mcg/mL based on the card, but is actually 0.094 mcg/mL based on the E-test. As such, the E-test is able to identify very susceptible isolates (MIC well below the breakpoint) and low-level resistant isolates (MIC just above the breakpoint). (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

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procedures, each well is inoculated with the cultured isolate. After standard incubation procedures, the test tube (or well) that contains the lowest concentration of drug and no detectable bacterial growth contains the minimum amount of antimicrobial necessary to inhibit in vitro isolate growth, that is, the MIC. As such, broth dilution procedures are quantitative, with the MIC reported in μg/mL (mg/L), the same units for which PK parameters (eg, maximum plasma drug concentration [PDC], or C_{max}) should be reported. The susceptibility report will include the MIC as well as either a 'S,' 'I,' or 'R' designation (Figure 2). However, for broth dilution procedures, the designation reflects the proximity of the isolate MIC to a susceptible and resistant threshold – or breakpoint MIC (MIC_{BP}) determined by CLSI for the drug (Table 2). An isolate inhibited at a concentration at or below the susceptible MIC_{BP} is designated 'S' whereas an isolate which is inhibited only at a concentration equal to or above the resistant MIC_{BP} will be designated 'R.' The susceptible MIC_{BP} is at least 1 dilution below the resistant MIC_{BP} for each drug. For some drugs, the susceptible MIC_{BP} is 2 or more dilutions below the resistant MIC_{BP}, allowing for an inter-

Table 2: Interpretive standards for minimum inhibitory concentration (MIC) breakpoints for selected antimicrobials. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

Drug	Breakpoint (μg/Ml)* Susceptible	Breakpoint MIC (μg/mL)* Resistant
Amikacin	≤ 16	≥ 64
*Amoxicillin with clavulanic acid	≤ 4/2†	≥ 8/4
	≤ 8/2‡	≥ 32/16
*Ampicillin§	≤ 0.25†	≥ 0.5
	≤ 8‡	≥ 32
	≤ 0.25¶	≥ 8
	≤ 8‖	≥ 16
Azithromycin	≤ 4	≥ 8
Carbenicillin	≤ 16	≥ 64
Cefazolin	≤ 8	≥ 32
Cefotaxime	≤ 8	≥ 64
Cefoxitin	≤ 8	≥ 32
Cefpodoxime	≤ 2	≥ 8
Ceftazidime	≤ 8	≥ 32
*Ceftiofur**	≤ 2	≥ 8
Ceftizoxime	≤ 8	≥ 32
Ceftriaxone	≤ 8	≥ 64
Cephalothin††	≤ 8	≥ 32
Chloramphenicol	≤ 8	≥ 32
	≤ 8¶	≥ 16
Ciprofloxacin‡‡ (see also enrofloxacin)	(≤ 1	≥ 4)
Clarithromycin	≤ 1	≥ 8
	≤ 8	≥ 32
*Clindamycin§§	≤ 0.5	≥ 4
*Difloxacin	≤ 0.5	≥ 4
Doxycyline	≤ 4	≥ 16
*Enrofloxacin	≤ 0.5	≥ 4
*Florfenicol**	≤ 2	≥ 8
Gentamicin*	≤ 4	≥ 16
Imipenem/cilastin	≤ 4	≥ 16
Kanamycin	≤ 16	≥ 64
Levofloxacin	(≤ 2	≥ 8)¶
Linezolid	≤ 4†	
	≤ 4‖	≥ 8
Marbofloxacin	≤ 1	≥ 4
Meropenem	≤ 4	≥ 16
Metronidazole	≤ 8	≥ 32
*Orbifloxacin	≤ 1	≥ 8
Penicillin G	≤ 8‡	≥ 16
	≤ 0.12†	≥ 0.25
Piperacillin	≤ 16†	≥ 128
	≤ 64¶¶	≥ 128
Rifampin	≤ 1	≥ 4
Tetracycline‖‖	≤ 4	≥ 16
	≤ 2¶	≥ 8
Ticarcillin	≤ 64¶¶	≥ 128
	≤ 16§	≥ 128
Ticarcillin with clavulanic acid	64/2¶¶	≥ 128/2
	16/2‡	≥ 128/2
Trimethoprim/sulfamethoxazole	≤ 2/38†††	≥ 4/76
Amethoxazole***	≤ 0.5/9.5¶	≥ 4/76
Vancomycin	≤ 4‖	
	≤ 1¶	≥ 32
	≤ 4	≥ 32

*Clinical and Laboratory Standards Institute. Interpretive standards that are based on animal pathogens are designated by an *asterisk.
†When testing *Staphyloccocus* organisms.
‡When testing gram-negative enteric organisms.
§Ampicillin is used to test amoxicillin.
¶When testing *Streptococcus* (*S. pneumoniae* for levofloxacin).
‖When testing enterococci.
**When testing pathogens associated with food animal respiratory disease.
††Cephalothin is used to test all first generation cephalsoprins. Does not represent cefazolin, which should be tested separately if a gram-negative organisms.
‡‡A human criteria, not adjusted to reduced oral bioavailability (mean of 40%) in dogs and negligible (0–20%) in cats.
§§Clindamycin is used to test lincomycin, which is less susceptible to *Staphylococcus*.
¶¶When testing *Pseudomonas*.
‖‖Used to test chlortetracycline, oxytetracycline, minocyclines, doxycycline.
***Trimethoprim-sulfamethoxazole is used to test trimethoprim-sulfadiazine and ormetoprim-sulfadimethoxine.
†††For soft tissue infections.
‡‡‡Oxacillin is used to treat methicillin, cloxacillin.
§§§For urinary tract infections.

