Antimicrobial susceptibility testing: is it driving your decisions or just driving you crazy?

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Abstract

There continues to be considerable confusion and debate regarding the utility of antimicrobial susceptibility testing (AST) to aid in the antimicrobial selection process. With the renewed emphasis on antimicrobial stewardship in both human and veterinary medicine, it is important to consider all disease preventive and diagnostic tools available. The scientific literature is equivocal regarding the correlation between the *in vitro* susceptibility test result and clinical outcome. These studies also highlight key points that practitioners should consider when using both individual animal AST results and cumulative diagnostic summaries to select antimicrobial therapeutics.

Key words: antibiotic, antimicrobial, susceptibility testing

Résumé

Il y a toujours beaucoup de confusion et des débats sur l'utilité des tests de sensibilité aux antimicrobiens (AST) pour aider à l'antimicrobien processus de sélection. Avec le nouvel accent mis sur l'intendance antimicrobiens chez l'humain et la médecine vétérinaire, il est important de tenir compte de toutes les mesures préventives des maladies et les outils de diagnostic disponibles. La littérature scientifique est équivoque en ce qui concerne la corrélation entre la sensibilité in vitro résultat d'essai et résultats cliniques. Ces études mettent également en évidence les points clés que les spécialistes devraient considérer lors de l'utilisation de l'animal individuel Résultats AST et cumulatifs des résumés de diagnostic pour choisir la thérapeutique des antimicrobiens.

Introduction

Antimicrobial stewardship has been described as "coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of optimal antimicrobial drug regimen"[7]. It has been proposed that these interventions include the use of patient-specific bacterial culture and susceptibility data to guide therapy, as well as cumulative susceptibility data for surveillance and investigations of outbreaks with resistant bacterial organisms. However, scientific studies investigating the correlation between antimicrobial susceptibility testing (AST) results and clinical outcomes have provided conflicting conclusions regarding the value of this test. This review will briefly discuss 2 of the studies conducted in veterinary medicine that did not demonstrate a correlation between the *in vitro* result and clinical outcome. The objective is not a complete critical appraisal, but rather to use these studies to highlight key points that practitioners can use to improve the value of AST in selecting antimicrobial therapy. This review will conclude by providing case examples to illustrate these key points.

Discussion

In 2011, McClary et al published the results of a retrospective analysis to evaluate the relationship between the tilmicosin minimum inhibitory concentrations of *Mannheimia haemolytica* and *Pasteurella multocida* isolates and clinical outcome of bovine respiratory disease.[11] The records from 16 clinical trials were pooled to evaluate the *in vitro–in vivo* correlation. The authors of this study reported no statistical association between clinical outcome and the categorical interpretation (susceptible or resistant) of the recovered isolate. One of the limitations of this study that would have impaired the *in vitro–in vivo* correlation was the diagnostic sampling method. The bacterial isolates in these clinical trials were recovered from deep nasopharyngeal swabs. Studies by Godinho et al,[9] Allen et al,[2] and DeRosa et al[8] have previously demonstrated that there can be significant differences between BRD isolates recovered from the upper and low respiratory tracts of cattle. The correlation between AST result and clinical outcome can only be fully realized if the isolates tested are representative of the isolates causing disease. Samples collected from the primary site of infection that limit overgrowth of contaminant organisms will improve the clinical value of AST.

In an earlier study, Constable and Morin concluded that AST results were of no value in predicting either duration of clinical signs or bacteriologic cure rates for mastitis.[6] Included in this study were the results of 121 episodes of clinical mastitis in 58 cows. A critical assessment of this study can be found elsewhere as part of a larger review on AST in mastitis therapy;[3] however, clinicians should be aware that the interpretive criteria (breakpoints) used in the study by Constable and Morin were not specific for bovine mastitis. Veterinary specific interpretive criteria, as developed by the Clinical and Laboratory Standards Institute–Veterinary Antimicrobial Susceptibility Testing subcommittee, relate the *in vitro* test result to clinical outcome, but only: for a specific disease, caused by that specific bacterial species, in that specific host species, treated with that specific antimicrobial, with a

