ANTIMICROBIAL DECISION MAKING: INTERPRETING CULTURE AND SUSCEPTBILITY TESTING

Dawn Merton Boothe, DVM, PhD, DACVIM, DACVCP
Auburn University, AL USA
boothdm@auburn.edu

INTRODUCTION For complicated infections, or in patients at risk for therapeutic failure or developing resistance, identification of the infecting organism most appropriately should be based on appropriately collected cultures with (ideally) tube dilution susceptibility testing to allow assessment of "how" susceptible the isolate is, in general, to other drugs, but specifically to the drug of choice, such that a dosing regimen can be designed for the bug in the patient. Basing antimicrobial selection on C&S data does not guarantee success, just as failing to use C&S as a basis for selection (or selecting a drug characterized by "R" on the data) does not guarantee failure. The "90-60 rule" implies that approximately 90% of infections treated based on C&S are likely to respond if an "S" drug is selected; yet, up to 60% will respond even if an "R" drug is selected. The most likely situations where the latter is true is if the infection is at a site in which drug much higher than that achieved in the test tube (i.e., much higher than the minimum inhibitory concentration [MIC]).

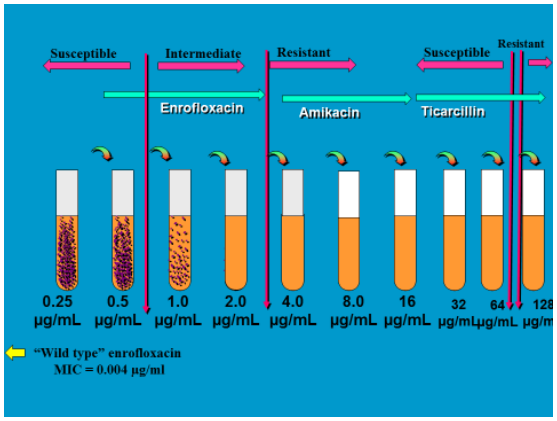
To treat or not to treat. Among the challenges to interpreting C&S data is determining whether or not the isolate cultured is a pathogen. The data is only as good as the sample collection and handling. For example, for UTI, free, midstream catch samples are unacceptable and even catheterized samples are less than ideal. Swabs are less ideal than tissue (let the lab macerate) because not only do organisms "hide" in the swab, but the swab itself can be an inhibitor. Just as absence of growth does not indicate absence of infection, growth does not necessarily indicate infection. Further, even if infection is present, the isolated organism may not be the actual pathogen. Clearly, (properly collected) culture of an organism from a tissue that is normally sterile indicates infection. However, discriminating between normal and infecting flora can be difficult. Purity, vibrant growth (meaning special media was not needed to coax the growth of the organism), and presence of a large number of colonies are indicators of infection. The isolation of three or more different organisms (including more than one strain of the same organism) may indicated contamination, and re-culture should be considered. The extent of growth should be strongly considered when deciding to treat. Generally, infection is considered to be present if there are >107 CFU/ml at the site. For C&S purposes, quantitative cultures can be helpful: the urinary tract is not considered infected until >105 CFU /ml are present whereas only > 10^3 is indicative of infection in the respiratory tract. Use of "urinary" paddles might be considered for samples that are being shipped, in order to increase accuracy of identification and numbers. Laboratories may also indicate "heavy, moderate or light" growth; isolates with the greatest amount growth might be targeted. For multiple organisms, that with the greatest growth should be the primary focus of therapy. A call to the diagnostic lab might be prudent before marked financial commitment is put into treating an organism that is not causing infection. This is particularly important if the organisms' presence is unusual (e.g., *Lactobacillus* sp. in urine).

To wait or not to wait? Frequently, antimicrobial therapy is begun before cultures are collected. This is particularly true and appropriate in critical patients or in patients for which clinical signs are evident and are detracting from quality of life (patient or parent). However, should therapy begin and the choice prove to be wrong once C&S data is received, the original C&S data collected before the drug was begun may no longer be relevant to the patient. The use of the drug may change the pattern of resistance versus susceptibility, or may result in higher MIC (see mutant prevention concentration). A re-culture may be indicated at that time, if possible. Certainly, dosing regimens with the appropriate drug should take into account the possibility that some level of resistance has developed toward the indicated drug.