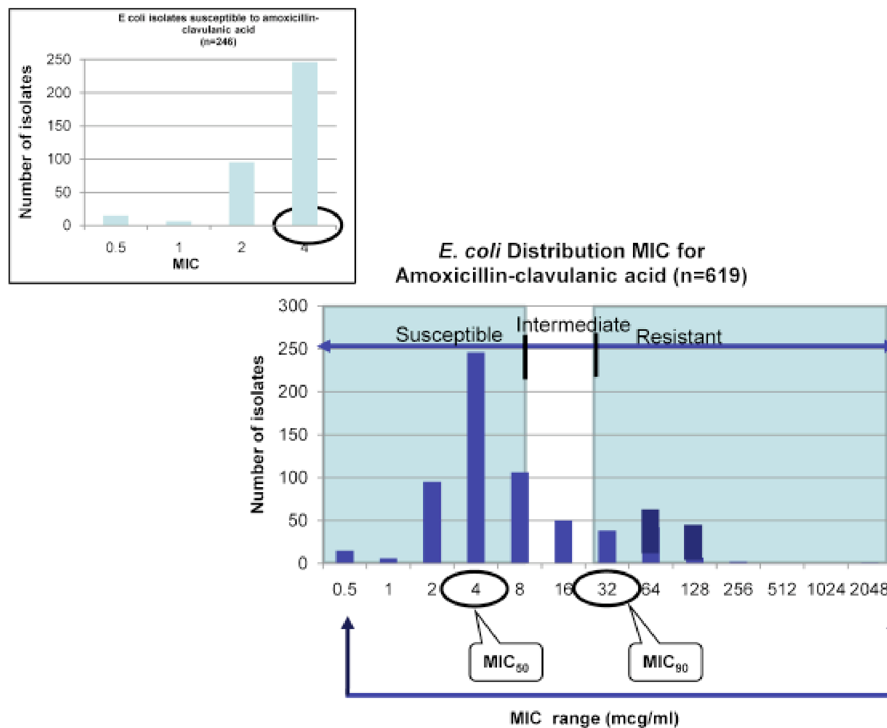

Figure 5: An example of a population minimum inhibitory concentration (MIC) distribution plot based on *Escherichia coli* cultured from dogs or cats ($n = 619$ isolates). The data represented here are summarized by the pharmacodynamic statistics in Table 4. The shaded areas represent the susceptible and resistant breakpoint MICs, respectively, based on CLSI interpretive standards. The distribution of all the isolates data are bimodal, characterized by a second population of resistant isolates. The MIC 50 or 90 (the latter is preferred) can serve as a basis for design of a dosing regimen. The upper left inset shows the same distribution data but includes only those isolates considered susceptible. If an infecting *E. coli* is known to be susceptible (eg, based on agar gel diffusion), then the MIC 50 or 90 of this population (4 μg/mL for both MIC statistics) would be an appropriate target for design of a dosing regimen. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

mediate or 'I' designation for those isolates whose MIC is inhibited at a concentration between the susceptible and resistant breakpoints.

Interpretive criteria established by CLSI for susceptibility testing are applicable nationwide to laboratories using CLSI protocols for testing. They are determined after careful and exhaustive review of PD (microbial response to drug) and PK (host handling of drug) data regarding each specific drug and microbe. Three criteria must be met for the MIC_{BP} established for each drug. The primary and initial consideration is the population MIC distribution of a large number (eg, 100) of isolates, including range (lowest and highest), mode (most common MIC), 50th (median) and 90th percentile, and character of distribution (single distribution versus bimodal distribution; Figure 5).[26,36] Obvious patterns of low versus high MIC can be used to identify the breakpoints. Secondly, the clinical pharmacology of the drug is considered, ideally in the target species. Among the more important PK parameters evaluated by CLSI are the peak and trough PDCs (C_{max} and C_{min}), area under the curve (AUC) for a 24-hour dosing period and the half-life as it relates to drug concentrations above the MIC ($T > MIC$).[1,11,37,38] The PK will be based on the recommended (labeled) dose. For the animal-approved fluoroquinolones, which were approved with flexible labels that allow a range of doses, susceptible breakpoints may be based on the lowest and resistant breakpoints on the highest approved dose. Newer drugs are no longer being approved with multiple dose options. The actual PK parameter considered by CLSI varies with each drug, depending on whether or not the drug acts in a time versus concentration-dependent manner. The third criteria for MIC_{BP} established by CLSI is that the threshold must be clinically relevant, that is, the microorganisms defined as susceptible should respond clinically to the drug, and in vitro data must correlate adequately with in vivo findings.[36,39]

Generally, CLSI determines one set of MIC_{BP} for each drug, in part because the PKs of the drug in the target species will not vary among infecting organisms (notable exceptions are penicillins susceptible to

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β-lactamase destruction). However, some organisms are much more susceptible to selected drugs than others, and in selected instances, CLSI will publish more than one set of interpretive criteria for a drug (Table 2). This is best exemplified for *Pseudomonas spp*., which are very susceptible to ticarcillin, compared with other enteric organisms. *Staphylococcus spp*. offers a similar example: its production of β-lactamases is more likely to destroy penicillins and a significant portion of the drug that reaches the site of infection is destroyed, resulting in a lower MIC_{BP} compared with non-*Staphylococcus* pathogens. Because they are less impacted by penicillinases, cephalosporins do not have 2 sets of MIC for *Staphylococcus spp*. (Table 2).

