Antimicrobials, Susceptibility Testing, and Minimum Inhibitory Concentrations (MIC) in Veterinary Infection Treatment

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KEYWORDS

- Antimicrobials • Minimum inhibitory concentration • Susceptibility testing
- Veterinary infection treatment

KEY POINTS

- If a laboratory does not adhere to a public standard, such as Clinical and Laboratory Standards Institute (CLSI), breakpoints may vary and interpretation may be inconsistent.
- The first question for the clinician is whether or not a culture and susceptibility test is needed for treatment.
- Bacterial culture and susceptibility tests are needed if the clinician suspects that the infection may be caused by organisms resistant to the empirically selected "first-tier" drugs.
- It is becoming more common for laboratories to directly measure the minimum inhibitory concentration (MIC) of an organism with an antimicrobial dilution test, rather than disk diffusion tests.
- Resistance and susceptibility are determined by comparing the organism's MIC to the drug's breakpoint as established by the CLSI.
- The next edition of CLSI M31 for veterinary drugs will be divided into one table for veterinary interpretive criteria and a separate table for drugs that still rely on human standards for interpretation.
- The changes and updates in interpretive criteria used to establish breakpoints illustrate the need for laboratories to use only the most updated document available for interpretation and standards.
- Susceptibility tests, even when appropriate standards are used, are not perfect.
- When evaluating a patient that has failed to respond to therapy, one must consider the many factors that contribute to antibiotic failure.

INTRODUCTION

Many veterinarians submit culture specimens to a laboratory without great thought about the test procedure or the interpretation. The most important information for the clinician is simply which drugs have an "S" and which ones have an "R." These results then guide their treatment. What really goes into this interpretation?

The standards for interpretation are available from the Clinical and Laboratory Standards Institute (CLSI) (http://www.clsi.org/).[1] Not all laboratories in the United States use CLSI standards. It is a voluntary program. However, if a laboratory does not adhere to a public standard, such as CLSI, breakpoints may vary and interpretation may be inconsistent from laboratory to laboratory, or among different regions of the country.

The collection of the specimen and appropriate handling and transportation is best discussed with microbiologists and diagnostic laboratory staff. Some guidelines were developed by the International Society for Companion Animal Infectious Diseases (ISCAID) and published online,[2] as well as on their Internet site (http://www.iscaid.org/).

TO CULTURE OR NOT TO CULTURE?

The first question for the clinician is whether or not a culture and susceptibility test is needed for treatment. The answer often is "no." The empiric choice for initial treatment can be highly reliable and guidelines are available in a variety of sources.[3,4] Empiric selection should be based on the assumption that the infection is not complicated and the infection is caused by wild-type bacteria. It is critically important at this state to define what is meant by wild-type and non–wild-type bacteria. Wild-type strains of bacteria are those that have an absence of acquired and mutational resistance mechanisms, whereas non–wild-type strains of bacteria are those that have the presence of an acquired or mutational resistance mechanism to the drug in question. Wild-type strains may include bacteria that have inherent resistance to antimicrobials. For example, wild-type anaerobic bacteria are inherently resistant to aminoglycosides by virtue of a lack of an oxygen-dependent drug entry to the bacteria. Gram-negative wild-type bacteria of the Enterobacteriaceae family and *Pseudomonas aeruginosa* are inherently resistant to macrolide antibiotics.

Wild-type strains of bacteria may or may not respond clinically to antimicrobial treatment. Likewise, non–wild-type strains may or may not respond clinically to antimicrobial treatment. The prediction of whether the bacteria will, or will not, respond to treatment is commonly referred to as the "90/60 rule."[5] The 90/60 rule was derived from the observation that, in general, bacteria treated with antimicrobials to which the strain is sensitive will have a favorable therapeutic response in approximately 90% of the patients. On the other hand, when the bacteria are resistant to the antimicrobial administered, despite the susceptibility result, approximately 60% of patients will respond to therapy. In veterinary medicine, we have no data to confirm or challenge the 90/60 rule. The investigators[5] emphasize that these observations apply to immunocompetent patients with infections caused by a single bacteria, when the drug is expected to penetrate to the site of infection adequately. Most clinicians would agree that these cases do not comprise all of their patients. Many patients have polymicrobial infections treated with more than one antibiotic, have pathologic changes that may affect drug distribution (eg, protein-binding changes), have received oral antibiotics that are insufficiently absorbed, are immune-compromised patients, or have infections at sites that are either poorly penetrated or diluted, or for which antibiotics are concentrated (for example, from topical treatment or by tubular concentration before clearance by the kidneys).