specific dosing regimen (dose, route, frequency and duration of therapy).[5] Extrapolation of any of these 5 conditions may negatively affect the correlation of susceptible/intermediate/resistant to clinical outcome. The correlation between AST result and clinical outcome can only be fully realized if the interpretive criteria used are specific to the bacterial organism, antimicrobial, host species, disease process and dosing regimen. When veterinary specific interpretive criteria are not available, clinicians should work with their diagnostic laboratory to consider the degree to which extrapolation of another interpretive criteria is reasonable. The antimicrobial–disease–pathogen combinations for which there are currently approved veterinary specific interpretive criteria in cattle can be found in Table 1.

While these studies were not able to demonstrate statistical associations between AST results and clinical outcome, there are other studies that do demonstrate the value of this diagnostic tool. Martineau et al were able to demonstrate a near perfect correlation between the resistance phenotype (using AST) and the presence of resistance genes for a collection of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates.[10] Carmeli et al reported significant increases in mortality and length of hospital stay when antimicrobial resistance (measured by AST) emerged during treatment of *Pseudomonas aeruginosa* infections in humans.[4] In a study evaluating clinical outcomes for *Bacteroides* bloodstream infections in humans, Nguyen et al reported that receiving inactive therapy (measured by AST) was significantly associated with clinical failure (OR = 38.0; CI = 4.2-337) and 30 day mortality ($P = 0.04$).[12]

Case Study #1 – Using Individual Animal AST results

Two pens of highly commingled, lightweight feeder calves received mass medication on arrival at a feedyard and are now experiencing an outbreak of respiratory disease. First treatment response rate is unusually low (~50%) and the veterinarian is concerned that antimicrobial resistance may be contributing to the poor therapeutic response. A gross necropsy is performed and lung tissue is submitted for aerobic culture and susceptibility testing. The culture

Table 1. Bovine veterinary specific interpretive criteria.

Bovine respiratory disease		Bovine mastitis	
Antimicrobial	**Pathogens**	**Antimicrobial**	**Pathogens**
Ceftiofur	*M. haemolytica* *P. multocida* *H. somni*	Ceftiofur	*Staph. aureus* *Strep. agalactiae* *Strep. dysgalactiae* *Strep. uberis* *E. coli*
Danofloxacin	*M. haemolytica* *P. multocida*		
Enrofloxacin	*M. haemolytica* *P. multocida* *H. somni*	Penicillin - novobiocin	*Staph. aureus* *Strep. agalactiae* *Strep. dysgalactiae* *Strep. uberis*
Florfenicol	*M. haemolytica* *P. multocida* *H. somni*		
Gamithromycin	*M. haemolytica* *P. multocida* *H. somni*	Pirlimycin	*Staph. aureus* *Strep. agalactiae* *Strep. dysgalactiae* *Strep. uberis*
Penicillin	*M. haemolytica* *P. multocida* *H. somni*		
Spectinomycin	*M. haemolytica* *P. multocida* *H. somni*		
Tetracycline – OTC injectable	*M. haemolytica* *P. multocida* *H. somni*		
Tildipirosin	*M. haemolytica* *P. multocida* *H. somni*		
Tilmicosin	*M. haemolytica*		
Tulathromycin	*M. haemolytica* *P. multocida* *H. somni*		

Adapted from: CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Second informational supplement. CLSI document VET01-S2. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

Table 2. AST report for Case Study #1.