Among the limitations of susceptibility testing is the range of concentrations tested. Standard card antibiograms generally used by microbiology laboratories facilitate rapid testing procedures, but are limited in the number of wells available for testing. The range of drug concentrations tested is thus narrow, generally varying by 2–4 tube dilutions, sometimes including only the susceptible or intermediate breakpoints, but other times extending one or 2 tube dilutions below the susceptible breakpoint (Figures 2 and 3). For isolates that are susceptible at concentrations below the lowest tested, the MIC will be reported as $<X$, where X is the lowest concentration tested or $\leq X$, where X is one dilution lower than the lowest concentration tested. Both numbers are reporting the same thing. For example, for *Proteus spp*. in Figure 2, no growth occurred in the well containing 8 μg/mL of amoxicilliln-clavulanic acid, which also might have been reported as $\leq$ 4 μg/mL. At the other end of the range, the highest concentration tested will always be one dilution below the resistant breakpoint. Growth in this well is generally reported as $>X$, where X is the highest concentration tested, or $\geq X$, where X is the next higher tube dilution, which is also the resistant breakpoint. For example, as is demonstrated for *Proteus spp*. in Figure 2, resistance to tetracycline would be reported as >8 or ≥ 16 μg/mL. Because the drug concentrations tested surround the breakpoint MICs, C&S testing is biased toward identification of resistant, rather than susceptible isolates. As such, a major limitation of broth dilution procedures for the clinician interested in avoiding resistance is an inability to assess how susceptible an isolate is to the drug of interest. Further, testing at ranges below the resistant breakpoint precludes identification of resistant isolates whose resistance is low level, meaning, it is at or just above the resistant breakpoint. Use of drugs in combination might reasonably target isolates with low-level resistance.

In addition to the limited ranges tested with broth dilution, the step-wise (logarithmic) nature of well dilutions can be difficult to apply clinically, particularly at higher concentrations. The actual MIC for any isolate is between the reported concentration and the next lowest dilution. Fine tuning of the MIC would allow better comparisons among isolate susceptibility (including repetitive sampling in the same patient) as well as facilitate the accuracy of dosing regimen. For example, targeting a dosing regimen for an MIC between 0.5 and 1 μg/mL is easier and cheaper than designing a dosing regimen if the MIC is between 128 and 256 μg/mL.

A third testing system that has been approved by the FDA for human testing circumvents to some degree the limitations presented by the logarithmic increase of drug concentrations of broth dilution. The E-test system, available only in a limited number of veterinary clinical microbiology laboratories. The rate of release of the drug into the surrounding agar results in Figure 4. Although more costly, selection of specific drugs allows an extended panel to be designed for the patient and its infecting organism. The drug of interest is incorporated in gradients into a strip; the rate of release of drug into the surrounding agar results in a pear-shaped zone of no growth, with the bottom of the pear indicating the MIC. In general, MIC generated by the E-test correlate well with MIC generated from broth dilution procedures and CLSI MIC criteria are used for interpretation of the E-test.[34,35] The E-test is preferred by the author for CCP for which resistance is a concern for 3 reasons. The range of concentrations tested ranges at least 1,200-fold, extending well below the CLSI susceptible breakpoints. For example, concentrations tested for enrofloxacin range from 0.06 to 32 μg/mL and for amoxicillin-clavulanic acid, from 0.25 to 256 μg/mL. As such, *how susceptible* an isolate is to a drug can be determined, allowing the clinician to choose a drug to which an isolate might be exquisitely susceptible. Secondly, because the concentrations exceed the resistant breakpoint, isolates with low-level resistance (within 2–3 dilutions of the MIC_{BP}) can be identified. For such drugs, increasing the dose (if sufficiently safe) may reasonably increase efficacy despite an 'R' designation, particularly if the drug is used in combination with another, complementary drug. Third, the increments of increase in drug concentrations tested of the E-test are more narrow than used in broth dilution procedures, allowing identification of discreet increases in MIC with subsequent cultures, and improving on the accuracy of dosing regimens.

Other Limitations of C&S

In addition to drugs approved for use in animals, CLSI may publish in its veterinary publications interpretive standards for drugs approved for use in humans.[30–33]

Those human drugs for which feline or canine criteria have not been determined reflects, in part, the lack of quality PK and PD data. Although the microbes might be similar (as research is increasingly demonstrating), extrapolation of PK data among the species is more questionable, particularly for drugs that are orally administered and lipid soluble (volume of distribution generally >0.6 L/kg). For these drugs, differences in disposition among species are more likely compared with those that are administered parenterally and are water soluble (volume of distribution generally <0.3 L/kg) drugs.[1,11] Care is particularly indicated for orally administered drugs, for which oral bioavailability can markedly differ. For example, the oral bioavailability of ciprofloxacin is approximately 40% in dogs,[40] compared with 80–100% in humans, and $<20\%$ in cats.[41] Accordingly, human breakpoints for ciprofloxacin should be set lower in the dog and may not apply to the cat if the drug is not administered IV. These differences may not be taken into account with CLSI and are not likely to appear in the interpretation of a culture report for ciprofloxacin and dogs or cats.

