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Evaluation of the Sensititre Aris® 2X and Vitek® 2 Automated Systems for Antimicrobial Susceptibility Testing of Contemporary Gram-Negative Pathogens Originating from Veterinary Sources

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REVISED ABSTRACT*

Background: Emergence of resistance among Gram-negative veterinary pathogens requires reliable detection for treatment, control and prevention. Given the widespread use of automated systems, periodic re-evaluation of performance characteristics with contemporary isolates is necessary. We evaluated the accuracy of the Sensititre ARIS 2X (TREK Diagnostic Systems) and Vitek 2 (bioMérieux) susceptibility systems when testing Gram-negative bacilli.

Methods: Clinically significant pathogens including Enterobacteriaceae (ENT; 143), *Pseudomonas aeruginosa* (PSA; 25), other non-fermentative bacilli (NFB; 29), and *Pasteurella/Actinobacillus* spp. (PAS; 8) were recovered in 2010 from companion and other animals. Susceptibility testing was performed according to the manufacturers' recommendations using veterinary specific Sensititre ARIS 2X (Part No. COMPAN1F) and Vitek 2 (Part No. GN38) panels. Results were compared with routine methods (primarily disk diffusion using CLSI M31-A3 breakpoints or the Vitek Legacy). Consensus was defined as ≥2 systems with matching categorical results. Ten agents common to all systems were compared when testing ENT, eight when testing PAS, and seven when testing PSA and NFB.

Results: A total of 3,756 categorical interpretations were generated for the 3 test systems with up to 11 antimicrobial agents. Overall agreement between the systems was 90.8%; agreement between the Sensititre ARIS 2X and Vitek 2 systems was 94.8%. Total error rates were 2.4%, 2.5% and 4.1% for Sensititre, Vitek 2 and the standard laboratory methods, respectively. Agreement and error rates are in the Table.

Conclusions: Acceptable categorical agreement (90.8% overall) was achieved between the susceptibility assay systems when testing veterinary-source isolates originating in 2010. Errors were more evident when testing certain species and antimicrobial agents with particular systems: standard methods (4.1%) > Vitek 2 (2.5%) > Sensititre (2.4%).

			% Error Rate		
Antimicrobial Agent	n	% Agreement All Systems	Std. Method	Sensititre ARIS 2X	Vitek 2
Amikacin	50	94.0	6.0	0	0
Amoxicillin/Clavulanic Acid	151	89.4	4.0	1.3	4.0
Ampicillin	141	89.4	6.4	1.4	1.4
Cefpodoxime	2	100	0	0	0
Ceftiofur	165	91.1	4.8	0	3.0
Chloramphenicol	33	72.7	0	15.2	12.1
Enrofloxacin	187	84.0	5.9	8.0	2.1
Gentamicin	176	93.2	4.5	0	2.3
Imipenem	27	92.6	0	3.7	3.7
Marbofloxacin	154	95.5	1.3	2.0	1.3
Trimethoprim/Sulfamethoxazole	166	94.6	2.4	1.2	1.8

*Revised to include additional agents.

INTRODUCTION

Automated instrumentation in the veterinary microbiology laboratory for the identification and susceptibility testing of bacterial pathogens is in widespread use due to convenience, accuracy and rapidity over that of traditional methods. Databases, database management and use of expert systems are common to most all systems and are periodically updated to expand identification abilities and for detection of newly emerging resistance profiles and mechanisms. Although no system is infallible, all should be reliable, especially with traditional pathogens, and minimize the number of repeat and off-line tests which may greatly lengthen time to reporting of results.

Clinical laboratories have the expectation that automated systems should be able to readily differentiate the most commonly occurring Gram-positive and -negative bacteria and perform reliable susceptibility testing. The over-arching goals of automation are to reduce turn-around times, increase efficiencies and, hopefully, reduce costs.

In addressing clinical needs, the primary goals of accurate bacterial identification and susceptibility testing are to provide veterinary practitioners with information that can be used to assist in selection of appropriate empiric therapy, assess prognosis and possible public health significance of the offending pathogen. Accurate identifications for the laboratorian allow us to properly interpret subsequent antimicrobial susceptibility testing results based upon consensus breakpoints.

Prior studies of automated systems have demonstrated individual organism-antimicrobial agent weaknesses (error rates) when performing antimicrobial susceptibility testing that. In this study we evaluated the accuracy of the Sensititre ARIS 2X (TREK Diagnostic Systems) and Vitek 2 (bioMérieux, Inc.) systems compared with existing laboratory methods (primarily disk diffusion) on a contemporary (2010) challenge collection of Gram-negative pathogens recovered at a regional veterinary diagnostic laboratory. All isolates originated from specimens collected from domestic and exotic animals.