WHEN IS IT TIME TO CULTURE?

Bacterial culture and susceptibility tests are needed if the clinician suspects that the infection may be caused by organisms resistant to the empirically selected "first-tier" drugs. Without a susceptibility test, the activity against these strains of bacteria is highly unpredictable. Bacteria most likely to be resistant are *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter* species, *Enterococcus* species, and *Staphylococcus pseudintermedius.* A more in-depth discussion of these organisms and their treatment is included in the article "Treatment of Resistant Infections" by M.G. Papich, elsewhere in this issue.

If the initial empiric treatment is unsuccessful, or if resistant strains of bacteria are suspected, a susceptibility test is advised. This test is important to (1) confirm the presence of a bacterial pathogen, (2) identify the species of bacteria so that virulence mechanisms are known, (3) guide treatment, and (4) monitor outcome (success or failure of treatment). Culture and susceptibility tests are advised if the patient has already been exposed to previous antibiotic therapy. Bacteria such as *E coli* are typically more resistant than other species of bacteria. There was a high incidence of resistance in *E coli* isolates collected from different regions of the United States.[6] The multidrug-resistant (MDR) isolates comprised 56% of the resistant isolates and more than half of these were resistant to amoxicillin, amoxicillin-clavulanate, and enrofloxacin. Previous antibiotic treatment is a known risk factor for methicillin-resistant *Staphylococcus*, as well as other resistant bacteria.[7–14] Fluoroquinolone activity may be especially unpredictable if the patient has previously been treated with this class of agents. Previous exposure to fluoroquinolones may select for resistant strains of *E coli* in dogs that can persist long after drug treatment has been discontinued.[15]

TYPES OF SUSCEPTIBILITY TESTS
Agar Disk Diffusion Test

Bacterial susceptibility to drugs has traditionally been tested with the agar-disk-diffusion test (ADD), also known as the Kirby-Bauer test. With this test, paper disks impregnated with the drug are placed on an agar plate and the drug diffuses into the agar. Activity of the drug against the bacteria correlates with the diameter of the zone of bacterial inhibition around the disk, measured in millimeters. In this test, a large zone of inhibition corresponds to a high degree of susceptibility. The larger the zone, the more susceptible the bacteria is to the drug in the disk. The size of the zone of inhibition has an inverse correlation to the minimum inhibitory concentration (MIC), but the size of the zone should not be used to derive an MIC value.

The inoculation variables must be well controlled and the test must be performed according to strict procedural guidelines.[1] The precise incubation time (usually 18–24 hours), selection and preparation of the agar, and interfering compounds should be known. The ADD test results are qualitative (that is, it determines only resistant vs sensitive) rather than providing quantitative information. If this test is performed using standardized procedures, it is valuable, even though it may sometimes overestimate the degree of susceptibility.

Microdilution Test for Determination of MIC

It is becoming more common for laboratories to directly measure the MIC of an organism with an antimicrobial dilution test. The test is usually performed by inoculating the wells of a plate with the bacterial culture and dilutions of antibiotics are arranged across the rows. The test is usually performed in modern laboratories using high-throughput plates, but individual tubes or plates can be used for dilution tests also.

Antibiotic drug concentrations are arranged in serial dilutions, with each concentration doubled from lowest to highest in a range. The MIC is not a measure of efficacy, but instead it is simply an in vitro measurement of drug activity and bacterial susceptibility. The lower the MIC value, the more susceptible the isolate is to that drug. The MICs are determined using serial twofold dilutions of drug to which is added a standardized inoculum that is incubated for a prescribed time. Concentrations are always listed in μg/mL. For example, if one were to start at a concentration of 256 μg/mL, the MIC dilution series would be as follows: 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06 μg/mL, and so forth. If, for example, bacterial growth occurs at a dilution of 0.12 μg/mL for a specific drug, but not at 0.25 μg/mL and above, the MIC is determined to be 0.25 μg/mL. Realistically, the true MIC lies somewhere between these values, but the MIC is recorded as the next highest value. Like the ADD test, the dilution test should be performed according to strict procedural standards, including quality control, such as those in CLSI documents M31.[1]