To trust or not to trust: One of the most important ways to "De-escalate" antimicrobial use is to not treat if not needed. The C&S procedures themselves are fraught with potential errors that may lead to inappropriate treating or inappropriate use of drugs. For practices that provide in-house susceptibility testing, care must be taken to follow guidelines established and published by (or comparable to) the Clinical and Laboratory Standards Institute (CLSI) or comparable standard-setting agency. Materials, including interpretive

standards, should be validated by the appropriate agency. Minor changes in pH, temperature, humidity, etc. can profoundly affect results. Personnel should be trained specifically in culture techniques and hospitals that provide this service (as do diagnostic labs) should maintain well designed and adequately collected quality control data to validate their procedures (CLSI indicates control organisms). Pitfalls of susceptibility testing are also reflected in the drugs selected for testing. Not all companies are interested in establishing interpretive criteria and as such, not all drugs are available for testing. Because automated systems cannot accommodate and laboratories (nor clients) cannot afford to test all potential drugs used to treat an infection, one drug often is tested as a model for other drugs in the class. For example, cephalothin models first generation cephalosporins, even though it is no longer used clinically. Note that it does not represent cefazolin well, the latter being more effective toward Gram negative (especially *E. coli*) isolates. No single cephalosporin can represent 2nd or beyond generations. Enrofloxacin often represents the fluoroquinolones. In general, cross resistance can be expected among the FQs, although differences in potency do exist (for example, ciprofloxacin is more potent toward *Pseudomonas* or *E coli*, but less to Gram positives compared to enrofloxacin). Culture does not take into account active metabolites of some drugs (e.g., enrofloxacin converted to ciprofloxacin). Note that if an organism is R to any FQ, FQ should be used only cautiously even if another is "S". Amikacin is often more effective than gentamicin toward many organisms, but less effective toward *Staphylococcus* sp. (hence both are often on a report). Note that the package inserts for aminoglycosides indicate that they should not be used by themselves to treat staphylococci, a fact often missed.

CLSI interpretive criteria are generated for specific species, and often for specific organisms and specific infections. Human laboratories will use human interpretive criteria, which often are not relevant to animals (e.g., ciprofloxacin). Ciprofloxacin (CIP) oral bioavailability in dogs is 30 to 40% of that in humans, and despite its increased potency compared to enrofloxacin (ENR) toward Gram negative organisms, its potential efficacy (MIC_{BP}) is equivalent to or less for many organisms. Susceptibility data also does not take into account active metabolites, again exemplified by ENR, which is metabolized to CIP: both C_{max} and area under the curve (AUC) of bioactivity of ENR may increase up to 50% or more by CIP; as such, C&S data may underestimate efficacy. MIC_{BP} generally are based on the highest labeled dose, but higher doses might be safely administered for many antibiotics. If recommended doses change, the manufacturer should provide CLSI with updated pharmacokinetic information so that interpretive criteria may change. One of the disadvantages of current susceptibility testing is that the concentrations tested are close to the MIC_{BP} and thus, does not allow identification of isolates that are very susceptible (that is, MIC are far away from the MIC_{BP}). As such, drugs may be chosen based on isolates that have already undergone first step mutations (see below). A final concern relates to the 3rd and 4th generation (extended spectrum) cephalosporins: they are susceptible to extended spectrum beta-lactamase (ESBL) that tends to be induced *in vivo* but often missed *in vitro*. If CLSI guidelines are followed, resistance to cefpodoxime indicates ESBL being produced. If CLSI



E coli UTI	Week 1		C_{max}:M	Week 4	
Amikacin	≤2	S	≥32	≤2	S
Amoxi-Clav	≤8	S		≥32	R
Ampicillin	≥32	R	≤1	≥32	R
Carbenicillin	≥512	R		≥512	R
Cephalothin	8	I		≥32	R
Ceftiofur	≤1	S	2.5	≥8	R
Chloramph	≥32	R		≥32	R
Ciprofloxacin	≤0.5	S		≥4	R
Enrofloxacin	≤0.25	S		≥4	R
Gentamicin	≤0.5	S	>5	1	I
Nitrofurantoin	≤32	S	>17	≤32	S
Piperacillin	≥256	R	>56	≥256	R
Tetracycline	≤1	S		≥16	R
Ticarcillin	≥256	R		≥256	R
Tobramycin	≤0.5	S		≤0.5	S
Trim-Sulfa	10	S	>7	≥320	R

Culture and susceptibility from a 3 yr. M Weimaraner with recurrent UTI. The second result was collected 2 weeks after 14 days of therapy of 5 mg/kg enrofloxacin. The table for Cmax is found below.

guidelines are not followed, therapeutic failure may occur. Carbapenems and clavulanic acid (e.g., amoxicillin-clavulanic acid) generally are not susceptible to these enzymes.