A major limitation of C&S testing is the disparity between the controlled environment of the in vitro test system and the dynamic in vivo environment of the host. It is important to remember that MIC are inhibitory, not killing concentrations of the drug. For bactericidal drugs, the MIC is similar to MBC, the minimum bactericidal concentration, which is the lowest concentration of drug that destroys the pathogen.[37] Further, the MIC is determined from a standard inoculum size; the rate of bacterial inhibition or killing may not be similar for larger or smaller inoculums. The in vitro test system cannot take into account the innumerable host-drug-microbial interactions that characterize the chemotherapeutic triangle. These interactions include those that are obvious – such as dynamic changes in drug concentrations in the patient versus static drug concentrations in the test tube, binding of drug to plasma proteins (eg, doxycycline, cefovecin), or formation of active metabolites (eg, enrofloxacin, clindamycin). However, less obvious factors are also critically important and are exemplified by the site of infection. Breakpoints determined by CLSI generally are based on anticipated PDCs. Whereas many drugs easily pass through capillary fenestrae such that concentration of free drug in interstitial fluid equals or exceeds[42] that in plasma, for other drugs, doses must be adjusted for the possibility of differing tissue concentrations.[1,2,11,43] Increasingly, tissue concentrations will be considered in the integration of PK and PD and these will differ from PDCs.[44,45] This is clearly true for sanctuary tissues characterized by specialized blood barriers (brain, prostate, eye, testicles, etc.). Note that, although treatment of UTI might be facilitated by treatment with renally excreted drugs, fluid therapy, diuretics, and renal disease will preclude concentration of the drug in the urine. Data from limited prospective studies generally demonstrate that PDCs may be a better predictor of therapeutic outcome for treatment of UTI.[36] Likewise, C&S cannot take into account the intracellular location of some organisms. Other host factors that can result in disparity between prediction from in vitro testing and actual in vivo response include host immune status, the degree of inflammatory response, microbial virulence factors, and the presence of biofilm. Of these, perhaps the most underappreciated in the CCP may be biofilm.[46,47] Bacteria exist in either a platonic (free floating) or sessile (attached) state. Whereas it is the former state that occurs with C&S testing, it is the latter state that enables persistence of the resident population, as well as the formation of biofilm. The impact of biofilm and its associated quorum sensing on antimicrobial efficacy can be profound. Additionally, many organisms are facultative anaerobes. Whereas staphyoloccoci and enterococci prefer low oxygen tension environments, *E. coli* prefer an oxygen-rich environment such as that associated with in vitro testing. Yet, growth in an anerobic environment in the host may decrease antimicrobial efficacy due to suppressed host immunity, decreased blood flow, and inherent deficiency in antimicrobial action (ie, aminoglycosides). Some factors not predicted by C&S actually facilitate efficacy beyond that predicted by in vitro testing. For example, enrofloxacin is converted to active metabolites (eg, ciprofloxacin) and some drugs are accumulated (eg, fluoroquinolones, clindamycin, or macrolides) in phagocytic WBCs such that killing of intracellular pathogens is enhanced and drug is more effectively distributed to the site of infection. Combination therapy can be a powerful tool for minimizing resistance,[17,18] but current C&S techniques do not provide guidance regarding additive or synergistic (or antagonistic) combination therapy.

Applying Culture and Susceptibility Data to the Patient

Using PD data

The CCP can benefit from 2 sources of PD data. The most ideal is that provided on a C&S report for a presumed pathogenic isolate cultured from a patient. The second is population data for the organism. The pertinent information provided for a patient on a C&S report is demonstrated in Figure 2 and includes both the susceptibility information (including MIC if broth dilution or E-test) and caveats to interpretation. The more accurate and detailed the history accompanying the

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patient sample, the more likely the veterinary microbiologist will provide an assessment that is relevant to the patient. These recommendations can make a difference between therapeutic success and failure.

Population PD data

Treatment of infections in CCP often must be initiated without the benefit of C&S data. Until such data are available, the empirical approach to drug selection can be supported by population PD data. Data might also support the design of a dosing regimen even after C&S data become available, particularly if susceptibility data do not include MIC. A nonquantitative but helpful summary of PD data is an antibiogram that indicates the proportion of isolates that are susceptible (or resistant) to the drug of interest (Figure 6). Although it does not provide information regarding the level of susceptibility, it can provide direction regarding drug selection. More useful are data generated from a summary of population statistics (Table 3). An important consideration of the statistical description of a population is the adequacy of sample size, which is ideally 100 or more different isolates. Among the important aspects of the distribution pattern is whether or not the data are normally distributed. A bimodal population indicates a second, more resistant population as is demonstrated for amoxicillin-clavulanic acid and *E. coli* isolated from dogs and cats in Figure 5; its statistical data are represented in Table 3. Among the most clinically relevant parameters describing the population are the range, representing the lowest and highest MICs recorded for any organism isolate; the mode, which is the most frequently reported isolate MIC for that organism; the median, or the middle MIC, the 50th percentile or MIC_{50}; and the MIC_{90} representing the 90th percentile of the MIC distribution. Although the mean is pertinent, the logarithmic nature of MICs results in noncontinuous data and means are often geometrically converted in scientific reports. Assuming the sample size population is sufficiently large, MIC_{90} might serve as a surrogate indicator for patient MIC when selecting among drugs or designing doses. Finding population PD information can be a difficult. Ideally, and not unreasonably, each hospital might develop their own ongoing antibiogram (Figure 6) based on cumulative data received from their patients. Several sources of PD data exist. Current literature is an important source, although journals in which such data are published generally are not those reviewed by most practitioners.[42,48] Package inserts of recently approved antimicrobial drugs (within the last 2 decades; eg, fluoroquinolones and beyond), include both agar gel diffusion and MIC

Cumulative Antibiogram Canine Isolates from 10/1/06 to 9/25/07

PERCENT SUSCEPTIBLE

(No. ISOLATES TESTED) a

	No. of Isolates	Amikacin	Amoxicillin/CA	Ampicillin	Cefazolin	Cefoxitin	Ceftiofur	Cephalothin b	Chloramphenicol	Clindamycin	Enrofloxacin	Erythromycin	Gentamicin	Marbofloxacin	Orbifloxacin	Oxacillin	Penicillin	Tetracycline	Ticarcillin	Ticarcillin/CA	Trimethoprim/Sulfa
Enterococcus faecalis	30		97	97					83		40	28 (29)		40 (15)	10		93	63			
Enterococcus faecium	13		15	13					100		0	15		0 (11)	0		15	23			
Escherichia coli	120	98	63	48	69	65	76 (103)	46	81		63		83	65	62 (119)			68	52	64	67 (119)
Klebsiella pneumoniae	30	80	70	0	70	70	63	70	80		77		77	77	73			73	0	70	77
Proteus mirabilis	32	100	97	94	94	97	100	94	94		97		91	100	91				94	100	81
Pseudaeruginosa	61	100							0		64		97	76 (50)	25				97	98	
Staphy aureus	8	100	50	13	50	50	29 (7)	50	100	50	75	50	88	75	75	50	13	88			88
Staph intermedius	102	92	66	17	64	64	60 (89)	64	97	43 (101)	55	43	87	63	60 (99)	72	18	42			60 (101)
S. schleferi ss coagulans	20	95	65	45	65	65	59 (17)	65	100	95 (19)	30	100	70	50	40	65	50	95			100
S. pseudointermedius	4	75	50	0	50	50	33 (3)	50	100	50	75	50	75	75	75	50	0	50			75

a. Numbers in parentheses represent actual number tested if different from total.
b. Cephalothin acts as a class drug representing cephalothin, cephapirin, cephalexin, cephradine, cefaclor, and cefadroxil.

