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# Evaluation of the Sensititre Aris® 2X and Vitek® 2 Automated Systems for Antimicrobial Susceptibility Testing of Staphylococci and Enterococci Originating from Veterinary Sources

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## REVISED ABSTRACT*

**Background:** Reliable detection of resistance in veterinary pathogens is critical for effective treatment, control and prevention. Given the widespread use of automated systems, continued performance assurance requires periodic re-evaluation with contemporary isolates. We evaluated the accuracy of the Sensititre ARIS 2X (TREK Diagnostic Systems) and Vitek 2 (bioMerieux automated susceptibility systems when testing Gram-positive pathogens.

**Methods:** Bacterial isolates (65 staphylococci and 45 enterococci) recovered in 2010 from companion animals (urine, 46.4%; skin and soft tissue, 33.6%; respiratory, 16.4%; others, 3.6%) were tested using veterinary specific Sensititre® (Part No. COMPAN1F) and Vitek 2 (Part No. AST-GP69) susceptibility panels. Results were compared with routine laboratory methods (primarily disk diffusion testing using CLSI M31 breakpoints or the Vitek Legacy). Consensus was defined as all three systems with matching categorical results; discordant results were considered as errors.

**Results:** A total of 1,737 categorical interpretations were generated for 10 antimicrobial agents. Overall agreement between the 3 systems was 82.6%; Sensititre ARIS 2X and Vitek 2 agreement was 93.9%. Those agents displaying >=95% agreement included ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole. Total error rates were 3.6% and 1.7% for the Sensititre and Vitek 2, respectively, compared with 11.4% for the standard laboratory methods. Agreement and error rates are in the Table.

| | | | % Error Rate | | |
|---|---|---|---|---|---|
| Antimicrobial Agent | n | % Agreement | Std. Method | Sensititre ARIS 2X | Vitek 2 |
| Ampicillin | 45 | 100 | 0 | 0 | 0 |
| Chloramphenicol | 24 | 95.8 | 4.2 | 0 | 0 |
| Clindamycin | 12 | 83.3 | 8.3 | 8.3 | 0 |
| Enrofloxacin | 107 | 68.2 | 25.2 | 4.7 | 1.0 |
| Erythromycin | 99 | 84.8 | 7.1 | 4.0 | 4.0 |
| Gentamicin | 64 | 89.1 | 1.6 | 4.7 | 3.1 |
| Marbofloxacin | 91 | 65.9 | 24.2 | 4.4 | 3.3 |
| Penicillin | 12 | 66.7 | 16.7 | 16.7 | 0 |
| Oxacillin | 64 | 93.8 | 3.1 | 3.1 | 0 |
| Trimethoprim/Sulfamethoxazole | 61 | 95.1 | 4.9 | 0 | 0 |

**Conclusions:** Sensititre ARIS 2X and Vitek 2 had low and acceptable error rates (3.6 and 1.7%, respectively) when testing this collection of contemporary Gram-positive veterinary pathogens, compared with an unacceptable 11.4% error rate using established laboratory methods. Most errors occurred when testing enterococci and fluoroquinolones by disk diffusion (25%), and were infrequent with the newer automated methods (<5%).

*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 bacilli 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 have demonstrated individual organism-antimicrobial agent weaknesses in automated systems when performing antimicrobial susceptibility testing. In this study we evaluated the accuracy of the Sensititre ARIS 2X (TREK Diagnostic Systems) and Vitek 2 (bioMérieux) systems compared with existing laboratory methods (primarily disk diffusion) on a contemporary (2010) challenge collection of Gram-positive 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 (110 total) were recovered largely from domestic and exotic animals by a USA midwest veterinary diagnostic laboratory in 2010, and included staphylococci (65) and enterococci (45). Isolates recovered originated from urine (46.4%); skin and soft tissue (33.6%); respiratory tract (16.4%); and other specimens (3.6%). Further details of species and numbers tested during this period are found in Table 1.