MATERIALS & METHODS

Bacterial isolates. Clinically significant veterinary pathogens (205 total) were recovered from domestic and exotic animals by a USA Midwest veterinary diagnostic laboratory in 2010, and included 143 Enterobacteriaceae (ENT; 47 *E. coli*; 16 *Klebsiella* spp.; 23 *P. mirabilis*; 57 others), 25 *Pseudomonas aeruginosa* (PSA); 29 other non-fermentative bacilli (NFB); and 1 *Pasteurella sp.* and 7 *Actinobacillus* spp. (8 total PAS). Isolates recovered originated from the respiratory tract (40.5%); urine (37.1%); skin and soft tissue (11.7%); feces (7.3%), urogenital tract (2.9%) and blood (0.5%). Further details of species and antimicrobial agents tested are found in Tables 1 and 2.

Identification and susceptibility testing methods. All isolates were tested with existing laboratory biochemical algorithms (rapid spot tests, biochemical strip tests, traditional tube biochemicals) with confirmation, where needed, by alternative methods (Sensititre GNID panels incubated and read by the ARIS 2X System; and Vitek 2 GN identification panels incubated and read by the Vitek 2 instrument according to the manufacturers' recommendations). Susceptibility testing was performed using veterinary specific Sensititre ARIS 2X (Part No. COMPAN1F) and Vitek 2 (Part No. GN38) susceptibility panels. Results were compared with routine laboratory methods (primarily disk diffusion [Kirby –Bauer] testing using CLSI M31 and M100-S20 breakpoints or the Vitek Legacy). Consensus was defined as ≥2 of the three test systems with matching categorical results; discordant results were considered as errors. Acceptability of errors was assessed as described previously (Jorgensen, 1993; CLSI, 2008); generally, total error should be kept to <10%, very major error to <1.5% and major error to <3%.

RESULTS

- A total of 3,756 categorical interpretations were generated for 11 antimicrobial agents when testing 205 contemporary Gram-negative veterinary pathogens by three susceptibility test systems.
- Overall agreement between the 3 systems was 90.8% and agreement between Sensititre ARIS 2X and Vitek 2 was 94.8%.
- Only four organism-antimicrobial agent combinations (0.3%) failed consensus (categorical agreement by ≥2 of the three test systems; Table 1).
- Total error rates were 2.4% and 2.5% for the Sensititre ARIS 2X and Vitek 2 automated systems, respectively, compared with 4.1% for the standard laboratory methods; agreement and total error rates are found in Tables 1 and 2.
- Rates for very major (VME), major (ME) and minor errors (MiE) for Sensititre were, respectively, 0.2%, 0.2% and 2.0% and for Vitek 2, 0.0%, 0.5% and 2.0%, all acceptable error rates by established criteria (Table 2).
- VME were detected with the standard method when testing combinations of *P. mirabilis*/trimethoprim-sulfa (4.3%), other ENT/gentamicin (5.3%), other NFB/ceftiofur (5.9%); ME with PSA/amikacin (8.3%)/gentamicin (4.0%) and other NFB/ampicillin (5.3%)/trimethoprim-sulfa (8.0%); and MiE exceeding threshold with certain ENT, PSA and other NFB for several agents.
- Sensititre VME occurred with combinations of other ENT/ampicillin (5.3%)/trimethoprim-sulfa (8.0%); ME with PAS/ampicillin (14.3%); and MiE exceeding threshold with other enteric bacilli, and, notably, PSA.
- No VME were found with the Vitek 2; MEs were seen with combinations of other NFB/trimethoprim-sulfa (12.0%); and MiE exceeding threshold were seen with certain ENT, PSA and other NFB (Table 2).

Table 1. Summary of susceptibility testing agreement and errors by antimicrobial agent and system when testing Gram-negative isolates originating from domestic animals.

			% Error Rate			No Agreement Between Methods(n)
Antimicrobial Agent	n	% Agreement All Systems	Standard Method	Sensititre ARIS 2X	Vitek 2	
Amikacin	50	94.0	6.0	0	0	0
Amoxicillin/Clavulanic Acid	151	89.4	4.0	1.3	4.0	1.3
Ampicillin	141	89.4	6.4	1.4	1.4	1.4
Cefpodoxime	2	100	0	0	0	0
Ceftiofur	165	91.1	4.8	0	3.0	0
Chloramphenicol	33	72.7	0	15.2	12.1	0
Enrofloxacin	187	84.0	5.9	8.0	2.1	0
Gentamicin	176	93.2	4.5	0	2.3	0
Imipenem	27	92.6	0	3.7	3.7	0
Marbofloxacin	154	95.5	1.3	2.0	1.3	0
Trimethoprim/Sulfamethoxazole	166	94.6	2.4	1.2	1.8	0
Total	**1252**	**90.8**	**4.1**	**2.4**	**2.5**	**4 (0.3%)**

Table 2. Error rate summary for 205 Gram-negative bacterial pathogens (year 2010) originating from domestic animals. Susceptibility testing was performed by two automated systems and the standard laboratory method.

