General Sessions:

Moderator: Lloyd Knight

Preparing for a Revolution in Antimicrobial Susceptibility Testing

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Introduction

Our understanding of the pharmacology of antimicrobials has taken major leaps in the past few years, as has the science of antimicrobial susceptibility testing. In addition, the pathogenic bacteria vying for existence have started to regain the edge they had before the introduction of antimicrobials. The result is that we need to revise our vision of how to use antimicrobials. Antimicrobial therapy should be viewed like cardiac therapy: tailored to the animal, drug and disease. The days of one drug/one dose/all diseases are over.

But how can you as practitioners prepare for these changes? As with any diagnostic test, an understanding of how susceptibility testing works is critical for you to make reasonable decisions based on the results. This is not meant to be a discussion of microbiology, but rather the application of microbiology to pharmacology. Most veterinarians are familiar with the disc diffusion (Kirby-Bauer, BIOMIC system) and tube or plate dilution (e.g., Sensititre system) methods of susceptibility testing, but a quick review is in order.

Discussion

Methods of susceptibility testing

The Kirby-Bauer method relies on antimicrobial-containing discs that are placed on a plate streaked with the isolated bacteria. After incubation, the so-called "zone of inhibition" is measured around each disc, with the zone sizes correlating with Minimum Inhibitory Concentrations (MICs). (MICs are correlated with expected clinical outcome; see discussion below.) Critical points regarding this method of– testing are: the bacteria must first be isolated from the diagnostic sample; only one isolate may be tested at a time; and methods must be scrupulously followed such as agar type, incubation period, and so on to permit accurate reading of results. Diagnostic laboratories perform quality assurance on a regular basis, including the use of stock organisms from the ATTC with known susceptibilities, and these procedures should be utilized in an in-house microbiology lab. Another caveat: "Dangerously misleading results can occur when certain antimicrobial agents are tested against specific organisms. These combinations include, but are not limited to: first- and second-generation cephalosporins and aminoglycosides against Salmonella spp.; ...cephalosporins against Listeria spp."[3]

The plate or tube-dilution method uses agar containing a given concentration of antimicrobial on which the organism is grown. The lowest concentration at which growth is inhibited is the MIC of the organism. This concentration can then be utilized in the construction of a regimen (dose, frequency, duration, withdrawal time). To simplify the testing and to minimize the materials required for testing–rather than testing a range of concentrations–most laboratories have utilized 1 or 2 concentrations known as "breakpoints", such that organisms that grow below the concentration are considered susceptible, and those above are resistant. In the case of disc diffusion methods of testing, relating MICs to zone diameter is usually performed via the error-rate bounding classification method.[2] This method allows for the selection of zone diameters based on allowable percentages of false susceptible and false resistant classifications. False susceptible results are considered "very major errors," since they could result in using an antimicrobial that is not effective; false resistant are

considered "major errors." "Minor errors" are isolates that are classified either resistant or susceptible by zone diameter but are intermediate by MIC. Error-rate bounding classification is illustrated in Figures 1 and 2 for a mock organism, adapted from Metzler and DeHaan.[2] Figure 1 is the distribution of the population of isolates among MICs. Figure 2 is the distribution of the MIC to zone diameter correlation with false susceptible and false resistant areas bounded by the MIC cutoff for resistance, and the 2 zone diameters that correspond to "susceptible" and "resistant."

And how are the breakpoints determined? This information is developed by microbiologists and clinicians working with the National Committee on Clinical Laboratory Standards (NCCLS). In theory, breakpoints are put together using 3 major pieces of information: pharmacokinetics of the antimicrobial in the species in question, historical information on the MICs of the organism in question, and clinical trials of the drug in the treatment of the disease caused by the organism. The susceptibility data are plotted on a scattergram correlating MICs with zones of inhibition as mentioned above, and the population of organisms in question is examined for clusters. These clusters can be related to the pharmacokinetics of the antimicrobial to evaluate the clinical likelihood of various concentrations. For example, if the serum concentration is not expected to reach the MIC of an organism, it would likely be categorized as resistant, although tissue concentrations of antimicrobial may be utilized if clinical correlations can be made.[5] The result is breakpoints that use an *in vitro* laboratory result to predict a clinical response. An important point should be made clear: breakpoints are established for a given organism in a given species for a given regimen. The practical implication of this is that if you change the regimen, or you use the drug in a different species or for a different application than the breakpoint was established, your clinical results may be very different than expected.