Figure 6: A cumulative antibiogram generated for the target species can be helpful in identifying drugs to which acquired resistance has emerged. The data will be specific to the facility (ie, hospital). The number in each cell refers to the number of tested isolates designated as susceptible to the drug. When present, the number in parentheses in each cell refers to the number of isolates tested for that drug; otherwise the number tested is indicated in the far left hand column. Note that the data indicate that speciation may be important, particularly for *Enterococcus* and *Staphylococcus* genera. (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

Table 3: Susceptibility data for feline and canine *Escherichia coli* pathogens ($n = 595$). (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Elsevier, 2011, in press. Reprinted with permission.[66])

Drug	Resistant breakpoint (µg/mL)	Mode	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range
Amoxicillin-clavulnate	>32/16	4	4	32	0.5–2048
Ampicillin	>32	2	4	512	0.25–512
Meropenem	>16	0.25	0.25	0.5	0.25
Cefotaxime	>64	1	1	16	1–2048
Cefoxitin	>32	4	4	32	0.5–2048
Cefpodoxime	>8	0.5	0.5	256	0.12–512
Ceftazidime	>32	0.5	0.5	16	0.25–512
Cephalothin	>32	8	16	2048	1–2,048
Gentamicin	>16	1	1	8	1
Enrofloxacin	>4	0.06	0.06	32	0.03–512
Ciprofloaxcin	(>4)	0.03	0.03	32	0.3–128
Trimethoprim-sulfamethoxazole	>4/76c	0.06	0.06	2	0.06
Azithromycin	>8	8	8	64	1–512
Chloramphenicol	>32	8	8	32	2–2048
Doxycycline	>16	1	2	32	0.25–1024

MIC, minimum inhibitory concentration; BP, breakpoint.

population data, although this may be restricted to the organisms approved for treatment. The data will have been extensively reviewed by the FDA. However, older drugs (eg, before fluoroquinolones) will be missing both PD and PK data. Further, the FDA unfortunately limits the data that can be included on the labels only to those organisms for which the drug is approved to treat. A handbook antibiogram is commercially available that provides a summary of data collected from one veterinary microbiological laboratory.[49]

Population PD data are impacted by the same limitations that impact C&S data. However, an additional limitation of any sample of population data is the relevance of the sample to the actual target population. Environmental factors such as geography, practice size, antimicrobial use practices, and patient factors such as gender, age, concomitant disease, will influence applicability of the sample to the actual population data. Perhaps the most important information to be assessed when applying population data to a patient is previous antimicrobial exposure of the patient. The utility of the data might be facilitated if the isolate in the CCP is known to be susceptible and the PD data include only susceptible isolates.[50]

Designing the dosing regimen: integration of PK and PD data

A variety of drug, microbial, and host factors impact therapeutic success (Figure 7). The extent to which these factors are considered by CLSI vary and should be taken into account by the clinician through the design of the dosing regimens by integrating PK/PD data. In doing so, the dynamic relationship between the drug concentration to which the organism is exposed is optimized throughout the dosing.[36,51–54] It is beyond the scope of this manuscript to discuss most of these factors. However, the approach to design of a dosing regimen varies markedly with drugs. The variability reflects, in part, the relationship between MIC, PDCs, and efficacy among drugs.

The Postantibiotic effect: The *postantibiotic effect* (PAE) is defined *as* the period of suppressed bacterial growth after a short exposure of the organism to the antimicrobial.[38,55–57] Clinically, this translates to inhibited bacterial growth even though the drug is no longer present, and thus is below the MIC of the infecting microbe. The impact of the PAE on antimicrobial efficacy can be profound, particularly for concentration-dependent drugs. It is the PAE that allows some drugs to be administered at long intervals despite short half-lives. The PAE may be absent for some organisms or some patients (eg, some immunocompromised patients). The duration of PAEs vary with each drug and each organism and the relationship between PDC and MIC. For example, it is the PAE of aminoglycosides toward gram-negative organisms that facilitates once daily therapy.[58] In general, concentration-dependent compared with time-dependent drugs appear to exhibit longer PAE, particularly for gram-negative organisms, with the duration of the PAE being proportional to the magnitude of the peak PDC (ie, longer with higher PDC).[55,59]

Concentration versus time-dependent drugs: The relationship between efficacy, MIC, and the magnitude and time course of PDC can be categorized, in vitro, as either *concentration dependent* (sometimes referred to as dose de-