			% Error Rate											
			Very Major Error			Major Error			Minor Error			Combined Error		
Isolate Species/Group	Antimicrobial Agent	n	Std. Method	Sensititre ARIS 2X	Vitek 2	Std. Method	Sensititre ARIS 2X	Vitek 2	Std. Method	Sensititre ARIS 2X	Vitek 2	Std. Method	Sensititre ARIS 2X	Vitek 2
Escherichia coli n=47	Amikacin	10	0	0	0	0	0	0	0	0	0	0	0	0
	Amoxicillin/Clavulanic acid	47	0	0	0	0	0	2.1	2.1	2.1	2.1	2.1	2.1	4.3
	Ampicillin	47	0	0	0	0	0	0	10.6	0	2.1	10.6	0	2.1
	Cefpodoxime	2	0	0	0	0	0	0	0	0	0	0	0	0
	Ceftiofur	46	0	0	0	0	0	0	0	0	0	0	0	0
	Chloramphenicol	10	0	0	0	0	0	0	0	0	20.0	0	0	20.0
	Enrofloxacin	47	0	0	0	0	0	0	0	0	0	0	0	0
	Gentamicin	47	0	0	0	0	0	0	0	0	0	0	0	0
	Marbofloxacin	38	0	0	0	0	0	0	0	0	0	0	0	0
	Trimethoprim/Sulfamethoxazole	47	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**342**	**0**	**0**	**0**	**0**	**0**	**2.9**	**1.8**	**2.9**	**1.2**	**1.8**	**2.9**	**1.5**
Klebsiella species n=16	Amoxicillin/Clavulanic acid	16	0	0	0	0	0	0	0	0	6.3	0	0	6.3
	Ampicillin	16	0	0	0	0	0	0	0	0	0	0	0	0
	Ceftiofur	15	0	0	0	0	0	0	0	0	13.3	0	0	13.3
	Enrofloxacin	16	0	0	0	0	0	0	0	0	0	0	0	0
	Gentamicin	16	0	0	0	0	0	0	0	0	0	0	0	0
	Marbofloxacin	15	0	0	0	0	0	0	0	0	0	0	0	0
	Trimethoprim/Sulfamethoxazole	16	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**112**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2.7**	**0**	**0**	**2.7**
Proteus mirabilis n=23	Amikacin	4	0	0	0	0	0	0	0	0	0	0	0	0
	Amoxicillin/Clavulanic acid	23	0	0	0	0	0	0	4.3	0	4.3	4.3	0	4.3
	Ampicillin	23	0	0	0	0	0	0	0	0	0	0	0	0
	Ceftiofur	22	0	0	0	0	0	0	4.5	0	0	4.5	0	0
	Chloramphenicol	4	0	0	0	0	0	0	0	0	25.0	0	0	25.0
	Enrofloxacin	23	0	0	0	0	0	0	0	0	0	0	0	0
	Gentamicin	23	0	0	0	0	0	0	0	0	0	0	0	0
	Marbofloxacin	19	0	0	0	0	0	0	0	0	0	0	0	0
	Trimethoprim/Sulfamethoxazole	23	4.3	0	0	0	0	0	4.3	0	0	0	0	0
	Total	**164**	**0.6**	**0**	**0**	**0**	**0**	**0**	**1.8**	**0**	**1.2**	**2.4**	**0**	**1.2**
Other Enterobacteriaceae n=57	Amikacin	6	0	0	0	0	0	0	0	0	0	0	0	0
	Amoxicillin/Clavulanic acid	45	0	0	0	0	0	2.2	6.7	0	2.2	6.7	0	4.4
	Ampicillin	29	0	0	0	0	0	0	3.4	0	0	3.4	0	0
	Ceftiofur	52	0	0	0	0	0	1.9	7.7	0	0	7.7	0	1.9
	Chloramphenicol	6	0	0	0	0	0	0	0	16.7	16.7	0	16.7	16.7
	Enrofloxacin	49	0	0	0	0	0	0	4.1	4.1	4.1	4.1	4.1	4.1
	Gentamicin	38	5.3	0	0	0	0	0	0	0	0	5.3	0	0
	Marbofloxacin	42	0	0	0	0	0	0	0	0	0	0	0	0
	Trimethoprim/Sulfamethoxazole	49	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**317**	**0.6**	**0**	**0**	**0**	**0**	**0.6**	**3.2**	**1.0**	**1.3**	**3.8**	**1.0**	**1.9**
Pseudomonas aeruginosa n=25	Amikacin	24	0	0	0	8.3	0	0	4.2	0	0	12.5	0	0
	Ceftiofur	6	0	0	0	0	0	0	0	0	0	0	0	0
	Chloramphenicol	6	0	0	0	0	0	0	0	66.7	0	0	66.7	0
	Enrofloxacin	25	0	0	0	0	0	0	20.0	48.0	4.0	20.0	48.0	4.0
	Gentamicin	25	0	0	4.0	0	0	0	20.0	0	12.0	24.0	0	12.0
