SUSCEPTIBILITY TESTING IN ANIMALS - HOW BREAKPOINTS ARE DERIVED AND INTERPRETATION OF SUSCEPTIBILITY DATA.
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SUSCEPTIBILITY TESTS

Antibacterial susceptibility testing is not necessary for every case in veterinary medicine because the majority of cases can be treated empirically. But performed properly, antibiotic susceptibility results are helpful to select proper antibacterial therapy for patients with refractory infections, seriously ill animals, or when resistant strains of bacteria are suspected. When MIC data is available, the test also is helpful to monitor trends in antibiotic activity. In order for those results to be reliable, appropriate test methods and quality control procedures should be performed by the laboratory. The laboratory should adhere to published standards (CLSI 2007). Factors such as depth of agar, size of inoculum, and time of incubation can affect the test result if these are not performed according to a standard. The standards are available from the Clinical and Laboratory Standards Institute – CLSI (http://www.clsi.org/) (formerly NCCLS – the National Committee for Clinical Laboratory Standards). 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.

Agar Disk Diffusion Test

The traditional and older method for performing the susceptibility test has been the agar-disk-diffusion-test (ADD test), also known as the Kirby-Bauer test. The Kirby-Bauer test measures inhibition of bacterial growth around an antimicrobial-impregnated disk that has been placed on a culture plate. In this test, a large zone of inhibition corresponds to a high degree of susceptibility. This test is performed on a fixed dose of drug in the paper disk, rather than a range of concentrations. Standards for concentrations of drug in each disk are listed by CLSI. 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 a MIC value.

Dilution Test (MIC Determination)

The MIC (minimum inhibitory concentration) is the lowest drug concentration that inhibits the growth of a bacterial isolate. The MIC drug concentration does not kill the bacteria; it is inhibitory. The lethal concentration – the minimum bactericidal concentration (MBC) – is rarely measured in diagnostic laboratories because it is a more difficult and time-consuming assay to perform. 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 two-fold dilutions of drug to which is added a standardized inoculum that is incubated for a prescribed time. 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, etc. 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 lowest value. Like the agar-disk-diffusion test, the
dilution test should be performed according to strict procedural standards, including quality control, such as those in CLSI (NCCLS) documents M31A2 (2004).

INTERPRETIVE CRITERIA FOR SUSCEPTIBILITY TESTS

Once an MIC for a bacterial isolate is determined for a particular antibiotic, it is then compared to published breakpoints from the CLSI (NCCLS) that indicate whether it is Susceptible, Resistant or Intermediate. These breakpoints have been submitted by the drug sponsor and are evaluated by a panel. 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.

MIC breakpoints are specific to the drug, but for some drugs there may be unique breakpoints established for different organisms. For example, for enrofloxacin the susceptible breakpoint for bovine respiratory bacteria is less than for canine bacteria (< 0.25 µg/mL vs < 0.5 µg/mL). Ceftiofur has a breakpoint of ≤ 2.0 µg/mL for cattle and swine respiratory pathogens, but a breakpoint of ≤ 0.25 µg/mL for equine respiratory pathogens. Human-labeled cephalosporins have a breakpoint of ≤ 8.0 µg/mL for most isolates, which is much higher than those listed for cefiofur. The CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) uses three criteria to determine veterinary-specific breakpoints (CLSI M37): 1) MIC data from populations of bacteria collected in the field (population distribution) or in clinical efficacy studies, 2) pharmacokinetic-pharmacodynamic properties (such as bactericidal or bacteriostatic activity, time- or concentration-dependency) appropriate for the antibiotic, and 3) clinical efficacy of the drug during field trials at the standard dose.

How many drugs have breakpoints?

For companion animals, veterinary-specific MIC breakpoints have been established for only the four licensed fluoroquinolones (enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin), gentamicin, cefpodoxime proxetil, and clindamycin (dogs only). For large animals, interpretive criteria exist for ceftiofur (horses and cattle), tulathromycin, danofloxacin, enrofloxacin, florfenicol, spectinomycin, tilmicosin, gentamicin (horses only), and ampicillin (horses only). Until veterinary-specific breakpoints are established for other antibiotics, we will continue to rely on the human breakpoints for drugs such as amikacin, amoxicillin-clavulanate, other cephalosporins, chloramphenicol, erythromycin, carbapenems (imipenem), penicillins, sulfonamides, potentiated sulfonamides, and tetracyclines. Similarities in pharmacokinetics and pathogen susceptibilities between humans and animals allow for a reasonable approximation to extrapolate human breakpoints to animal situations for some drugs until veterinary-specific standards are available. However, there may be infections for which human-derived breakpoints are not appropriate for interpretation. For example, if one is using ampicillin-trihydrate injection (Polyflex), the concentrations are low compared to the sodium salt injection and oral dose formulations used in people (Gehring et al, 2005). Subsequently, an organism considered “sensitive” by a human standard breakpoint, may not be susceptible in vitro if the injectable ampicillin trihydrate is used.

CATEGORIES OF SUSCEPTIBILITY

After a laboratory determines an MIC or zone, it may use the CLSI “SIR” classification for breakpoints (S, susceptible; I, intermediate, or R, resistant) to assign susceptibility. If 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 assure success; drug failure is still possible owing to other drug or patient factors (e.g., underlying disease, or immunosuppression), and interactions. If the isolate 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.