**Identification and susceptibility testing methods.** All isolates were tested with existing laboratory biochemical algorithms (rapid spot tests, biochemical strip tests, traditional tubed biochemicals) with confirmation, where needed, by alternative methods (Sensititre GPID panels incubated and read by the ARIS 2X System; and Vitek 2 GP 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. AST-GP69) 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). Acceptability of errors was assessed as described previously (Jorgensen, 1993; CLSI M23-A3, 2008); generally, total error should be kept to <10%, very major error to <1.5% and major error to <3%.

## RESULTS

- A total of 1,737 categorical interpretations were generated for 10 antimicrobial agents when testing 110 contemporary Gram-positive veterinary pathogens.
- Only four organism-antimicrobial agent combinations (0.7%) failed consensus (categorical agreement by ≥2 of the three test systems; Table 1).
- Overall agreement between the 3 systems was 82.6%; Sensititre and Vitek 2 agreement was 93.9%.
- Total error rates were 3.6% and 1.7% for the Sensititre ARIS 2X and Vitek 2 automated systems, respectively, compared with 11.4% for the standard laboratory methods; agreement and total error rates are in Tables 1 and 2.
- Rates for very major (VME), major error (ME) and minor errors (MiE) for Sensititre were, respectively, 0.7%, 0.0% and 2.9% and for Vitek 2, 0.0%, 0.0% and 1.7%, all very acceptable.
- Most Sensititre ARIS 2X VME occurred when testing the combinations of staphylococci/penicillin (22.2%); and MiE when testing staphylococci/clindamycin (33.3%)/erythromycin (9.5%), and enterococci/fluoroquinolones (8.9-11.4%)/erythromycin (5.7%).
- All Vitek 2 errors (MiE only) occurred when testing staphylococci/gentamicin (4.7%) and enterococci/fluoroquinolones (8.6%)/erythromycin (11.4%).
- Those agents displaying ≥95% agreement included ampicillin, chloramphenicol, oxacillin and trimethoprim/sulfamethoxazole.

**Table 1. Summary of susceptibility testing agreement and errors by antimicrobial agent and system when testing animal-origin Gram-positive (staphylococci and enterococci) isolates.**

| | | | % Error Rate | | | No Agreement Between Methods |
|---|---|---|---|---|---|---|
| Antimicrobial Agent | n | % Agreement All Systems | Standard Method | Sensititre ARIS 2X | Vitek 2 | |
| Ampicillin | 45 | 100 | 0 | 0 | 0 | 0 |
| Chloramphenicol | 24 | 95.8 | 4.2 | 0 | 0 | 0 |
| Clindamycin | 12 | 83.3 | 8.3 | 8.3 | 0 | 0 |
| Enrofloxacin | 107 | 68.2 | 25.2 | 4.7 | 1.0 | 1 |
| Erythromycin | 99 | 84.8 | 7.1 | 4.0 | 4.0 | 0 |
| Gentamicin | 64 | 89.1 | 1.6 | 4.7 | 3.1 | 1 |
| Marbofloxacin | 91 | 65.9 | 24.2 | 4.4 | 3.3 | 2 |
| Penicillin | 12 | 66.7 | 16.7 | 16.7 | 0 | 0 |
| Oxacillin | 64 | 93.8 | 3.1 | 3.1 | 0 | 0 |
| Trimethoprim/Sulfamethoxazole | 61 | 95.1 | 4.9 | 0 | 0 | 0 |
| **Total** | **579** | **82.6** | **11.4** | **3.6** | **1.7** | **4 (0.7%)** |

**Table 2. Susceptibility testing summary for 110 Gram-positive bacterial pathogens (all from year 2010) originating from companion and other animals when tested by two automated systems and the standard laboratory algorithm.**