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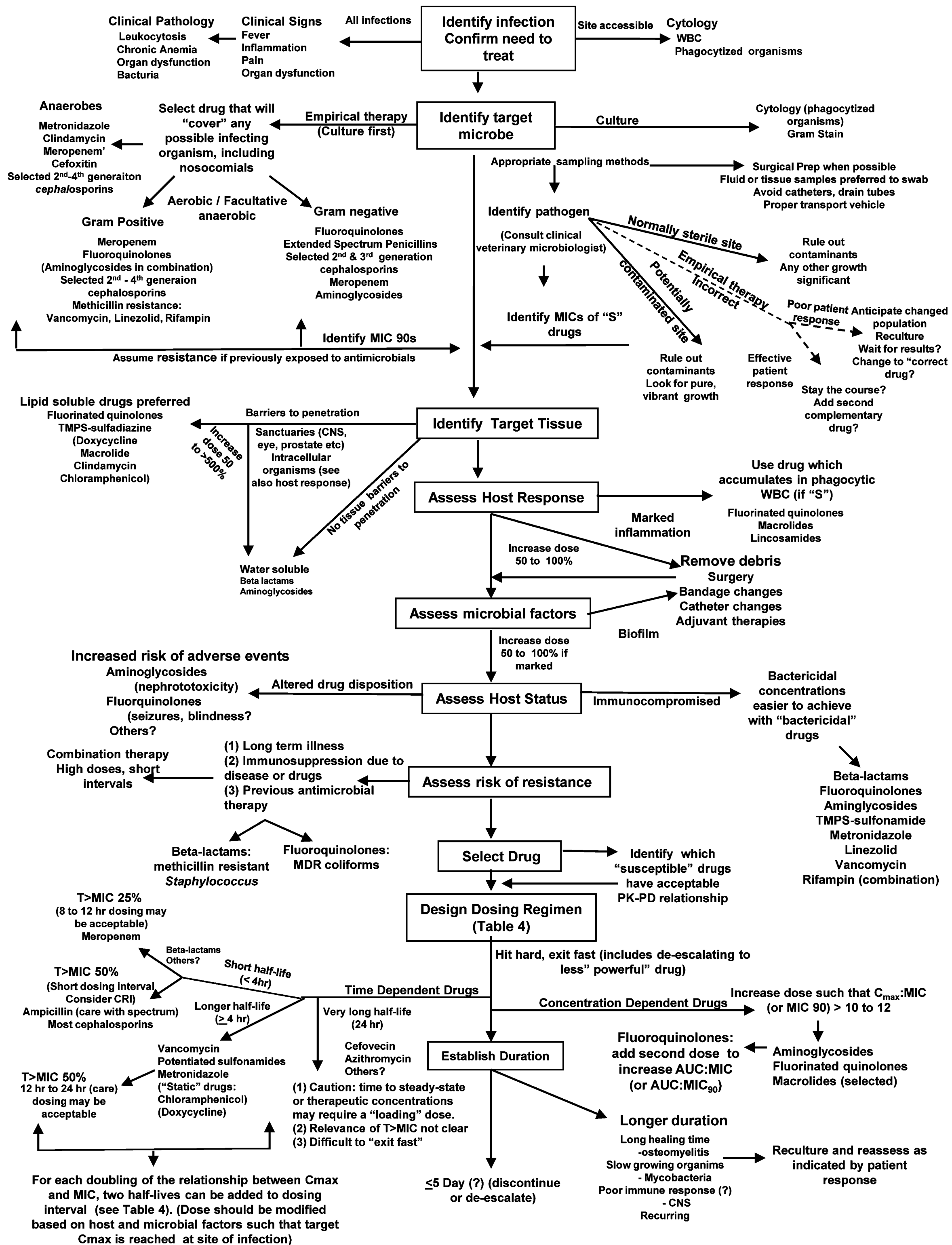

Figure 7: An algorithmic approach to antimicrobial selection in the critical care patient, with an emphasis on the role of culture and susceptibility data in the selection of the drug and the design of a dosing regimen.

pendent) or *time dependent* (sometimes referred to as concentration independent).[37,53,54] A third class of drugs has emerged with characteristics from each of these classes.

Concentration-dependent drugs are best represented by the fluoroquinolones and aminoglycosides.[60] Efficacy is enhanced by C_{max} to MIC ratios of 10–12, with a higher

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Table 4: Integration of population pharmacodynamic (PD) and pharmacokinetic (PK) data and its role in the design of dosing regimens (Adapted from Boothe DM. Small Animal Clinical Pharmacology and Therapeutics. 2 ed. Elsevier, 2011, in press. Reprinted with permission.[66])

Drug	Organism	MIC_{90} µg/mL	Route	Interval (h)	Dose (mg/kg)	C_{max} (µg/mL)	Half-life (h)	Half-lives $T>MIC$	Interval (h)
Time-dependent drugs ($T>MIC$ 50%)									
Amoxicillin-clavulanate	SIG	<0.5	PO	12	12.5	5.5	1	3.46	3.46
	S. aureus	4						0.46	0.46
	E. coli	32						0.00	0.00
Cephalexin	SIG	2	PO	12	22	20	1.3	3.32	4.32
	S. aureus	8						1.32	1.72
	E. coli	26						0.32	0.42
Cefovecin	SIG	0.25	SC	168	8	4.2*	133	4.07	541.48
	S. aureus	2						1.07	142.39
	E. coli	1						2.07	275.42
Cefpodoxime	SIG	0.5	PO	24	5	8.2	5.6	4.04	22.60
	S. aureus	NA						NA	
	E. coli	0.5						4.04	22.60
Meropenem†	SIG	NA	SC	12	20	26	0.75	NA	
	S. aureus	0.25						6.7	5
	E. coli	0.5						8.8	7
	P. aerug	2						3.7	3

Drug	Organism	MIC_{90} µg/mL	Route	Interval (h)	Dose (mg/kg)	C_{max} (µg/mL)	C_{max}/MIC
Concentration-dependent drugs ($C_{max}/MIC>10$–12)							
Enrofloxacin	SIG	0.25	PO	24	20	7.1‡	28
	S. aureus	64					0.11
	E. coli	64					0.11
	P. aerug	0.5					14
Marbofloxacin	SIG	1	PO	24	5.5	4.2	4.20
	S. aureus	64					0.07
	E. coli	64					0.07
	P. aerug	0.5					8.40
Orbifloxacin	SIG	2	PO	24	2.5	2.3	1.15
	S. aureus	64					0.04
	E. coli	64					0.04
	P. aerug	16					0.14
Ciprofloxacin	SIG *o*	0.125	PO	24	20	2.8	22
	S. aureus	0.25					11
	E. coli	64					0.04
	P. aerug	2					1.40

Gentamicin§	SIG		IM	24	3	27	NR
	S. aureus	1					27
	E. coli (7)	2					13.50
	P. aerug	4					6.8
Amikacin§	SIG		SC	24	10	14	NR
	S. aureus						NR
	E. coli	8					1.75
	P. aerug	8					1.75

*The concentration of unbound (free and active) drug.