The *Intermediate* category is intended as a buffer zone between susceptible and resistant strains. This category reflects the possibility of error when an isolate borders between susceptible and resistant. The intermediate category is not intended to mean “moderately susceptible.” 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 concentrations 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. 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 to 20 mg/kg/day, rather than the minimum dose of 5 mg/kg/day.)

**Note:** The CLSI no longer uses the “flexible” category for fluoroquinolones when the MIC for these drugs falls in the *Intermediate* category.

### TISSUE DRUG CONCENTRATIONS

At times, veterinarians would like information on the tissue concentration rather than the plasma concentration or would prefer that the MIC breakpoints were interpreted according to the ability (or inability) of a drug to penetrate the tissue that is affected by the bacteria. This is not possible. The penetration into a tissue is not taken into account when interpretive criteria are used to determine breakpoints. However, some drugs attain low concentrations in tissues because of a barrier to penetration such as in the eye, prostate, or central nervous system. Otherwise, drug concentrations at the site of infection (interstitial space) are correlated with the plasma concentrations (Cars, 1991, Nix et al, 1991). Fenestrations in capillaries allow rapid equilibrium between unbound plasma drug concentrations and the fluid of tissues – where the infection occurs. A frequent mistake in MIC interpretation is to compare the MIC with published tissue concentrations that are derived from whole-tissue homogenized samples. Tissue concentration data is 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.

### Interpretation of Susceptibility for Urinary Tract Infections

Some laboratories have reported susceptibility breakpoints that are higher for urinary tract infections than for infections at other sites. Urinary tract pathogens do not have different breakpoints because these have never been standardized by the CLSI (exception for ampicillin, M31 A3). Thus, MICs should be interpreted using the same criteria as discussed above. One shouldn’t 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 (Frimodt-Møller, 2002).

REVISING INTERPRETIVE CRITERIA

The Generic Working Group (GWG) of the CLSI has been working to revise the breakpoints for older drugs. Drugs that do not have CLSI approved standards established include drugs such as penicillins, tetracyclines, aminoglycosides, potentiated sulfonamides, and several cephalosporins. The CLSI document M-37 provides guidelines for establishing interpretive criteria for older drugs such as these (M 37, section 3.7). The data needed for developing interpretive criteria include: 1) MIC data from a population of clinically relevant isolates (for example, collected in a field study or survey, 2) pharmacokinetic data for the drug in the species of interest, 3) pharmacokinetic-pharmacodynamic (PK-PD) indices for the drug or class, and 4) efficacy data. The data that has been the most difficult for the GWG to obtain has been efficacy data. In many instances these drugs are old and although efficacy is accepted by veterinarians, it may not have been established in controlled clinical studies. Regulatory approval of a drug has been considered for sufficient proof of efficacy. Pharmacokinetic has been available through published studies and the GWG also has used the FARAD database as a source of pharmacokinetic data for some drugs (Craigmill et al, 2004). With pharmacokinetic data and a range of MIC values, simulated plasma concentrations have been derived. The GWG then used Monte Carlo simulations to determine the probability of meeting PK-PD indices for these drugs (Ambrose 2006). Criteria used were: ampicillin (β-lactam) time above MIC (T>MIC) for 50% of the dosing interval, tetracycline AUC/MIC ratio > 40, and gentamicin CMAX/MIC > 10. If PK-PD criteria can be met for a MIC value in an acceptable proportion of the treated population as shown through Monte Carlo simulations, that MIC value will be considered within the Sensitive range.

Using the process described above, the GWG has revised the breakpoints for three classes of drugs (see below). Drugs listed for future evaluation include: 1<sup>st</sup> generation cephalosporins, amoxicillin-clavulanate and penicillin G.

<table>
<thead>
<tr>
<th>Revised Breakpoints for CLSI M31-A Table 2</th>
<th>Breakpoint MIC µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
</tr>
<tr>
<td>Previous breakpoint (human)</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Dogs 10 mg/kg q24h, IM.</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Horses 6.6 mg/kg q24h, IM.</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Tetracycline (class representative)</td>
<td></td>
</tr>
<tr>
<td>Previous breakpoint (human)</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Cattle (for BRD) for oxytetracycline at 20 mg/kg IM</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Swine (for SRD) for oxytetracycline at 20 mg/kg IM</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Ampicillin (including amoxicillin)</td>
<td></td>
</tr>
<tr>
<td>Previous breakpoint (human) Enterobacteriaceae</td>
<td>≤ 8</td>
</tr>
<tr>
<td>Previous breakpoint (human) Staphylococcus</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td>Dogs (SST) Staphylococcus, for amoxicillin 22 mg/kg q12h, oral</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td>Dogs Enterobacteriaceae, for amoxicillin 22 mg/kg q12h, oral</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td>Horses (respiratory) Streptococcus 22 mg/kg IM, q12h</td>
<td>≤ 0.25</td>
</tr>
</tbody>
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REFERENCES


KEY WORDS
Susceptibility, Antibiotics, MIC, PK-PD, Resistant, Ampicillin, Tetracycline, Gentamicin