In some laboratories, other methods to measure the MIC are being used, such as the E-test (epsilometer test) by bioMérieux SA (bioMérieux, F-69280 Marcy l'Etoile, France, http://www.biomerieux-diagnostics.com). The E-test is a quantitative technique that measures the MIC by direct measurement of bacterial growth along a concentration gradient of the antibiotic contained in a test strip.

WHY REPORT ONLY THE MIC?

The MIC is the lowest concentration that inhibits visible bacterial growth. Frequently this is expressed as MIC_{50} or MIC_{90}, which is the MIC that inhibits 50% or 90% of the bacteria, respectively. It is sometimes cited in error that the MIC_{50} and MIC_{90} are the average concentrations for 50% and 90% efficacy. These values should not be confused with clinical efficacy (more on that later).

The MBC is the Minimum Bactericidal Concentration, which is the lowest concentration that kills 99.9% of the bacteria. Standards are available to measure the MBC, but the test is more complicated and difficult to perform than the MIC determination. Therefore, the MBC is rarely measured or reported in clinical laboratories.

The MPC is the Mutant Prevention Concentration. This is lowest antibiotic concentration that prevents growth of the least-susceptible first-step resistant mutant among a large bacterial population (eg, 10^7 or 10^{10} colony-forming units).[16] It also may be defined as the MIC of the most resistant first-step cell present in a bacterial population. The mutant selection window (MSW) is the concentration between the MIC of susceptible organisms, and the MPC. The MPC test is not standardized and is more difficult to perform in a clinical laboratory. Large inoculums are required. The interpretation of the MPC value for clinical dose determinations is difficult and has not been established for veterinary antimicrobials.

INTERPRETATION OF SUSCEPTIBILITY TESTS

Resistance and susceptibility are determined by comparing the organism's MIC to the drug's breakpoint as established by the CLSI, formerly known as the National Committee for Clinical Laboratory Standards (NCCLS).[1] An example of approved breakpoints is provided in **Table 1**. After a laboratory determines an MIC, it may use the CLSI "SIR" classification for breakpoints (S, susceptible; I, intermediate; or R, resistant). In practice, if the MIC for the bacterial isolate falls in the *susceptible category*, there is a greater likelihood of successful treatment (cure) than if the isolate were classified as resistant. It does not ensure success; drug failure is still possible owing to other drug or patient factors (for example, immune status, immaturity, or severe illness

Table 1
Susceptibility breakpoints for antimicrobials used in animals (CLSI M31-A3, 2008)

Antimicrobial	Susceptible (μg/mL)[a]	Resistant (μg/mL)[a]
Amikacin	≤16[b]	≥64
Ampicillin	≤0.25	≥0.5
Amoxicillin/Clavulanate	≤0.25/0.12	≥1/0.5
Cefazolin	≤2	≥8
Cefotaxime	≤8[b]	≥64
Cefpodoxime	≤2	≥8
Cephalothin[c]	≤2	≥8
Chloramphenicol	≤8[b]	≥32
Cefoxitin	≤8[b]	≥32
Ciprofloxacin	≤1[b]	≥4
Clindamycin	≤0.5	≥4
Difloxacin	≤0.5	≥4
Enrofloxacin	≤0.5	≥4
Erythromycin	≤0.5[b]	≥8
Gentamicin	≤2 (≤500 for Enterococci)	≥8 (≥500 for Enterococci)
Imipenem	≤1[b]	≥4
Marbofloxacin	≤1	≥4
Orbifloxacin	≤1	≥8
Oxacillin (veterinary)	≤0.25	≥0.5
Penicillin G (equine)	≤0.5	≥2.0
Rifampin	≤1[b]	≥4
Tetracycline[d]	≤4[b]	≥16
Ticarcillin	≤64 (*Pseudomonas*)[b] ≤16 (for others)[b]	≥128 ≥128
Trimethoprim/Sulfa	≤2/38[b]	≥4/76
Vancomycin	≤2 (Staphylococci)[b]	≥16 (Staphylococci)