BRIDGING PHARMACODYNAMIC (PD) AND PHARMACOKINETIC (PK) DATA. So, once you have the data, what do you do with it? Simplistically, susceptibility data represents "what is needed" in the patient to facilitate antimicrobial efficacy. Care must be taken with this simplistic approach: susceptibility data is generated from in vitro methodologies, yet it is applied to in vivo (and abnormal) conditions. This caveat should be foremost in the clinician's selection. Note that population susceptibility data can be helpful with empirical selection of antimicrobials (see below); mind, the data can be useful to antimicrobial selection.

Pharmacodynamic (microbiological) data: what you need. PD data includes data generated both from agar gel disc diffusion (e.g., Kirby Bauer: zone diameters) as well MI tube dilution (MIC) methods of susceptibility testing. What is tube dilution data and why is it so useful? In contrast to disk diffusion, tube dilution methods involve inoculation of a series of test tubes with a standard number of organisms. The test tubes contain increasing concentrations of the drug of interest in two-fold dilutions whose range varies with the drug, reflecting concentrations achieved in the patient for that drug at the recommended dose. Following a standard time, the tubes are evaluated for detectable growth. The test tube that contains the lowest concentration of drug and no visible growth contains the minimum amount of drug necessary to inhibit (not kill) the growth of the organism cultured from the patient (the MIC). Ideally, this concentration must be achieved at the site of infection. Adaptation to computerized/automated systems allows much more accurate testing in short time periods. For either method of susceptibility testing, simplistically, the likelihood of a drug being effective in the patient is based on whether or not the recommended dose on the label is likely to generate plasma drug concentrations (PDC) that equal or surpass the MIC of the infecting organism. Diagnostic laboratories indicate the likelihood of susceptibility by the "SIR" letter designation. Understanding the basis of that designation will facilitate antimicrobial selection. The SIR designation reflects whether or not the MIC of the infecting organism is less than ("S"), close or equal to ("I") or greater than ("R") the breakpoint MIC (MIC_{BP}) of the drug. CLSI determines the breakpoint, based in part, on peak plasma drug concentrations (C_{max}) of the drug (population data). Because dose and C_{max} varies for each drug (e.g., at 20 mg/kg, C_{max} of enrofloxacin is 4 mcg/ml; at 22 mg/kg, C_{max} of amikacin is 65 mcg/ml), the concentrations of drugs tested by the laboratory vary, and the breakpoint will also vary. Thus, one should not compare an MIC for enrofloxacin (e.g., 0.25 mcg/ml) to an MIC for amikacin (e.g., 4 mcg/ml) and assume the former is better. Rather, "how far" that MIC is from the C_{max} determines how susceptible the isolate is to each drug. Note also that the range of each drug tested is very narrow, leading to "≤" on reports. For example, for the culture report below and amikacin, ≤ 4 means no growth occurred in the test tube containing 8 mcg/ml, which was the lowest concentration tested by the lab,

2016 Cumulative Antimicrobial Susceptibility Report
Canine Isolates

PERCENT SUSCEPTIBLE
(No. ISOLATES TESTED) [a]

	No. of Isolates	Amikacin	Amoxicillin/CA	Ampicillin	Cefpodoxime	Cefovecin	Chloramphenicol	Clindamycin	Doxycycline	Enrofloxacin	Erythromycin	Gentamicin	Marbofloxacin	Nitrofurantoin	Oxacillin + 2% NaCl	Penicillin	Rifampin	Tetracycline	Trimethoprim/Sulfa
Enterococcus faecalis	68		100 (19)				93			40 (67)	6 (31)		34 (67)	100 (36)		100 (58)	12 (42)	55 (64)	
Enterococcus faecium	24		29 (7)				88			0	5 (17)		0	29 (7)		24 (21)	27 (15)	18 (22)	
Escherichia coli	261	92 (260)	59 (250)	51 (251)	71 (241)	70 (248)	56			76 (260)		92	81 (260)	98 (174)				81 (250)	82 (257)
Klebsiella pneumoniae	40	100	50 (36)	0 (37)	72 (39)	69 (39)	95			83		85	83	12 (24)				85 (39)	75
Proteus mirabilis	59	100	80 (54)	71 (55)	98 (50)	98 (50)	63			95		98	98	0				0	84 (58)
Pseudomonas aeruginosa	93	84 (87)								31 (87)		84	81 (88)						
Staphylococcus aureus	19	94 (18)	56 (18)		60 (15)	53 (16)	84	85 (13)		67 (18)	50 (14)	95	72 (18)	100 (5)	53	27 (15)	89 (18)	84	94 (17)
Staphylococcus intermedius[b]	255	99 (244)	52 (227)		54 (195)	54 (222)	87 (252)	56 (198)		59 (239)	49 (217)	65 (254)	62 (238)	100 (39)	44	13 (216)	99 (238)	52 (250)	58 (238)
Group G Beta Streptococci	73	2 (61)	100 (59)	100 (59)			100 (70)	58 (57)	78 (72)	57 (61)	66	48 (71)	78 (59)				100 (58)		

a. Numbers in parentheses represent actual number tested if different from total.
b. Represents what is presently known as *S. intermedius* group and includes *S. intermedius*, *S. pseudintermedius*, and *S. delphini*.

