

RATIONAL USE OF BACTERIAL CULTURE/SENSITIVITY DATA

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INTRODUCTION

Bacterial susceptibility testing information is often treated as if it came from a magic "black box" that automatically takes into account patient and pathogen species and location of infection. The results, consisting of S, I, and R, are then used to select an antimicrobial that is put into clinical use with one's favorite dosing regimen or an apparently universal regimen pulled from a reference.

In reality, bacterial susceptibility testing data must be interpreted based on an understanding of the testing methods, the interpretive criteria applied to derive S, I, and R, (the breakpoints) and how different classes of antimicrobials are optimally dosed in relation to these breakpoints. This paper presents the basic components of susceptibility testing and a brief overview of the pharmacodynamic principles needed to apply susceptibility results in a clinical setting.

TESTING METHODS

The microbiological methods for performing antimicrobial susceptibility testing by either Kirby-Bauer (disk diffusion) or micro well dilution methods are contained in the National Committee for Clinical Laboratory Standards publication (NCCLS) M31-A, *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard*. The NCCLS is an international, interdisciplinary, nonprofit, standards-developing and education organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. The M-31A document was developed by the Veterinary Antimicrobial Susceptibility Testing subcommittee of the NCCLS and contains information on:

1. performance standards for disk diffusion antimicrobial susceptibility tests for bacteria that grow aerobically and anaerobically;
2. zone size interpretive standards, equivalent MIC breakpoints, and methodologies for disk diffusion susceptibility tests;
3. indications and methodologies for performing broth and agar dilution susceptibility tests;
4. quality control guidelines; and
5. additional information including selection of antimicrobial agents for routine testing and reporting, and beta-lactamase tests.

To purchase a copy of the M31-A document, contact the NCCLS at 610-688-0100 or Exoffice@nccls.org. The mission of this organization and document is to provide standardized testing methods. The first step in evaluating susceptibility results from the laboratory you use is to ask if they are adhering to NCCLS guidelines, including the use of NCCLS zone interpretive criteria or breakpoints. This document is especially critical if you are doing in-house susceptibility testing utilizing Kirby-Bauer disk diffusion. Deviation from standardized testing and interpretation procedures will result in unreliable susceptibility results, which are worse than no results at all!

THE DIFFERENCE BETWEEN A MIC AND A BREAKPOINT

Susceptibility test interpretation is based on breakpoints for susceptible "S", Intermediate "I", and Resistant "R", designations. A breakpoint is a specific MIC value, but a MIC is not always a breakpoint.

An MIC is the concentration of an antimicrobial that inhibits growth of a bacterial isolate *in vitro* under standardized conditions. The culture is not sterilized. Samples removed from this culture and placed in an antimicrobial-free medium will grow again. An antimicrobial concentration that inhibited growth of 50% of the isolates tested is termed the MIC₅₀. The concentration that inhibited growth of 90% of the isolates is termed the MIC₉₀. Reporting an MIC for an organism indicates what happened in the laboratory, with no prediction of clinical efficacy implied.

A breakpoint is used to predict clinical efficacy based on *in vitro* susceptibility results. Breakpoints for use in veterinary medicine are specified in the NCCLS M31-A document. A "susceptible" breakpoint is the MIC at which clinical efficacy is expected. An "intermediate" result is an indicator that the MIC of the organism is on the edge of the bacterial population for which clinical efficacy is predicted. A "resistant" result indicates that the MIC of the tested organism is high enough that clinical efficacy is unlikely. Now, the biggest take home point of this presentation.

VALIDATED BREAKPOINTS ARE SPECIFIC FOR AN ANTIMICROBIAL, A SPECIFIC REGIMEN OF THE ANTIMICROBIAL (DOSE, ROUTE, DURATION, FREQUENCY), AN ANIMAL SPECIES (THIS MAY INCLUDE AGE OR ANOTHER SUBCLASSIFICATION), A SPECIFIC PATHOGEN, AND A SPECIFIC DISEASE ENTITY.

To establish a veterinary breakpoint, the NCCLS VAST subcommittee works in cooperation with the sponsoring company. The specific requirements are published in NCCLS M37-A document, *Development of in vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents, Approved Guideline*. The committee considers data on MIC distribution of the pathogen population, clinical trial data with the label regimen, and pharmacokinetic/pharmacodynamic data prior to setting a breakpoint. These data are specific to the application criteria listed above. For antimicrobials where this information is not available for a veterinary application, the NCCLS VAST Committee has adopted breakpoints from human medicine that are reasonable for veterinary applications. It is important to know the applications for which veterinary breakpoints have been validated by the NCCLS VAST subcommittee. Table 1 lists all breakpoints included in the M31-A document that have been validated for veterinary applications. Note that different applications for a drug may have different breakpoints, although these breakpoints are not detailed here.