Antimicrobial	Interpretation	MIC	Test range
Ampicillin	S	≤0.25	0.25-16
Ceftiofur	S	≤0.25	0.25-8
Chlortetracycline	NI	4.0	0.5-8
Clindamycin	NI	16.0	0.25-16
Danofloxacin	NI	>1.0	0.12-1
Enrofloxacin	R	>2.0	0.12-2
Florfenicol	S	1.0	0.25-8
Gentamicin	R	>16.0	1-16
Neomycin	NI	8.0	4-32
Oxytetracycline	R	>8.0	0.5-8
Penicillin	I	0.5	0.12-8
Spectinomycin	R	>64.0	8-64
Sulphadimethoxine	NI	≤256	256
Tiamulin	NI	8.0	0.5-32
Tilmicosin	I	16.0	4-64
Trimeth/sulfa	I	≤2.0	2/38
Tulathromycin	R	>64.0	1-64
Tylosin tartrate	R	32.0	0.5-32

is positive for *Mannheimia haemolytica*. The susceptibility report is listed in Table 2.

- Interpretation
  In this case study, the following antimicrobials are legal for therapy of BRD caused by *Mannheimia haemolytica*: ceftiofur, danofloxacin, enrofloxacin, florfenicol, oxytetracycline, penicillin, spectinomycin, tilmicosin, and tulathromycin. Based on the AST result for this particular isolate, ceftiofur and florfenicol would carry a reasonable expectation of efficacy and the practitioner would be justified in selecting between these 2 antibiotics.

Case Study #2 – Using Cumulative AST results

The same veterinarian is re-evaluating health protocols in this feedyard. This veterinarian is going to evaluate cumulative AST data to determine which antimicrobials would be reasonable empiric choices given historical antibiotic resistance trends. The graphs in Figures 1-3 are available for this purpose.

- Interpretation
  In this case, rates of multidrug antimicrobial resistance are high for *Mannheimia haemolytica*, making empirical drug choices quite limited. For *Pasteurella multocida,* only oxytetracycline shows significant levels of antibiotic resistance, while there are high levels of resistance to oxytetracycline, fluoroquinolones, and spectinomycin for *Histophilus somni.*

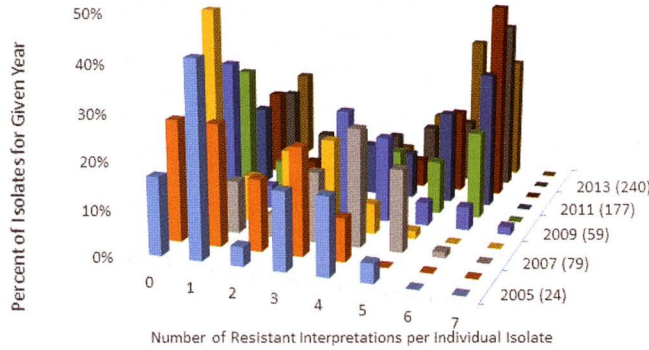

Figure 1. Multidrug resistance prevalence of *Mannheimia haemolytica* isolates recovered at Kansas State Veterinary Diagnostic Laboratory.

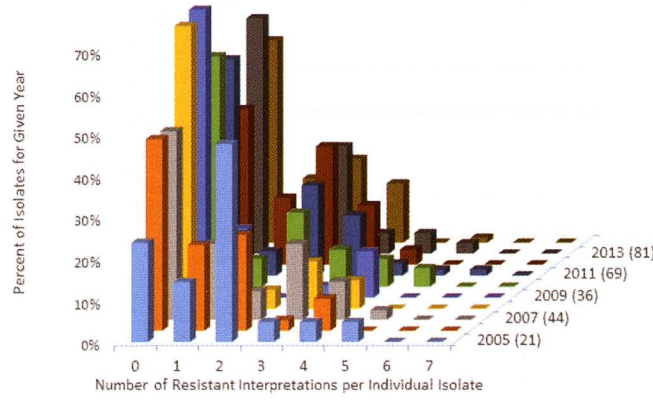

Figure 2. Multidrug resistance prevalence of *Pasteurella multocida* isolates recovered at Kansas State Veterinary Diagnostic Laboratory.