[a] Values between the susceptible and resistant range are interpreted as "intermediate."
[b] Some of the breakpoints listed are derived from human standards listed in M100.
[c] Cephalothin is used to test for other first-generation cephalosporins (eg, cephalexin).
[d] Tetracycline is used to test for other tetracyclines (doxycycline and minocycline).
 Data from Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard. 3rd edition. CLSI document M31-A3. Wayne (PA): Clinical and Laboratory Standards Institute; 2008.

that compromises the action of antibacterial drugs), and interactions. If the MIC is in the *resistant category*, bacteriologic failure is more likely because of specific resistance mechanisms or inadequate drug concentrations in the patient. However, a patient with a competent immune system may sometimes eradicate an infection even when the isolate is resistant to the drug in the MIC test.

The *intermediate category* is intended as a buffer zone between susceptible and resistant strains. This category reflects the possibility of error when an isolate has an MIC that borders between susceptible and resistant. If the MIC value is in the intermediate category, therapy with this drug at the usual standard dosage is discouraged because there is a good likelihood that drug concentrations may be inadequate for a cure. However, successful therapy is possible when drug concentrates at certain sites (in urine, or as the result of topical therapy, for example) or at doses higher than the

minimum effective dose listed on the label. Prescribing guidelines for some antimicrobials allow for an increase in dose when susceptibility testing identifies an organism in the intermediate range of susceptibility. For example, fluoroquinolone antimicrobials have been approved with a dose range that allows increases in doses when susceptibility testing identifies an organism in the *intermediate* range of susceptibility. In these cases, higher drug concentrations make a cure possible if the clinician is able to safely increase the dose above the minimum labeled dose. (For example, in the case of enrofloxacin in dogs, this would be equivalent to a dose of 10–20 mg/kg/d, rather than the minimum dose of 5 mg/kg/d.)[17]

MIC data should not be used in isolation, but by coupling the MIC from a laboratory report with CLSI breakpoints and other important information, such as the virulence of the bacteria and the pharmacology of the antibiotics being considered, the clinician can make a more informed selection of an antibacterial drug.

Does the Susceptibility Test Provide Tissue-Specific Interpretation?

The susceptibility interpretation is based on plasma/serum concentrations. No tissue-specific interpretation can be provided that accounts for differences in drug distribution among tissues. For example, even though it is anticipated that many antibiotics concentrate in the urine, which may be beneficial for treating a urinary tract infection, the susceptibility interpretation is based on achieving adequate concentrations in the blood. (There are 2 exceptions to this because amoxicillin and amoxicillin-clavulanate interpretations allow for high concentrations in urine.) One should not assume that concentrations in urine, even when they are high due to concentration by the nephrons, are sufficient to eradicate infections of the urinary tract. Infections may involve the deeper layers of the mucosa, the renal tissue, or the prostate tissue. In these instances, it is the tissue concentration, which is correlated to the plasma concentration, that will be predictive of a bacteriologic cure.[18]

A frequent mistake in MIC interpretation is to compare the MIC with published tissue concentrations that are derived from whole-tissue homogenized samples.[19] Tissue concentration data are often published by pharmaceutical companies in their product information. These concentrations may be misleading because they may either underestimate or overestimate (depending on the drug's affinity for intracellular sites) the true drug concentration at the site of infection.

In most instances, the clinician should not be concerned with the question of whether or not there are tissue-specific susceptibility interpretations. For most tissues, antibiotic protein-unbound drug concentrations in the serum or plasma approximate the drug concentration in the extracellular space (interstitial fluid). This is because there is no barrier that impedes drug diffusion from the vascular compartment to extracellular tissue fluid.[20,21] There is really no such thing as "good penetration" and "poor penetration" when referring to most drugs in most tissues. Pores (fenestrations) or microchannels in the endothelium of capillaries are large enough to allow drug molecules to pass through unless the drug is restricted by protein binding in the blood. Tissues lacking pores or channels may inhibit penetration of some drugs (discussed later in this article).