Table 1: Antimicrobials with NCCLS Veterinary Antimicrobial Susceptibility Testing subcommittee validated breakpoints and interpretive criteria

Ceftiofur	Bovine (<i>Pasteurella haemolytica</i> , <i>Pasteurella multocida</i> , <i>Haemophilus somnus</i>) and swine respiratory disease (<i>A. pleuropneumoniae</i> , <i>P. multocida</i> , <i>S. choleraesuis</i> , <i>Strep. suis</i>)
Tilmicosin	Bovine respiratory disease (<i>Pasteurella haemolytica</i> , the M31-A document notes that these break points also a I to <i>Pasteurella multocida</i>)
Enrofloxacin	Bovine respiratory disease (<i>Pasteurella haemolytica</i> , <i>Pasteurella multocida</i> , <i>Haemophilus somnus</i>), Canine and feline dermal, URI, UTI , (Gram-negative enteric bacilli, <i>Staphylococcus</i> spp., other susceptible organisms), Chickens and Turkeys (<i>Pasteurella multocida</i> , <i>Escherichia coli</i>)
Sarafloxacin	Colibacillosis in broiler chickens and growing turkeys
Pirlimycin	Clinical and subclinical mastitis in dairy cattle. (<i>Staph. aureus</i> , <i>Strep. agalactiae</i> , <i>Strep. dysgalactiae</i> , <i>Strep. uberis</i>)
Penicillin/novobiocin	Bovine mastitis (<i>Satph. aureus</i> , <i>Strep. agalactiae</i> , <i>Strep. dysgalactiae</i> , <i>Strep. uberis</i>)
Amoxicillin/clavulanic acid	Canine dermal/UTI , (separate interpretive criteria for <i>Staphylococci</i> and other organisms)

BREAKPOINTS VALIDATED BY THE NCCLS BUT NOT YET INCLUDED IN THE M31.A DOCUMENT

Spectinomycin hydrochloride	Bovine respiratory disease (<i>Pasteurella haemolytica</i> , <i>Pasteurella multocida</i> , <i>Haemophilus somnus</i>)
Florfenicol	Bovine respiratory disease (<i>Pasteurella haemolytica</i> , <i>Pasteurella multocida</i> , <i>Haemophilus somnus</i>)

CONFIDENCE IN BREAKPOINTS

Susceptibility results for label applications in Table 1 should be thought of as giving the most reliable estimates of the potential for clinical efficacy. The breakpoints have gone through a validation process and the S, I, or R reported on your isolate has much more predictive value than other situations. What are the other situations? A diagnostic laboratory will use the best breakpoint available for the isolate you have sent in. For example, a typical food animal panel would include ceftiofur with the breakpoints of ≤ 2 mcg/ml (susceptible), ≤ 4 mcg/ml (intermediate), and ≥ 8 mcg/ml for the applications outlined in Table 1. If you submitted an enteric *Escherichia coli* or *Salmonella* spp., this isolate would be tested against the same breakpoints, resulting in an S, I, or R being reported. You should know that this result is not based on validated breakpoints and though it may be used as a guide, it should not be interpreted in the same light as a ceftiofur susceptibility report for a *Salmonella choleraesuis* isolate from swine respiratory disease. Another situation is where the antimicrobial in question has no validated veterinary breakpoints. Table 2 presents antimicrobials for which the susceptibility testing interpretive criteria included in the M31-A document have been adapted from human NCCLS interpretive criteria.

Table 2: Antimicrobials with interpretive criteria included in the M31.A document for which the interpretive criteria are adapted from human interpretive criteria

Beta-lactams	Penicillins - ampicillin, oxacillin, penicillin G, ticarcillin Cephalosporins - cephalothin, cefazolin, cefoxitin Carbapenems - imipenem
Aminoglycosides	amikacin, gentamicin, kanamycin
Macrolides	Erythromycin
Tetracyclines	Oxytetracycline
Lincosamides	Clindamycin
Others	Chloramphenicol, rifampin, sulfonamides, vancomycin, trimethoprim/sulfamethoxazole

Susceptibility testing based on these interpretive criteria can be a valuable part of designing and antimicrobial regimen.

