Antimicrobial susceptibility testing - Beyond S/I/R

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Abstract

Antimicrobial susceptibility testing and interpretation is a critical component of antimicrobial stewardship in bovine practice, yet it remains 1 of the most poorly understood diagnostic tests in veterinary medicine. Improved practitioner understanding of the process of antimicrobial susceptibility testing, clinical breakpoint determination, and the limitations of this critical diagnostic tool are important in ensuring appropriate results interpretation and improving clinical decision making in the context of antimicrobial stewardship.

Key words: antimicrobial susceptibility testing, bovine, antimicrobial stewardship

Introduction

Bovine practitioners are not immune from the rise in antimicrobial resistance that currently threatens both human and animal health, and antimicrobial stewardship is critical to preserving the efficacy of antimicrobials for treatment of bacterial diseases in both humans and animals. One of the key tenets of antimicrobial stewardship is the use of culture and antimicrobial susceptibility testing (AST) to help inform clinical decision making regarding antimicrobial use. Just like any other diagnostic test, however, it is critical that the bovine practitioner understand the appropriate application and limitations associated with the use of AST to be able to utilize the information gained to improve antimicrobial decision-making in clinical practice and to “think beyond S/I/R”.

Rethinking the Definition of “S” and “R”

As busy practitioners, we tend to see an “R” on the diagnostic lab report and automatically assume that the bacteria in question is resistant to the antibiotic, and move on to the next antibiotic on the list that says “S” without giving it more thought. To understand what “S” and “R” might really mean, however, we have to understand how the lab arrived at that interpretation. Veterinary diagnostic laboratories in the United States utilize clinical breakpoints that have been established by the Clinical Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST). A true clinical breakpoint is specific to the bacterial species of interest, location in the body of the infection, the animal species being treated, and the drug dosage regimen being utilized. From a clinician’s perspective, clinical breakpoints are important because they consider the clinical picture including the dosage, route, and frequency of administration of drug utilized to treat the patient. In regards to clinical breakpoints, “susceptibility” refers to the clinical condition whereby the infection is expected to be susceptible to the antimicrobial administered at a specific dosage and route, while “resistance” indicates that at the dose and route given, the infection is not expected to respond as favorably as it is less susceptible or non-susceptible to the antimicrobial treatment. As clinicians, this is the reason we perform AST, to be able to more appropriately select an antimicrobial for treatment of a disease either in an individual animal or in a group of animals.

Clinical breakpoints can be derived via multiple approaches including microbiological characteristics, pharmacokinetic and pharmacodynamics (PK/PD) parameters, and/or clinical outcome data. It is the application of the clinical breakpoints to the AST result that allows reporting of results as susceptible, intermediate or resistant (S/I/R) to aid in clinical decision-making. There are 2 primary methodologies currently employed to perform AST: disc diffusion and microbroth dilution. Disc diffusion (often referred to as the Kirby-Bauer [KB] test), is a dynamic test involving the diffusion of antibiotics from disc on a plate and yields a qualitative result in the form of a zone diameter. If veterinary clinics choose to perform their own AST, it is most frequently via this method; however, there are several drawbacks to this approach. There are fewer CLSI-approved breakpoints for this approach, and the qualitative result cannot be used for dosage calculations or monitoring for increased resistance development. The most commonly employed method of AST in both human and veterinary diagnostic laboratories is broth microdilution, which is performed using standardized testing panels provided in 96-well plates which contain varying dilutions of antibiotic concentrations. Broth microdilution testing allows for generation of a quantitative result in the form of an MIC (minimum inhibitory concentration) which represents the minimum concentration of an antimicrobial agent that prevents visible growth of a microorganism (typically 90% of the organisms, or MIC90). The MIC or zone diameter datapoint generated from AST are then interpreted using the clinical breakpoints established by CLSI to provide the clinician with the S/I/R designation.