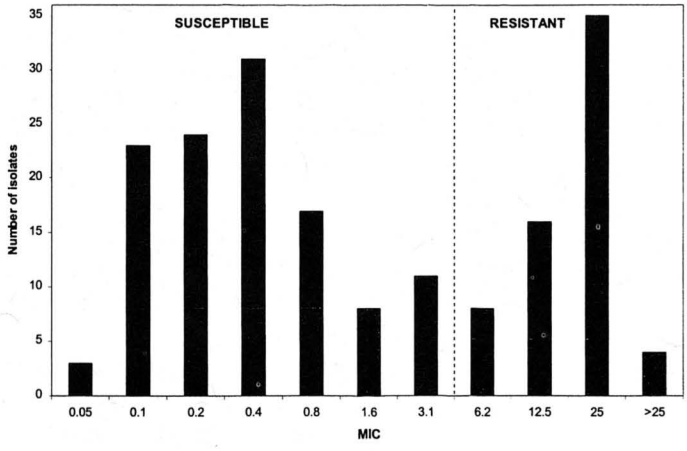

Figure 1. Mock organism–Number of isolates by minimum inhibitory concentration.

The drugs for which breakpoints have been established in cattle are in Table 1. Considerable progress has been made by the Veterinary Subcommittee of the NCCLS on establishing breakpoints. However, the absence of veterinary-derived breakpoints for antimicrobials commonly used in bovine practice such as penicillin, oxytetracycline, ampicillin and erythromycin should be noted. For those drugs, human-derived breakpoints are used. To illustrate the errors that can be encountered using these breakpoints, the pharmacokinetics of oxytetracycline found in 1 study are shown in Figure 3. The breakpoints are shown on this graph, and it becomes clear that a veterinary pathogen could be falsely labeled "susceptible" by the lab. One important result of this discrepancy is that the use of susceptibility results alone may not be justification for extra-label use.

Changes in susceptibility testing

To combat this problem, at least one commercial plate dilution testing system, Sensititre, has made a major change in the testing plates available to diagnostic labs. Rather than using 2 or 3 wells representing breakpoints, they have developed plates with extended dilutions, allowing the practitioner or the diagnostician to make the call of "susceptible" or "resistant," rather than relying on S, I and R (see Table 2). Practically speaking, the laboratory may just give the results in terms of minimum inhibitory concentrations, rather than that the pathogen was susceptible. The responsibility now becomes the practitioner's to understand what the MICs mean and to track MICs and clinical case response. This is an area that will require vigilance on the part of practitioners.

Help is on the way in dealing with these changes in susceptibility testing and reporting in the decision support system being developed by collaborators at the Iowa State University, Virginia Tech, and Mississippi

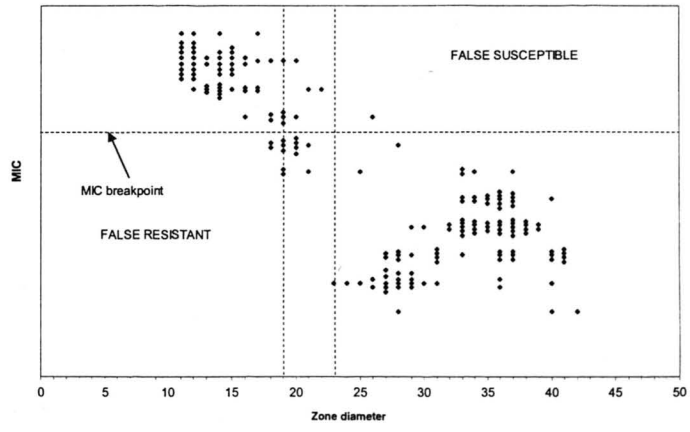

Figure 2. Mock organism–Comparison of zone diameters and minimum inhibitory concentration.

Table 1. Drugs for which breakpoints have been established for cattle by the NCCLS[3].

Drug	Indication	Organisms
Ceftiofur	Bovine Respiratory Disease	*Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus*
Tilmicosin	Bovine Respiratory Disease	*Pasteurella haemolytica*
Enrofloxacin	Bovine Respiratory Disease	*Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus*
Florfenicol	Bovine Respiratory Disease	*Pasteurella haemolytica, Pasteurella multocida*
Penicillin/ novobiocin	Mastitis	*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* Other organisms
Pirlimycin	Mastitis	None specified
Spectinomycin sulfate	Bovine Respiratory Disease	*Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus*

State University colleges of veterinary medicine. The system has been described in detail.[1] The role of the Veterinary Antimicrobial Decision Support system is to provide information and decision support in developing antimicrobial regimens particularly for extra-label use, whether the veterinarian is treating empirically or has susceptibility results in hand.