	Imipenem	20	0	0	0	0	0	0	0	5.0	5.0	0	5.0	5.0
	Marbofloxacin	20	0	0	0	0	0	0	5.0	15.0	5.0	5.0	15.0	5.0
	Total	**126**	**0**	**0**	**0**	**2.4**	**0**	**0**	**9.5**	**15.9**	**4.8**	**11.9**	**15.9**	**4.8**
Other Non-fermentative GNB n=29	Amoxicillin/Clavulanic acid	19	0	0	0	0	0	0	5.3	0	0	5.3	0	0
	Ampicillin	19	0	5.3	0	5.3	0	0	10.5	0	5.3	15.8	5.3	5.3
	Ceftiofur	17	5.9	0	0	0	0	0	11.8	0	11.8	17.6	0	11.8
	Enrofloxacin	20	0	0	0	0	0	0	20.0	5.0	5.0	20.0	5.0	5.0
	Gentamicin	20	0	0	0	0	0	0	0	0	5.0	0	0	5.0
	Marbofloxacin	19	0	0	0	0	0	0	5.3	0	5.3	5.3	0	5.3
	Trimethoprim/Sulfamethoxazole	25	0	8.0	0	8.0	0	12.0	0	0	0	8.0	8.0	12.0
	Total	**140**	**0.7**	**2.1**	**0**	**2.1**	**0**	**2.1**	**7.1**	**0.7**	**4.3**	**10.0**	**2.9**	**6.4**
Pasteurella/Actinobacillus species n=8	Amikacin	5	0	0	0	0	0	0	0	0	0	0	0	0
	Ampicillin	7	0	0	0	0	14.3	0	0	0	0	0	14.3	0
	Ceftiofur	7	0	0	0	0	0	0	0	0	0	0	0	0
	Chloramphenicol	5	0	0	0	0	0	0	0	0	0	0	0	0
	Enrofloxacin	7	0	0	0	0	0	0	0	0	0	0	0	0
	Gentamicin	7	0	0	0	0	0	0	0	0	0	0	0	0
	Imipenem	5	0	0	0	0	0	0	0	0	0	0	0	0
	Trimethoprim/Sulfamethoxazole	6	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**51**	**0**	**0**	**0**	**0**	**3.9**	**0**	**0**	**0**	**0**	**0**	**3.9**	**0**
	Total Error	**1252**	**0.3**	**0.2**	**0**	**0.5**	**0.2**	**0.5**	**3.3**	**2.0**	**2.0**	**4.1**	**2.4**	**2.5**

CONCLUSIONS

- A total of 3,756 categorical interpretations were generated for 11 antimicrobial agents when testing 205 contemporary Gram-negative veterinary pathogens; only four organism-antimicrobial agent combinations (0.3%) failed consensus (categorical agreement by ≥2 of the three test systems).
- Sensititre ARIS 2X and Vitek 2 had low and acceptable total error rates (2.4 and 2.5%, respectively), compared with 4.1% by the standard method; concordance between the two automated systems was 94.8%).
- VME, ME and MiE for Sensititre were, respectively, 0.2%, 0.2% and 2.0% and for Vitek 2, 0.0%, 0.5% and 2.0%, all acceptable by established criteria.
- Despite overall performance, certain organism/antimicrobial agent combinations proved to be unacceptable, and mostly involved *P. aeruginosa* and other NFB.
- While limitations with each system were apparent and require consideration with implementation consistent with the animal population being served and prevalence of commonly occurring pathogens, the automated systems provide a high level of concordance and low total error rates.
- Critical veterinary-specific antimicrobics were not common on all systems and precluded their assessment; comparisons of drugs available on each system and client needs are therefore required.
- Comparative data using frozen-form microbroth dilution panels remains to be performed to adjust final error rate analyses.

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