In an ideal world, true clinical breakpoints would exist for all pathogens of cattle isolated from all locations in the body for all possible antimicrobial agents; however, due to a number of limiting factors, this is not the case. Table 1 demonstrates the currently available clinical breakpoints established by CLSI VAST that are used by veterinary diagnostic
laboratories for interpretation of non-mastitis cattle specimens\textsuperscript{5}. As demonstrated in Table 1, all but 1 of the clinical breakpoints available for parenteral antibiotics in cattle are specific only to bovine respiratory disease pathogens such as \textit{M. haemolytica}, \textit{P. multocida}, and \textit{H. somni}. Why is this important? Because clinical breakpoints have been established for many antibiotics for treatment of bovine respiratory disease pathogens, there is a higher level of confidence in performing AST on these organisms for this condition\textsuperscript{5}. In contrast, however, for other bacteria isolated from other disease processes, the confidence in the interpretation of the results of the AST decreases as the clinical breakpoint utilized to determine susceptible vs resistant must be extrapolated from other locations, animal species, and bacterial species. While this information can still be valuable to the practitioner; it is important to recognize this limitation when interpreting these results. As clinical breakpoints are reliant on drug dosing and pharmacokinetic parameters, a breakpoint established in humans or dogs may not necessarily correlate well to use of the same drug at a different dosage or route in cattle. In some cases, no reasonable breakpoint exists in other species from which to extrapolate, and the results may be reported as “no interpretation.” This is not meant to frustrate the practitioner, although it frequently does, but is meant to highlight the fact that not enough information is available to make a reasonable recommendation in that case.

Table 2 demonstrates the most common bovine specimens that received culture followed by AST testing at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) from 2003-2018. While the majority of specimens were unfortunately classified as “assorted” due to collection and submission of multiple tissue types at necropsy, the most common specified specimen location was respiratory tract followed by gastrointestinal, with all other reported locations making up less than 8% of the total isolates (this includes specified locations such as eye, milk, urinary, CNS, etc). As demonstrated in Table 2, the 5 most common isolates overall representing 86% of the dataset are (from most common to least): \textit{Escherichia coli}, \textit{Mannheimia haemolytica}, \textit{Salmonella enterica}, \textit{Pasteurella multocida}, and \textit{Histophilus somni}. Of these isolates, only \textit{M. haemolytica}, \textit{P. multocida} and \textit{H. somni} have clinical breakpoints established in cattle, while the first

<table>
<thead>
<tr>
<th>Antibiotics routinely tested via AST for bovine isolates</th>
<th>Dosage regimen used for breakpoint determination\textsuperscript{**}</th>
<th>Bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>5 mg/lb (11 mg/kg) IM q 24 hrs</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1 mg/lb (2.2 mg/kg) IM (sodium or hydrochloride)</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Clindamycin (test for lincomycin)</td>
<td>2.7 mg/lb (6 mg/kg) SQ twice 48 hrs apart</td>
<td>Yes Yes</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>3.4 mg/lb (7.5 mg/kg) SQ once</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.7 mg/lb (6 mg/kg) SQ once</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Neomycin</td>
<td>10,000 IU/lb (22,000 IU/kg) IM q 24 hrs</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>4.5 mg/lb (10 mg/kg) SQ q 24 hrs</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Tetracycline (test for oxytetracycline)</td>
<td>9.1 mg/lb (20 mg/kg) IM once for oxytetracycline (may be cautiously applied to SQ dosing)</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>1.8 mg/lb (4 mg/kg) SQ once</td>
<td>Yes Yes Yes Yes</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>4.5 mg/lb (10mg/kg) SQ once</td>
<td>Yes Yes</td>
</tr>
<tr>
<td>Trimethoprim / sulfamethoxazole</td>
<td>1.1 mg/lb (2.5 mg/kg) SQ once</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>Tylosin tartrate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{*} information summarized from CLSI documents VET08\textsuperscript{5} and VET09\textsuperscript{5}  
\textsuperscript{**} SQ = subcutaneous; IM = intramuscular; q = every

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Table 2. Most common bovine specimens that received culture followed by AST testing from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) 2003-2018.

<table>
<thead>
<tr>
<th>Location from which culture was taken</th>
<th>Number of ASTs performed</th>
<th>Most common isolates (number in parenthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assorted (i.e., multiple tissues taken at necropsy)</td>
<td>7163</td>
<td>--</td>
</tr>
</tbody>
</table>
| Respiratory tract | 4588 | M. haemolytica (1729)  
P. multocida (1477)  
H. somni (943)  
Salmonella sp (309)  
B. trehalosi (74) |
| Gastrointestinal tract/fecal | 2621 | E. coli (2090)  
Salmonella sp (478) |
| All other (eye, CNS, joint, etc.) | 1205 | -- |
| TOTAL | 15577 | E. coli (5259)  
M. haemolytica (2847)  
Salmonella sp (2447)  
P. multocida (2500)  
H. somni (1453)  
All others (2101) |

and third most common isolates on which AST was performed do not have established clinical breakpoints (the single exception being E. coli and ampicillin, which will be further discussed below). This makes interpretation of AST results challenging for both the diagnostic laboratory as well as the practitioner, as all interpretations for these isolates must be extrapolated from data in other species. As both E. coli and Salmonella are most frequently isolated from the gastrointestinal tract, this also presents several additional issues.