One example of the information being used in the VADS system is a study such as that performed by Shpigel and others[4]: Cows with field cases of clinical mastitis were treated with a trimethoprim-sulfa combination, and recovery rates were compared between organisms that were susceptible and resistant *in vitro*. The investigators found that 89.1% of cows recovered if the organism was susceptible, and 74.6% recovered if the organism was resistant, with an odds ratio of recovery from a sensitive compared to a resistant organism being 2.75. (The breakpoints were those derived in humans.)

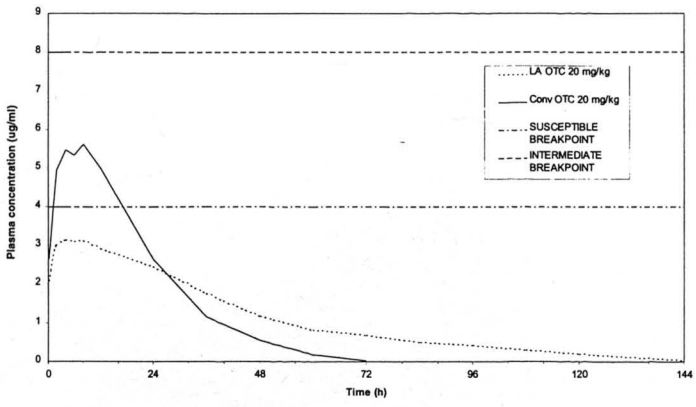

Figure 3. Plasma concentrations of long-acting vs. conventional oxytetracycline IM in steers (188-212 kg; 414-466 lb) (adapted from Davey *et al*, 1985).

One of the other pieces of the puzzle in designing regimens is an understanding of how antimicrobials work and how best to apply them. One of the major areas of interest of the VADS system is in the pharmacokinetics of antimicrobials, since the way they are handled by the body has a large impact on their ability to inhibit bacterial growth.

Practical pharmacokinetics

A basic understanding of pharmacokinetics requires the review of only a few simple concepts. Pharmacokinetics are used to describe how a drug is absorbed, distributed, metabolized and excreted, i.e., how the drug moves through the body. One method of graphically displaying the pharmacokinetic parameters is the plasma concentration curve. Figures 4 and 5 show example curves: one for intravenous (IV) administration and one for intramuscular (IM)/subcutaneous (SQ)/per os (PO) administration. The major difference between the two graphs is that IV drugs are immediately available in the serum, whereas the other administration routes require absorption into the serum/plasma. Absorption kinetics may become a significant issue for drugs administered IM, SQ or PO. With some preparations of drugs, such as long-acting products, the rate of absorption may be slower than the rate of elimination.

Table 2. Extended dilutions of antimicrobials (μg/ml) commercially available in the Sensititre® plates from Trek Diagnostic Systems, Inc., Westlake, Ohio.

Antimicrobial	Also tests for	Food Animal	Mastitis
Ampicillin	Amoxicillin	0.25-16	0.12-8
Apramycin		4-32	
Ceftiofur		0.5-8	0.5-4
Cephalothin			2-16
Chlortetracycline		0.5-8	
Clindamycin	Lincomycin	0.25-2	
Enrofloxacin		0.12-2	
Erythromycin		0.25-4	0.25-4
Florfenicol		0.25-8	
Gentamicin		1-8	
Neomycin		4-32	
Oxacillin			2-4
Oxytetracycline		0.25-8	
Penicillin		0.12-8	0.12-8
Penicillin/Novobiocin			1-8
Pirlimycin			0.5-4
Spectinomycin		8-64	
Sulfachlorpyridazine		32-256	
Sulfadimethoxine		32-256	32-256
Sulfathiazole		32-256	
Tetracycline			1-8
Tiamulin		4-32	
Tilmicosin		4-32	
Trimethoprim/ Sulfamethoxazole		0.5-2	
Tylosin tartrate		2.5-20	

These drugs display flip-flop kinetics: elimination of the drug is limited by the rate of absorption. Typically, these drugs will reach a lower peak than the same product administered IV, and the drugs will exhibit a much longer T_{max}, that is, it will take longer to reach the highest concentration.