| | | | % 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 |
| Coagulase Positive *Staphylococcus* | Chloramphenicol | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Clindamycin | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Enrofloxacin | 41 | 2.4 | 0 | 0 | 4.9 | 0 | 0 | 0 | 2.4 | 0 | 7.3 | 2.4 | 0 |
| | Erythromycin | 43 | 0 | 0 | 0 | 4.7 | 0 | 0 | 0 | 0 | 0 | 4.7 | 0 | 0 |
| | Gentamicin | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 2.3 | 4.7 | 4.7 | 2.3 | 4.7 | 4.7 |
| | Marbofloxacin | 38 | 2.6 | 0 | 0 | 5.3 | 0 | 0 | 0 | 0 | 0 | 7.9 | 0 | 0 |
| | Penicillin | 9 | 0 | 22.2 | 0 | 11.1 | 0 | 0 | 0 | 0 | 0 | 11.1 | 22.2 | 0 |
| | Oxacillin | 43 | 2.3 | 4.7 | 0 | 2.3 | 0 | 0 | 0 | 0 | 0 | 4.7 | 4.7 | 0 |
| | Trimethoprim/Sulfamethoxazole | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 5.0 | 0 | 0 | 5.0 | 0 | 0 |
| | **Total** | **275** | **1.1** | **1.5** | **0** | **2.9** | **0** | **0** | **1.3** | **1.1** | **0.7** | **5.1** | **2.5** | **0.7** |
| Coagulase Negative *Staphylococcus* | Chloramphenicol | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Clindamycin | 3 | 33.3 | 0 | 0 | 0 | 0 | 0 | 0 | 33.3 | 0 | 33.3 | 33.3 | 0 |
| | Enrofloxacin | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 4.8 | 0 | 0 | 4.8 | 0 | 0 |
| | Erythromycin | 21 | 0 | 0 | 0 | 4.8 | 0 | 0 | 4.8 | 9.5 | 0 | 9.5 | 9.5 | 0 |
| | Gentamicin | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.8 | 0 | 0 | 4.8 | 0 |
| | Marbofloxacin | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 5.6 | 0 | 0 | 5.6 | 0 | 0 |
| | Penicillin | 3 | 0 | 0 | 0 | 33.3 | 0 | 0 | 0 | 0 | 0 | 33.3 | 0 | 0 |
| | Oxacillin | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Trimethoprim/Sulfamethoxazole | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 4.8 | 0 | 0 | 4.8 | 0 | 0 |
| | **Total** | **132** | **0.8** | **0** | **0** | **1.5** | **0** | **0** | **3.0** | **3.0** | **0** | **5.3** | **3.0** | **0** |
| *Enterococcus* species | Ampicillin | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Chloramphenicol | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 8.3 | 0 | 0 | 8.3 | 0 | 0 |
| | Enrofloxacin | 45 | 0 | 0 | 0 | 2.2 | 0 | 0 | 48.9 | 8.9 | 2.2 | 51.1 | 8.9 | 2.2 |
| | Erythromycin | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 8.6 | 5.7 | 11.4 | 8.6 | 5.7 | 11.4 |
| | Marbofloxacin | 35 | 0 | 0 | 0 | 2.9 | 0 | 0 | 48.6 | 11.4 | 8.6 | 51.4 | 11.4 | 8.6 |
| | **Total** | **172** | **0** | **0** | **0** | **1.2** | **0** | **0** | **25.0** | **5.8** | **4.7** | **26.2** | **5.8** | **4.7** |
| | **Total Error** | **579** | **0.7** | **0.7** | **0** | **2.1** | **0** | **0** | **8.6** | **2.9** | **1.7** | **11.4** | **3.6** | **1.7** |

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## CONCLUSIONS

- A total of 1,737 categorical interpretations were generated for 10 antimicrobial agents when testing 110 contemporary Gram-positive veterinary pathogens; only four organism-antimicrobial agent combinations (0.7%) failed consensus (categorical agreement by ≥2 of the three test systems).
- Sensititre ARIS 2X and Vitek 2 had low and acceptable total error rates (3.6 and 1.7%, respectively), compared with an unacceptable 11.4% error rate using established laboratory methods (primarily disk diffusion).
- VME, ME and MiE for Sensititre were, respectively, 0.7%, 0.0% and 2.9% and for Vitek 2, 0.0%, 0.0% and 1.7%, all within accepted limits.
- Most errors occurred when testing enterococci and fluoroquinolones by disk diffusion, and were less frequent with the newer automated methods.
- While limitations with each system were apparent and require consideration with implementation depending upon the animal population being served and prevalence of commonly occurring pathogens, the automated systems provided a high level of concordance (93.9%) 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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