so the MIC must be lower than 8 or ≤ 4 mcg/ml, (the next lowest concentration). The isolate is susceptible. An MIC of > X is accompanied by an "R" because the organism was not susceptible to the highest concentration tested. CLSI updates MIC_{BP}, generally yearly, particularly as new data is provided regarding organism susceptibility. Increasing resistance to organisms may lead to changes in the MIC_{BP} such as has recently occurred for amoxicillin and doxycycline. For older antibiotics approved decades ago, originally labeled doses may be inappropriate for all except very sensitive organisms. Again, a good laboratory will follow CLSI guidelines.

Population data: Population data can be used for empirical antimicrobial selection. For example, clinicians can use an antibiogram which indicates the proportion of isolates resistant vs. susceptible to a drug. Similarly, the Target® Antimicrobial Handbook indicates not only the most likely organisms cultured (but not necessarily pathogenic) from selected sites, but also provides a "scoring" system of susceptibility. For antibiograms (see Auburn University Canine Cumulative Antimicrobial Susceptibility Report), drugs to which >75% or more of isolates are susceptible might be wiser selections. A patient that has not been previously exposed to antimicrobials is more likely to be represented by the "susceptible" isolates whereas an "at risk" patient (e.g., previously exposed to antimicrobials, immunosuppressed) may be better represented by the resistant proportion. Likewise, package inserts for newer antimicrobials include susceptibility data (MIC) and as such, can guide not only the selection of a drug, but the design of a dosing regimen. The MIC data on a label may include: 1. the range of MIC for susceptible organisms; 2. the mode of MIC (the most frequently cited MIC); 3. or the MIC_{50} and the MIC_{90}. The data are population statistics; the latter two reflect, respectively, the MIC below which 50% and 90% of the isolates (by genus and species) are inhibited (not killed). However, the MIC_{50} and MIC_{90} should be based on a large number of microorganisms to assure accurate sample representation of the population (ideally >300). Organisms with MIC_{90} that are low are more susceptible than organisms with higher $MIC_{90;}$ organisms whose MIC_{90} is approaching the C_{max} of the drug (also on a package insert) prudently should not be treated with that drug (see also concentration and time dependency). An example of population MIC data is demonstrated from a fluorinated quinolone package insert. Those organisms most susceptible to the drug have the lowest MIC whereas organisms with higher MIC are less likely to respond.

Pharmacokinetic (PK) Data: What you get. The selection of an antimicrobial should be based on the likelihood that therapeutic (effective) concentrations will be achieved at the tissue site. What is needed for therapeutic efficacy for infections is determined largely by the susceptibility (pharmacodynamic data) of the organism. If you have a C&S from your patient with MIC, the MIC for the drug of interest is how much you need. For populations of microbes, the MIC_{90} provides an indication of what is needed. Efficacy of an antibiotic is most likely to occur when the pharmacodynamic data is coupled with what is achieved in the patient. For the clinician seeking to improve antimicrobial efficacy, the further the MIC of the infecting organism is from the C_{max} (or MIC_{BP}) of the drug, the more likely effective concentrations will be reached at the site of infection. If a number of drugs are designated as "S", the selection of which "S" is best might be narrowed by focusing on those drugs for which the MIC is furthest from the MICBP or C_{max}. The most susceptible, lowest tier drug should be selected.

Selecting a drug: patient data: Compare the MIC (what is needed) to the peak drug concentration

Fluoroquinolone A

Parameter *For oral use in dogs and cats only*	Dose		
	Dog Mean ± SD* (2.5mg/lb) n=6	Dog Mean ± SD* (5.5mg/lb) n=6	Cat Mean ± SD* (5.5mg/lb) n=7
Time of maximum concentration, T_{max}(h)	1.5±0.3	1.8±0.3	1.2±0.6
Maximum concentration, C_{max}, (μg/mL)	2.0±0.2	4.2±0.5	4.8±0.7
AUC0-inf (μg•h/mL)	31.2±1.6	64±8	70±6
Terminal plasma elimination half-life, $t_{1/2}$(h)	10.7±1.6	10.9±0.6	12.7±1.1