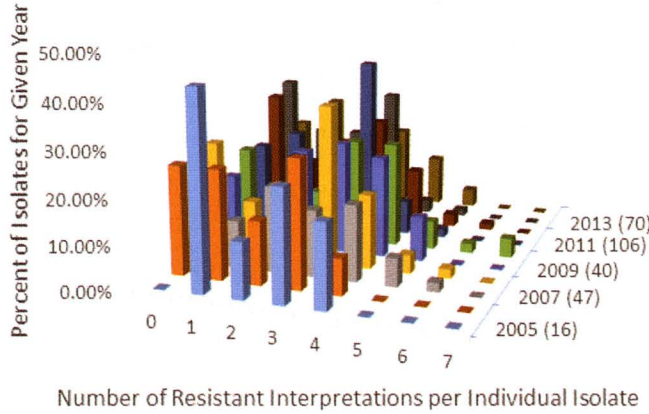

Figure 3. Multidrug resistance prevalence of *Histophilus somni* isolates recovered at Kansas State Veterinary Diagnostic Laboratory.

For the latter 2 pathogens, co-resistance will be discussed.

Case Study #3 – Using Individual Animal and Cumulative AST results together

A veterinarian is consulted to investigate an outbreak of pinkeye in an 80 cow herd in the midwestern United States. The owner reports "poor" response to treatment with long-acting oxytetracycline. The veterinarian has submitted a number of conjunctival swabs from acutely affected animals for aerobic culture and AST. The cultures are positive for *Moraxella bovoculi* and the AST report can be found in Table 3.

- Interpretation
  In this case, fluoroquinolones are not reported as this constitutes extra-label drug use, which is strictly prohibited for this antimicrobial class. Gentamicin and neomycin are not illegal, however, they would require a substantial withdrawal period and as such, the American Association of Bovine Practitioners has a position statement against such use.[1] According to the Animal Medicinal Drug Use Clarification Act of 1994, a practitioner should first consider a drug which has been labeled for that purpose in that particular species and production class. In this case, there are 2 such antimicrobials, oxytetracycline and tulathromycin, labeled for treatment of pinkeye. Neither have veterinary specific interpretive criteria for treatment of infectious bovine keratoconjunctivitis. Using cumulative AST data, the practitioner may be able to separate out MIC values of isolates with and without resistance genes (see Figure 4). That is indeed the case here where an MIC of ≤1 for tulathromycin likely represents an isolate without a resistance gene. This concurs with the current interpretive criteria for BRD pathogens, thus making tulathromycin a reasonable therapeutic choice.

Table 3. AST report for Case Study #3.

Antimicrobial	Interpretation	MIC	Test range
Ampicillin	NI	≤0.25	0.25-16
Ceftiofur	NI	≤0.25	0.25-8
Chlortetracycline	NI	2.0	0.5-8
Clindamycin	NI	1.0	0.25-16
Florfenicol	NI	0.5	0.25-8
Gentamicin	S	≤1.0	1-16
Neomycin	NI	≤4.0	4-32
Oxytetracycline	I	8.0	0.5-8
Penicillin	NI	≤0.12	0.12-8
Spectinomycin	NI	16.0	8-64
Sulphadimethoxine	NI	≤256	256
Tiamulin	NI	≤0.5	0.5-32
Tilmicosin	NI	≤4.0	4-64
Trimeth/sulfa	S	≤2.0	2/38
Tulathromycin	NI	≤1.0	1-64
Tylosin tartrate	NI	8.0	0.5-32

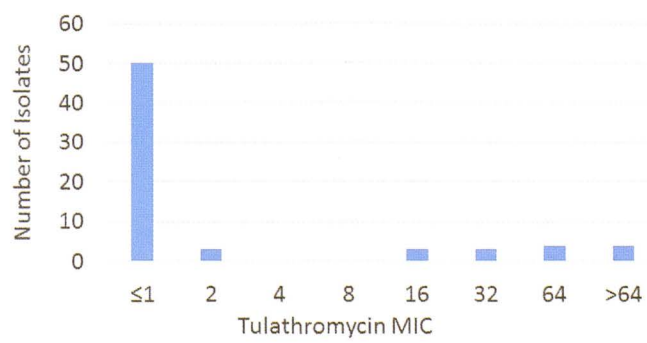

Figure 4. Distribution of minimum inhibitory concentrations for *Moraxella* sp recovered at the Kansas State Veterinary Diagnostic Laboratory from 2012-2015.