†$T>$MIC 25% generally acceptable for carbepenems.

‡Includes approximately 25% ciprofloxacin formed from metabolism of enrofloxacin.

§Aminoglycosides should not be used as sole agents when treating *Staphylococcus spp.*

$T>$MIC, time that plasma drug concentrations are above the MIC (or MIC_{90}) of the infecting microbe; C_{max}/MIC, maximum plasma drug concentration/minimum inhibitory concentration (or MIC_{90}) of the infecting microbe; PO, orally; SIG, *Staphylococcus intermedius* group; *S. aureus*, *Staphylococcus aureus*; *P. aerug*, *Pseudomonas aeruginosa*; *E. coli*, *Escherichia coli*.

index targeted for more difficult infections (eg, *P. aeruginosa*, or infections caused by multiple organisms).[53] For concentration-dependent drugs, a dose that is too low is particularly detrimental, and decreasing doses in the face of altered renal function may not be prudent.[61] However, for some, efficacy may be both dose- and time-dependent, with the best predictor of efficacy being AUC/MIC. For example, efficacy of fluorinated quinolones can be predicted by both a C_{max}/MIC (target 10–12) or AUC/MIC (100–125 for gram-negative organisms).[26,62] The optimum AUC/MIC also varies with the organism, ranging from as low as 30–40 for *Streptococcus pneumoniae* and levofloxacin to >350 for *P. aeruginosa* and ciprofloxacin. Concentration-dependent drugs generally can be administered at longer intervals (ie, once a day). Indeed, efficacy may be enhanced by a drug-free period (ie, a long interval between doses) for aminoglycosides,[63] whereas the addition of a second dose (at the originally high dose) may improve efficacy and minimize emergent resistance for fluoroquinolones. The impact of the relationship C_{max}/MIC$>$10–12 is demonstrated in Table 4.

In contrast to concentration-dependent drugs, efficacy of time-dependent drugs (eg, β-lactams, vancomycin), is best predicted by the time that PDC remain above the ($T>$MIC), that is, the duration of exposure.[51] For such drugs, PDC should be 2–4 times the MIC of the infecting microbe throughout the dosing interval ($T>$MIC = 100%). However, the duration of $T>$MIC varies from a low of 25% for carbapenems to 50–70% for extended spectrum penicillins to 100% for penicillin and aminopenicillins.[51,53] With time-dependent drugs, increasing the dose may be necessary to assure PDC are above (ideally several fold) the MIC. However, maintaining $T>$MIC may be problematic for drugs with a short half-life. Because drug concentrations decrease by 50% every drug half-life, a C_{max}/MIC of 2 will result in PDC that reach the MIC in 1 half-life; the dosing interval for $T>$MIC 50% would then be 2 half-lives. For each additional half-life to be added to the duration that $T>$MIC (or for the addition of 2 half-lives on the dosing interval), concentrations must be doubled again (ie, quadrupuled if $T>$MIC = 2 half-lives, 8-fold if $T>$MIC = 3 half-lives). The impact of C_{max} and half-life for time-dependent drugs is demonstrated in Table 4. Constant rate infusion might be ideal for time-dependent drugs with short half-lives.[64] Caution is recommended with slow release products both in regards to time of onset and duration of therapy; the former may be contraindicated in the CCP and the latter may preclude the *get out quick* goal of therapy. Examples might include penicillins in ester forms (procaine, benzathine) or even cefovecin, which is tightly bound and slowly released from plasma proteins. Other examples include those drugs that accumulate in selected tissues (ie, macrolides, clindamycin, or drugs that accumulate in phagocytes). The optimal rela-

tionship between PDC and MIC and the parameter that best predicts antimicrobial efficacy (eg, C_{max}/MIC, AUC/MIC, $T>$MIC) have not been established definitively for all antimicrobials. However, for drugs characterized by inhibition (bacteriostatic drugs), $T>$MIC may best predict efficacy. Finally, some drugs (eg, macrolides) are characterized by time dependency for some organisms but concentration dependency for others.

Drug selection

An advantage of broth dilution data is the potential ability to choose among several drugs designated 'S.' If patient MIC data are not available, population data (ie, MIC_{90}) might be used as a surrogate. The efficacy of drugs cannot be compared by directly comparing MIC (or MIC_{90}) among the 'S' drugs unless the breakpoints of the drugs are numerically the same, which is more likely for drugs in the same class. For example, the MIC_{BP} of several β-lactams are similar as are the breakpoints of several fluoroquinolones. However, the breakpoints for fluoroquinolones are markedly lower than for β-lactams. The MIC is a measure of potency, and differs among drugs toward a susceptible microbe for a variety of reasons. This includes, but is not limited to differences in molecular weight, the number of molecules necessary to neutralize the target, microbial penetrability of the drugs, affinity of the molecule for the target, and differences in the mechanisms of action. Inherent mechanisms of resistance that vary among drugs will influence MIC. As such, relative susceptibility among drugs is determined for each drug by normalizing the MIC (an indicator of what is needed) to the concentration that will be obtained in the patient when the drug is given at a known dose (what is achieved). For what is achieved, one option is to use the CLSI resistant breakpoint (Table 2) which is based, in part, on PK such as peak PDC, or C_{max}. However, because breakpoints do not always equal the C_{max} and because the dose upon which breakpoints are based are not necessarily known, actual C_{max} is preferred. For C_{max}, the most ideal data are determined in the infected patient. However, this is likely to be available only for antimicrobials amenable to therapeutic drug monitoring (eg, aminoglycosides, vancomycin, and fluoroquinolones). More commonly, population PK data will be used (Table 4). The choice of PK parameter to use as an estimate of what is achieved depends on whether or not the drug is time versus concentration dependent. For the latter, the ratio of C_{max} to MIC (target of 10–12) is compared. For the latter, $T>$MIC must be compared, which must take into account elimination half-life.