The terms used to describe drug movement through the body include C_{max}, T_{max}, AUC, $T_{1/2}$, MRT, and Vd. $\mathbf{C_{max}}$ is the maximum serum concentration reached; units are usually μg/ml, meaning μg of drug per ml of serum. Serum concentrations are the most common and easiest way to measure how much drug is in the body at a given time if we know the volume of distribution. $\mathbf{T_{max}}$ is the time at which the maximum serum concentration is reached; units are usually minutes or hours. **AUC** (Area under the curve) is a mathematical description of how much total drug is available in the serum or plasma over the time period measured. The 2 most common ways we talk about AUC are $AUC_{0-\infty}$ and AUC_{0-24}. $AUC_{0-\infty}$ is an estimate of the total amount of available drug in the body; AUC_{0-24} is the amount of drug available for the first 24 hours after dosing. The units for AUC are mg*ml/hr. $\mathbf{T_{1/2}}$, or apparent elimination half-life, is the amount of time it takes for the serum concentration of drug to be reduced by half. What this means is that for most drugs, which exhibit first order kinetics, a constant fraction or proportion of the drug is eliminated per time period. It takes about 7 half lives for 99% of a drug to be eliminated. In some references or abstracts, the half-life will not be reported but the elimination rate constant (K_{el}) will be. The elimination constant is the slope of the elimination portion of the plasma concentration curve, and to convert it to half-life, the following equation can be used:

$$T_{1/2} = 0.693/K_{el}$$

or

$$K_{el} = 0.693/T_{1/2}$$

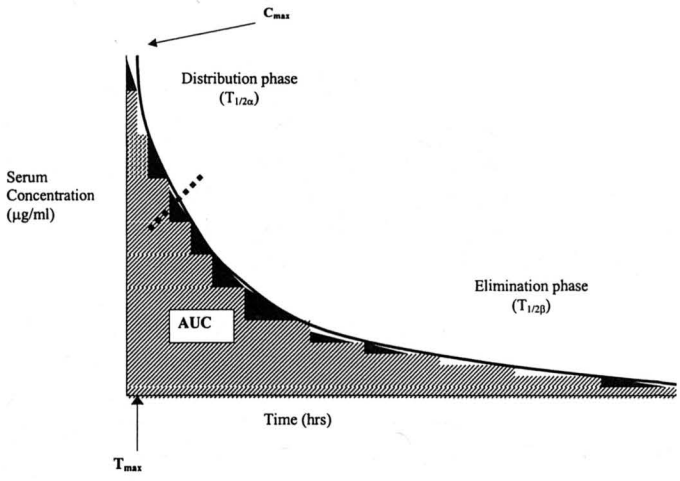

Figure 4. Plasma disappearance curve (IV bolus).

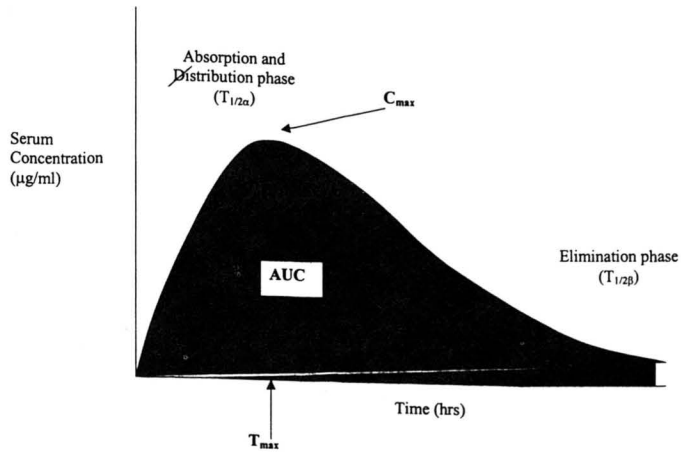

Figure 5. Plasma disappearance curve (IM, SQ, PO).

In addition, for the purposes of extrapolating withdrawal intervals, the following relationship is important:

K_{el} = Vd/Clearance

Therefore, the elimination half-life will be affected by diseases or changes in physiology that affect the volume of distribution of the drug in the animal or the clearance of the drug.

MRT (Mean Residence Time) is another way of describing how long a drug stays in the body. It is an estimate of the mean length of time a given molecule of drug will remain in the animal. It is calculated using mathematical manipulations of the plasma concentration curve. **Vd**, the volume of distribution, is the volume of plasma at a given drug concentration required to account for all of the drug in the body. It is a theoretical concept that is useful as a way of evaluating whether a particular drug tends to stay in plasma or tends to move into tissues. Very high volumes of distribution may even indicate that the drug binds to certain tissues. Low volumes of distribution indicate that the drug tends to stay in plasma. As a general rule, lipid-soluble drugs have higher volumes of distribution, and water-soluble drugs have lower volumes of distribution, in the absence of tissue binding. It is usually expressed as L/kg.

Conclusion

The purpose of understanding these concepts and learning the terminology is so that the literature of antimicrobials makes sense. This understanding will assist the practitioner in making decisions about antimicrobial regimens. The next article in these proceedings will cover the concepts of regimen design, utilizing pharmacokinetic information and antimicrobial susceptibility results so as to optimize the treatment of bacterial infections.

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