As introduced above, a true clinical breakpoint is specific not only to the bacterial species of interest and animal species being treated, but it is also specific to the location in the body of the infection. This is because the ability of an antibiotic to access the site of infection is critical in determining its clinical effectiveness at that location. There are unfortunately no clinical breakpoints that have been established for cattle for enteric infections. So, while bovine practitioners frequently request AST for enteric isolates, there are no established breakpoints to assist the diagnostic lab and practitioner in selecting appropriate antimicrobial therapy for treatment of infections in the gastrointestinal tract. In fact, no clinical breakpoints have been established in ANY veterinary species for ANY gastrointestinal Enterobacteriaceae infections.1,15 Therefore, the use of AST to determine appropriate antimicrobial treatment of enteric infections with pathogens such as E. coli and Salmonella is not recommended, as the concentrations of antimicrobials achievable and needed for disease resolution in the gastrointestinal tract are generally considered to be unknown. This poses significant challenges to veterinary clinicians who are regularly faced with patients suffering from bacterial gastroenteritis and choose to perform AST, yet have little appropriate bovine-specific guidance with which to direct therapy.

Because clinical breakpoints take into consideration patient and drug factors, it is critical to understand that just because an organism tests “resistant” to a particular antibiotic does not necessarily mean that it actually has acquired resistance (i.e., not inherently present or intrinsic) to that antibiotic. Up to this point, we have exclusively looked at resistance from the clinical standpoint and how likely the infection is to respond to the proposed treatment, which is heavily dependent on drug dosing and pharmacokinetic factors. However, when we look at resistance from a bacteriologic standpoint, the terms “susceptible” and “resistant” are instead used to differentiate between 2 populations of bacteria: 1 population that does not typically harbor acquired resistance to an antimicrobial and is thus “susceptible” (i.e., wild type population), and another that does typically harbor acquired resistance mechanisms and is thus considered “resistant” to the antimicrobial (i.e., non-wild type population). The bacteriologic difference between wild type (i.e., do not typically harbor resistance genes) and non-wild type (i.e., typically harbor acquired resistance genes) populations of bacteria is termed an epidemiologic cut-off value (ECOFF or ECV).6 While clinical breakpoints are used to assist in clinical decision-making, the ECV is recommended to be used to measure resistance development in bacterial species over time, as well as monitor the success of interventions in preventing resistance development. This is because the ECV is determined exclusively from the distribution of observed MIC values in a population of bacteria and is independent of host species or pharmacologic information.

On the surface, it seems that the clinical breakpoint and ECV should be the same value, and in many cases they are. However, the newly adopted (2018) CLSI clinical breakpoint for susceptibility of E. coli to ampicillin in cattle provides an excellent example of where this is not the case. The current E. coli ampicillin bovine breakpoint is <0.25 µg/mL,6 while the current human E. coli ampicillin breakpoint is <8 µg/mL. The bacteria does not differ between humans and cattle, so why is there such a large difference in clinical breakpoints? In cattle, this breakpoint was derived from PK/PD data and the labeled dose of ampicillin trihydrate given IM once daily; in humans, the breakpoint is for ampicillin sodium given IV 4 times daily. From a bacteriologic standpoint, the human breakpoint also matches the ECV value for E. coli, while using the cattle breakpoint means that almost all wild-type bacteria with no acquired resistance will still be classified as “R”. If these results were only reported and compared using the “S/I/R” designation, it would appear that there was extensive resistance in E. coli in cattle when compared to humans, which is incorrect. This risks the false interpretation that antimicrobial use in livestock species leads to higher rates of antimicrobial resistance in bacteria from animals, which

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then may be interpreted as the cause for resistance seen in human infections. Therefore, it is critical that clinical breakpoint interpretation data alone not be utilized to assess for acquired resistance development in bacteria across species. In this context, clinical resistance is better thought of as non-susceptibility to the antibiotic due to the drug formulation, dosage, and frequency, and not always as true bacterial resistance to the antibiotic. In this example, from a clinical standpoint, the use of increased frequency, higher dosages, and different formulations of ampicillin in cattle are likely to move the clinical breakpoint closer the ECV, however, additional research is necessary to support the extent to which this occurs. Thus, it is critically important to know what drug dosage was used to determine a clinical breakpoint when evaluating its utility for interpretation of treatment options for a particular condition.