Amoxicillin offers an extreme example of why C_{max} is preferred to MIC_{BP} as an indicator of what is achieved when selecting drugs. The resistant MIC_{BP} of amoxicillin-clavulanic for all *Staphylococcus spp.* is $\geq 8/4\,\mu g/mL$ and for *E. coli* $\geq 32/16\,\mu g/mL$. Using the MIC_{90} as an indicator for what is needed for each organism, the ratio of MIC_{BP}/MIC_{90} (rather than C_{max}/MIC_{90}) yields ratios of 2 and 8, respectively. However, based on the literature, the actual C_{max} achieved in dogs at the labeled dose of 13.5 mg/kg is (for amoxicillin) at most, 6 μg/mL.[65] The ratio of C_{max}/MIC_{90} is thus 1.5 for *S. pseudointermedius* but only 0.75 for *E. coli*. Clearly, as a time-dependent drug, amoxicillin (with a half-life of 1 to 1.5 h) would need to be administered as a CRI at a higher dose if the dual goals of efficacy and avoidance of resistance are to be achieved. It is not clear why CLSI originally established a breakpoint for amoxicillin-clavulanic acid that is so high compared with PDCs. However, the institute is in the process of reevaulating the MIC_{BP} for amoxicillin and amoxicillin-clavulanic acid, and it is likely that many isolates previously considered susceptible to these drugs will be designated as resistant.[a]

As with PD data, finding population PK data for the target species can be problematic. Sources again include textbooks[66] and current literature. Among the most comprehensive source of PK data for dogs and cats can be found in the Antimicrobial Monographs, published by the American Academy of Pharmacology and Therapeutics through the United States Pharmacopeia.[65] Although data are available only for a limited number of doses, C_{max} and AUC are dose dependent such that, in general, each changes proportionately with a change in dose, particularly with IV administration. It is important to remember that population PK data generally reflect normal, healthy patients at a specific dose (Table 4) and applicability, particularly to the CCP must be made cautiously. It is beyond the scope of this manuscript to address how changes in drug disposition will impact PK. However, in general, elimination half-life of renally excreted drugs will change directly and proportionately with clearance, in turn dependent upon glomerular filtration rate.[1,11,61] This change may be offset by changes in volume of distribution: changes in both C_{max} (inversely) and half-life (directly) will be proportional to changes in volume of distribution.

Designing the Dosing Regimen

Designing dosing regimens based on PD data

Table 4 demonstrates the impact of dose, C_{max} and elimination half-life on the design of a dosing regimen for time- or concentration-dependent drugs. Although the MIC_{90} of selected organisms as reported in current literature was used for this demonstration, the MIC collected from a patient can be substituted. Design of the dosing regimen is relatively easy for concentration-

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dependent drugs. If the target C_{max}/MIC_{90} (or patient isolate MIC) of 10–12 is not achieved with the chosen dose, the dose can be modified by simply multiplying it by the proportional difference (ie, if the ratio at the chosen dose is 5, the dose needs to be increased by 10/5 or 2-fold). Designing dosing regimens for time-dependent drugs ($T > MIC$) is more difficult because elimination half-life is the primary determinant of change. Dosing intervals are much more reasonable for drugs with longer half-lives (eg, cefpodoxime, cefovecin). The dosing regimen begins with determination of the C_{max}/MIC_{90} such that the number of half-lives (X) that lapse before $T > MIC$ is reached can be determined ($X = [\ln(C_{max}/MIC_{90})]/0.693$). Once the number of half-lives is known, then $T > MIC$ (in h) = (X)(drug half-life). The duration of the dosing interval then depends on the duration of $T > MIC$. For most β-lactams, the *minimum* $T > MIC$ is 50%; an exception is the carbapenems for which $T > MIC$ 25% may be acceptable. Thus, for $T > MIC$ 50%, the dosing interval becomes (X)(drug half-life)/(0.5) (Table 4). Alternatively, the interval can be "common sensed." If the MIC for any isolate for amoxicillin-clavulanic acid is 2, and the extrapolated C_{max} is 12 μg/mL (at a dose of 27 mg/kg), 2 half-lives can lapse before the MIC is reached in plasma as concentrations (μg/mL) decline from 12 to 6 to 3. By the third half-life, concentrations would reach 1.5 μg/mL, which is below the MIC. The half-life of amoxicillin approximates 1.25 hours, indicating $T > MIC$ for 2.5 hours. If $T > MIC$ 50% is targeted, a minimum of a 5-hour dosing interval is indicated; a 6-hour dosing interval may be acceptable, particularly in a patient that is able to overcome an emerging resistant population. To use the drug at an 8-hour dosing interval, 2 hours would need to be added to the dosing regimen, or approximately 1 half-life for $T > MIC$. The dose of 25 mg/kg would thus need to be doubled, which is not likely to be tolerated by the patient. Note, however, that these doses and intervals for both time- and concentration-dependent drugs would need to be modified even further to account for host and microbial factors previously discussed (eg, distribution to the site, biofilm, etc.).

In conclusion, C&S data can be useful for not only their traditionally important role in identification of the target organism and the drugs to which it is susceptible, but also to select among a number of potentially susceptible drugs. Just as important is the use of susceptibility data – whether from the patient or from a sample population – in to the design a dosing regimen that is individualized for the patient. Among the challenges facing the emergency/critical care specialists is the generation of PD ('what is needed' to effectively kill pathogens) and PK data – including changes associated with the pathyphysiology of illness – that support the empirical selection and design of dosing regimens. Until such information is available, dosing regimens should be designed such that they err on the side of too much rather than too little, particularly in the fact of challenging host and microbial factors. Such an approach facilitates a *hit hard-exit fast* approach to antimicrobial therapy that should help minimize antimicrobial resistance.

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Footnotes

[a] Papich M, Chair of Veterinary Standards Subcommittee, CLSI, Personal communication, August 2009.

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