If adequate drug concentrations can be achieved in plasma, it is unlikely that a barrier in the tissue will prevent drug diffusion to the site of infection as long as the tissue has an adequate blood supply. Clinicians should be concerned when treating tissues that have poor or impaired blood supply. Drug diffusion into an abscess or granulation tissue is sometimes a problem, because in these conditions, drug penetration relies on simple diffusion and the site of infection lacks adequate blood supply. In an abscess, there may not be a physical barrier to diffusion (that is, there is no impenetrable

membrane) but low drug concentrations are attained in the abscess and drug concentrations may be slow to accumulate.

In some tissues, a lipid membrane (such as tight junctions on capillaries) presents a barrier to drug diffusion. In these instances, a drug must be sufficiently lipid-soluble, or be actively carried across the membrane to reach effective concentrations in tissues. These tissues include the central nervous system, eye, and prostate. A functional membrane pump (p-glycoprotein) also contributes to the barrier. There also is a barrier between plasma and bronchial epithelium (blood-bronchus barrier).[22] This limits drug concentrations of some drugs in the bronchial secretions and epithelial fluid of the airways. Lipophilic drugs may be more likely to diffuse through the blood-bronchus barrier and reach effective drug concentrations in bronchial secretions.

Is Susceptibility Interpretation by CLSI Specific for Veterinary Species?

In past years, the veterinary diagnostic laboratories had to rely heavily on the CLSI interpretation from the human standards. There were not enough veterinary-specific interpretive criteria available to establish breakpoints for veterinary drugs and veterinary species. This is now changing. The next edition of CLSI M31 for veterinary drugs will be divided into 2 tables: one table for veterinary interpretive criteria, and a separate table for drugs that still rely on human standards for interpretation. In the past several years, CLSI has tremendously expanded the list of drugs for which there are veterinary-specific breakpoints. For companion animals, veterinary-specific MIC breakpoints have now been established for the 4 licensed fluoroquinolones: enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin, but not ciprofloxacin. There are also veterinary breakpoints for gentamicin, cefpodoxime proxetil, ampicillin/amoxicillin, amoxicillin-clavulanic acid, first-generation cephalosporins (cephalexin and cefazolin), and clindamycin (dogs only). Important changes were also made for the interpretation of *Staphylococcus* resistance. This is illustrated in more detail later in this article. Until other veterinary-specific breakpoints are established for other antibiotics used in companion animals, we will continue to rely on the human breakpoints for drugs such as amikacin, chloramphenicol, erythromycin, carbapenems (imipenem), penicillins, sulfonamides, potentiated sulfonamides, and tetracyclines. Revised breakpoints for some of these drugs may be available in the next year. But in the meantime, similarities in pharmacokinetics and pathogen susceptibilities between humans and animals allow for an acceptable approximation to extrapolate human breakpoints to animal situations for many drugs until veterinary-specific standards are available.

Veterinary-specific testing issues for Staphylococcus

The previous standards published by the CLSI (CLSI M31-A3, 2008) did not differentiate the interpretive criteria of *Staphylococcus aureus* from that of *Staphylococcus pseudintermedius or Staphylococcus intermedius*; however, this has been corrected in the new edition (M31-A4) to be published in 2013. This edition will indicate that the *S aureus* interpretive criteria uses an MIC breakpoint of greater than or equal to 4.0 μg/mL to define resistance. However, for non–*S aureus* isolates from animals, *Staphylococcus* spp should be considered resistant when the MIC is greater than or equal to 0.5 μg/mL. This interpretation differentiates *S pseudintermedius* from *S aureus*.[23] The current CLSI standard instructs laboratories to report non–*S aureus* isolates from animals that are oxacillin resistant as positive for *mecA*, or that produce PBP 2a, the *mec*A gene product. Laboratories should report *mecA*-positive and/or PBP 2a producing methicillin-resistant *Staphylococcus* as resistant to all other penicillins, carbapenems, cephalosporins (cephems), and β-lactam/β-lactamase inhibitor combinations, regardless of in vitro test results with those agents.