However, one cannot place the same level of confidence in the resulting S, I, or R as if they were validated for veterinary use. Utilizing susceptibility results for antimicrobials in Table 2, or results for nonvalidated applications for those in Table 1 require knowing the MIC values associated with the susceptibility result and coupling this MIC with pharmacokinetic and pharmacodynamic knowledge about the antimicrobial.

LINKING SUSCEPTIBILITY RESULTS TO REGIMEN DETERMINATION

First, it is necessary to convert the susceptibility result to a "worst case" MIC for the organism. For example, a susceptible result for ceftiofur using the breakpoints discussed above would mean that the MIC of the organism is ≤ 2 mcg/ml. The MIC could actually be 0.06 mcg/ml, but if the lab is using zone interpretive criteria or dilutional testing utilizing only the breakpoint values, there is no way of knowing. Some diagnostic laboratories are now conducting extended-range susceptibility testing using micro well dilution systems. These systems are utilizing test plates with additional dilutions below the susceptible breakpoint to give a better indication of the actual MIC of the organism if it tests as susceptible. Some laboratories use a system designed to extrapolate back from a zone diameter to a specific MIC. The laboratory should be questioned as to the validation parameters for the specific antimicrobial/pathogen combination that has been tested. The Kirby-Bauer zone interpretive criteria in the M31-A document are linked to an MIC in the form of breakpoint criteria.

Next, the optimal pharmacodynamic presentation of the antimicrobial to the pathogen should be considered. There are numerous review and research articles available on the subject of antimicrobial pharmacodynamics, some of which are cited below.¹⁻⁵ For purposes of discussion; antimicrobials may be divided into two main groups.

1. Concentration-dependent killing: aminoglycosides, fluoroquinolones, metronidazole. These antimicrobials demonstrate improved efficacy as the concentration of the antimicrobial in relation to the pathogen MIC is increased. Antimicrobial concentration may be expressed as the area under the plasma concentration curve (AUC) or as the peak concentration (C_{max}). For fluoroquinolones, a C_{max}:MIC ratio of 8-10: 1 has been correlated with the best suppression of selection for isolates with resistance mutations in some studies, while a AUC to MIC ratio (the AUIC) of ≥ 125 has been most closely correlated with efficacy. A similar C_{max}:MIC ratio is an efficacy target for the Aminoglycosides. The Q24H dosing regimens now common for the aminoglycosides and fluoroquinolones are based on concentration dependent killing coupled with a significant post-antimicrobial effect (PAE) against many pathogens. For the aminoglycosides, low trough concentrations between dosing are also required to minimize toxicity.

2. Time of exposure-dependent killing: Beta-lactams, macrolides (some studies classify the azolides and ketolides as concentration dependent), lincosamides, vancomycin, quinupristin/dalfopristin, tetracyclines. In this group, exceeding concentrations above 45 times the pathogen MIC gives little benefit in efficacy. For Beta-lactams, the main focus of constructing a regimen is time that the plasma or serum concentration curve exceeds the pathogen MIC. A prolonged PAE is expected for susceptible *Staphylococcus* isolates, so 50% of the dosing interval is often a target for time > MIC. However, for many *Streptococcus* isolates and most Gram (-) isolates, the lack of a significant PAE has led to a recommendation of a time > MIC target of 70-80% for these isolates. From this discussion it is apparent how important the elimination half time is for constructing a Beta-lactam dosing regimen. Although the tetracyclines and quinupristin/dalfopristin are not considered to exhibit concentration-dependent killing, the AUIC has been demonstrated to be a better predictor of efficacy than the time above MIC.

Before accepting the above guidelines as definitive science, it is important to realize that the type of killing and the best presentation of the antimicrobial may vary depending on the pathogen genus, species, and even the isolate! When you throw in the fact that the MIC of a pathogen may differ in different substrates (milk ultra filtrate vs. plasma) and that normal laboratory variation is considered to be +/- one dilution for MICs, then it is clear that all of these techniques are useful for

eliminating unreasonable antimicrobial regimens, but not for pinpointing the exact ideal regimen. Susceptibility testing results may be applied to label regimens where validated breakpoints are available. Outside of this relationship, you need to know what the S, I, or R is telling you and then be able to couple this information with an understanding of the pharmacodynamics of the antimicrobial selected for therapy. The final step is to utilize pharmacokinetics reported for the species of the patient to construct a reasonable regimen and monitor the outcome.

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