Conclusions

There is, and will likely continue to be, discussion regarding the utility of AST as a predictive tool. The test itself cannot fully account for all of the variables that, in sum, lead to a treatment success or failure; however, AST can provide the clinician with some expectation of the ability of the antibiotic to contribute to clinical outcome as one component of the antibiotic selection process.

As the use of antibiotics in food production comes under further scrutiny, the use of AST in justifying antimicrobial selection becomes clear. However, in order to realize the full value of this diagnostic tool, practitioners will require an understanding beyond S/I/R. When applying AST results to individual animal therapy, 1) proper sample submission, leading to testing an isolate that is representative of the disease process, and 2) awareness of which interpretive criteria are being applied will aid in result interpretation. Awareness of the patient population from which cumulative summaries is derived can aid the clinician in making empiric therapy choices for similar populations of animals.

References

1. AABP Position Statement. Aminoglycoside use in cattle. Available at: http://www.aabp.org/resources/aabp_position_statements/AABPaminoglycoside.pdf. Accessed June 20, 2016.
2. Allen JW, Viel L, Bateman KG, Rosendal S, Shewen PE, Physick-Sheard P. The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. *Can J Vet Res* 1991; 55:341-346.
3. Barlow J. Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. *J Mammary Gland Biol Neoplasia* 2011; 16:383-407.
4. Carmeli Y, Troillet N, Karchmer AW, Samore MH. Health and economic outcomes of antibiotic resistance in *Pseudomonas aeruginosa*. *Arch Intern Med* 1999; 159:1127-1132.

5. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, 4th edition. CLSI document VET01-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
6. Constable PD, Morin DE. Use of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis to predict response to treatment with cephapirin and oxytetracycline. *J Am Vet Med Assoc* 2002; 221:103-108.
7. Dellit TH, Owens RC, McGowan JE, Gerding DN, Weinstein RA, Burke JP, Huskins WC, Paterson DL, Fishman NO, Carpenter CF, Brennan PJ, Billeter M, Hooton TM. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America Guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clin Infect Dis* 2007; 44:159-177.
8. DeRosa DC, Mechor GD, Staats JJ, Chengappa MM, Shryock TR. Comparison of *Pasteurella* spp simultaneously isolated from nasal and transtracheal swabs from cattle with clinical signs of bovine respiratory disease. *J Clin Microbiol* 2000; 38:327-332.
9. Godinho KS, Sarasola P, Renoult E, Tilt N, Keane S, Windsor GD, Rowan TG, Sunderland SJ. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. *Vet Rec* 2007; 160:22-25.
10. Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M, Bergeron MG. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis. Antimicrob Agents Chemother* 2000; 44:231-238.
11. McClary DG, Loneragan GH, Shryock TR, Carter BL, Guthrie CA, Corbin MJ, Mechor GD. Relationship of in vitro minimum inhibitory concentrations of tilmicosin against *Mannheimia haemolytica* and *Pasteurella multocida* and in vivo tilmicosin treatment outcome among calves with signs of bovine respiratory disease. *J Am Vet Med Assoc* 2011; 239:129-135.
12. Nguyen MH, Yu VL, Morris AJ, McDermott L, Wagener MW, Harrell L, Snydman DR. Antimicrobial resistance and clinical outcome of Bacteroides bacteremia: findings of a multicenter prospective observational trial. *Clin Infect Dis* 2000; 30:870-876.