A Practical Approach to Utilizing AST Results in Bovine Practice

With the knowledge provided above, we can now discuss a practical approach to utilizing AST results in clinical practice. First, it is critical that AST be focused on only potential pathogens. When a bacterial organism is isolated from a diseased animal, we first must ask if it is reasonable to expect that this bacteria is playing a role in the disease process prior to asking what antimicrobial drugs could be used to treat the infection. For many body sites commonly cultured by bovine practitioners (including the upper respiratory tract and gastrointestinal tract), normal flora exist and are routinely able to be cultured. The ability to culture bacteria from these sites, however, does not ensure that they are playing a role in the disease process. In addition, contamination is common when culturing clinical samples and must always be taken into consideration when mixed growth is present instead of pure growth of a bacterial organism. Thus, the bacteriology lab and/or practitioner must frequently determine which of the isolated bacteria are most likely to play a role in the disease process to determine which isolates warrant AST. Requesting or performing AST on isolates that are likely to be normal flora or contaminants has several issues, 1 of which is a lack of clinical breakpoints for these organisms, as discussed above. This, combined with the common existence of both intrinsic and acquired resistance in many of these organisms, may falsely lead the clinician to interpret an infection to be more difficult to treat than necessary, and hinder rather than assist antimicrobial stewardship efforts. When presented with AST results of several organisms isolated from the same location, focusing on the results from the organism(s) most likely to be able to cause clinical disease is likely more beneficial than attempting to find an antibiotic that is listed as “susceptible” for all organisms present. It is also important to keep in mind that MIC values should not be compared across antibiotics (i.e., selection of the lowest numerical value of all of the susceptible results), as the MIC is drug-specific due to the pharmacokinetics of that antibiotic and not an indicator of success across drug classes.

As presented in Table 1, the disease condition for which bovine practitioners can have the most confidence in AST testing interpretation is bovine respiratory disease (BRD) caused by either *M. haemolytica*, *P. multocida* or *H. somni*. Antimicrobial resistance in these pathogens is of serious concern, as available evidence does suggest that resistance to several antibiotic classes has increased over the past several decades. However, it is important to keep in mind that most of the published literature focuses on results from diagnostic laboratory submissions obtained from dead cattle that have been treated multiple times with multiple different antimicrobials. It is unclear at this time the significance of isolation of highly resistant pathogens from these chronic cases. Were these resistant bacteria present in high numbers at the start of the infection, and thus responsible for the treatment failure? Or, do they simply represent the only bacteria that are able to be cultured after multiple rounds of antimicrobial therapy failed, due to other factors such as decreased immune function? The universally accepted gold standard for performing culture and AST is to utilize it on untreated, newly diagnosed animals, yet this is not how AST in BRD is typically approached. To truly understand the impact of antimicrobial resistance on treatment outcome, we as a profession likely need to move closer to this ideal through more targeted collection of nasal swabs, deep nasopharyngeal swabs, transtracheal washes or bronchoalveolar lavage from clinical animals prior to treatment. While minimal published work evaluating the impact of antimicrobial resistance on clinical outcome in cattle with BRD is available, what has been published does suggest that the presence of resistance at the time of initial treatment affects outcome.

One additional caveat in regard to the application of clinical breakpoints for BRD pathogens is that all of the current clinical breakpoints were developed using dosages of the antibiotics administered parenterally; these breakpoints should not be extrapolated to in-feed antibiotic administration, and there are currently no clinical breakpoints available in cattle for in-feed administration of antimicrobials.

From a practical standpoint, AST should only be performed on clinical specimens and bacterial isolates when it has a high likelihood of providing useful results to instruct either antimicrobial use, or for monitoring trends in populations of pathogenic bacteria. As discussed above, AST testing on enteric isolates presents significant challenges for interpretation due to a lack of clinical breakpoints for enteric infections. When CLSI bovine-specific breakpoints are not available, there is no standard for which breakpoints should be used for a given organism and antibiotic combination, therefore, isolates sent to different labs can receive different interpretations for the same MIC. Beyond interpretation challenges, *E. coli* also represents normal flora of the bovine fecal environment. When we perform AST, the results are generated from a single colony with the assumption that clinical

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Antimicrobial susceptibility testing is a valuable tool for antimicrobial stewardship, but only when used with the understanding of its benefits and limitations in regards to bovine medicine. Improved understanding of key concepts of AST should assist the practitioner in making improved decisions regarding antimicrobial therapy in bovine patients.

**References**