Organism	Number of Isolates	MIC_{50}	MIC_{90}	MIC Range
Staphylococcus intermedius	135	0.25	0.25	0.125–2
Escherichia coli	61	0.03	0.06	0.015–2
Proteus mirabilis	35	0.06	0.125	0.03–0.25
Beta-hemolytic Streptococcus, (not Group A or Group B)	25	1	2	0.5–16
Streptococcus, Group D enterococcus	16	1	4	0.008–4
Pasteurella multocida	13	0.015	0.06	≤0.008–0.5
Staphylococcus aureus	12	0.25	0.25	0.25–0.5
Enterococcus faecalis	11	2	2	0–4
Klebsiella pneumoniae	11	0.06	0.06	0.01–0.06
Pseudomonas spp.	9	**	**	0.06–1
Pseudomonas aeruginosa	7	**	**	0.25–1

Table: MIC Values* (μg/mL) of FQA against pathogens isolated from skin, soft tissue and urinary tract infections in dogs enrolled in clinical studies conducted during 1994-1996

Fluoroquinolone B

Bacteria Name	Number of Isolates	MIC_{50}	MIC_{90}	MIC Range
Enterobacter spp.	9	0.11	3.66	≤0.05–3.66
Escherichia coli	28	≤0.05	0.11	≤0.05–7.3
Klebsiella spp.	8	0.11	0.11	0.11–0.23
Pasteurella spp.	8	≤0.05	≤0.05	≤0.05
Proteus spp.	15	0.92	1.83	0.11–1.83
Pseudomonas spp.	5	0.11	0.92	≤0.05–0.92
Staphylococcus spp.	193	0.23	0.46	≤0.05–1.83
Streptococcus spp.	56	1.83	3.66	0.11–7.3

Table: MIC values* (μg/mL) of FQB for bacterial pathogens isolated from skin and soft tissue infections and urinary tract infections in dogs enrolled in clinical studies conducted during 1991–1993.

Pharmacokinetic Measure	Mean Value
Peak plasma concentration (C_{MAX})	1.8 μg/mL
Time to reach C_{max} (T_{MAX})	2.8 hours
Elimination half-life ($T_{1/2}$)	9.3 hours
Area under the plasma curve (AUC0–∞)	14.5 μg•hr/mL
Total body clearance/F[a] (CL/F)	375 mL/kg/hr
Steady state volume of distribution/F[b]	3.8 L/kg
Volume of distribution (area)/F[c]	4.7 L/kg

Table: Plasma pharmacokinetics following administration of FQB tablets (5 mg/kg body weight) to dogs (n=20).

achieved at the recommended (or modified) dose: the higher the C_{max} is compared to the MIC, the greater the chance of therapeutic success and the less the chance of resistance. Once the "best" drugs are identified based on C&S, then the list can be narrowed down based on other factors. The same approach can be used for population data. P**ackage insert data**: Using P*roteus* as an example, comparison of C_{max} to MIC_{90} reveals a ratio 2:0.125 or 16 for marbofloxacin compared to 1.8:1.8 or 1 for difloxacin, using the low dose for each drug. For *E. coli,* the numbers are 2:0.06 or 33 for marbofloxacin compared to 16 for difloxacin. For either organism, marbofloxacin offers the best ratio. For *E. coli,* the low does might be acceptable for marbofloxacin, and potential for difloxacin, although the latter might not be prudent. For *Proteus*, again, the low dose of marbofloxacin might not be prudent, and difloxacin should not be used to treat *Proteus.*

For complicated infections, drugs and doses ideally will be based on C&S. Note that the sample must be collected properly cystocentesis; do NOT use free-catch data. It also must be handled properly: with a doubling time as short as 20 minutes, a small colony count indicative of no infection can rapidly become a high colony count (>10(5) CFU/ml), indicative of infection. Consider using a "paddle" that maintains colony counting capability during shipping. Finally, it must be performed properly: find a laboratory that uses guidelines and interpretive criteria delineated by the Clinical Laboratory Standards Institute and specifically, those for animals. Tube dilution (minimum inhibitory concentration) data is most helpful: the further the MIC is from the drug concentration achieved at the site, the more likely the drug is to be effective. Remember that an "S" designation does NOT indicate that the isolate has not developed resistance; it only means that effective concentration are likely to be achievable in the patient at the recommended dose. The more at risk the patient is for developing a resistant residual inoculum, however, the less confident the clinician should be in using a drug for which the MIC of the infecting organism is approaching the susceptible breakpoint. The more chronic the infection, the more likely the infection is in the deeper layers of the uroepithelium and protected by biofilm and thus the more important the drug be lipid soluble. This leads us to the next step: considering the impact of host and other factors on the design of the dosing regimen.