If the previous criteria of greater than or equal to 4.0 μg/mL is used, resistant staphylococci from animals may be misidentified. In the next published supplement of the CLSI standards, this recommendation will change to reflect this new evidence. Until then, diagnostic laboratories should adopt the recommendation that if any non-*aureus* coagulase-positive *Staphylococcus* isolated from animals has an MIC value greater than or equal to 0.5 μg/mL (corresponding to a zone diameter of ≤17 mm), it should be considered methicillin resistant, *mec-A* positive, and resistant to all β-lactam antibiotics. The cefoxitin disk is no longer recommended for testing *S pseudintermedius*, as it was in older editions of CLSI M31.

The need for current standards

These changes and updates in the interpretive criteria used to establish breakpoints illustrate the need for laboratories to use only the most updated document available for interpretation and standards. The previous edition of CLSI-VAST M31[1] will be replaced by a new supplement in 2013. Human breakpoints also are being revised. Because of concerns for misidentifying extended-spectrum β-lactamase–producing Enterobacteriaceae, the cephalosporin breakpoints have been lowered compared with previous criteria.[24,25] Carbapenem breakpoints also have been recently lowered.

HOW ARE BREAKPOINTS DERIVED?

The CLSI subcommittee for Veterinary Antimicrobial Susceptibility Testing (VAST) uses strict criteria to establish and evaluate breakpoints. Sponsors are required to follow guidelines provided by CLSI and must submit data to support a proposed breakpoint. The data include pharmacokinetic data in the target species, MIC distributions for the pathogens targeted, clinical data from the drug used under field conditions at the approved dose, and pharmacokinetic-pharmacodynamic (PK-PD) analysis, using Monte Carlo simulations[26] to show that at the approved dose the drug attains PK-PD targets for the labeled pathogen.[27]

ARE THESE STANDARDS, OR GUIDELINES?

The CLSI is a consensus-driven process and after approval by the subcommittee the standards become public documents. The consensus process involves the development and public open review of documents, revision of documents in response to discussion, and, finally, the acceptance of a document as a consensus standard or guideline. The CLSI M31 document used for culture and susceptibility testing[1] should be regarded as a public standard, not a guideline.

A *standard* is a document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

A *guideline* is a document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

PITFALLS OF SENSITIVITY TESTING

Susceptibility tests, even when appropriate standards are used, are not perfect. The 90/60 rule discussed earlier reminds us that we are treating animals with uncertain underlying disease and immune status. Individual animals may vary in the drug pharmacokinetics, response to treatment, and immune status. There are many reasons

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Box 1
Reasons for antimicrobial failure

- Incorrect diagnosis.
- Incorrect dose, route, frequency of administration.
- Depressed patient immunologic status.
- Poor owner compliance.
- Antibacterial drug resistance.
- Presence of pus and a foreign body.[28]
- Presence of a biofilm.[29,30]
- Pharmacokinetic drug interactions.
- Antibiotic antagonism.
- Pharmacokinetic problems: poor oral absorption, pharmacokinetic antagonism, intestinal metabolism of drug.

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why susceptibility tests may not accurately predict an outcome. Among these are the following:

Susceptibility tests assume equal plasma and tissue concentrations. As indicated earlier, a susceptibility test will *over*estimate the antimicrobial activity in tissues difficult to penetrate, such as the central nervous system, prostatic fluid, eye, and respiratory tract. On the other hand, susceptibility tests *under*estimate activity of topical treatments, local infusions, and antibacterials that concentrate in the urine.

Susceptibility tests underestimate activity at concentrations below MIC (sub-MIC). Some drugs exhibit antibacterial effects at concentrations below the MIC, but this cannot be measured under the conditions of the usual susceptibility tests.

Susceptibility tests usually do not test for antibiotic combinations and may miss potentially synergistic combinations (exceptions that are measured include trimethoprim-sulfonamides and amoxicillin-clavulanate).

Susceptibility tests cannot consider the local factors that may affect antimicrobial activity, such as pus, low oxygen tension, or poor blood flow to tissue.

There are not standards available for interpretation of all veterinary-specific drugs. Human standards are used for interpretation for many drugs and may not be equivalent.

Veterinarians are quick to attribute an unsuccessful antimicrobial treatment to a failure of the culture and susceptibility test. There are many reasons why antimicrobial treatment fails. When evaluating a patient that has failed to respond to therapy, one must consider any of the many factors that contribute to antibiotic failure, such as the factors listed in **Box 1**.

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