

NOTES

Antimicrobial Susceptibility Testing of Veterinary Clinical Isolates with the Sceptor System

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The Sceptor System (Becton Dickinson) was compared with an agar dilution method for antimicrobial susceptibility testing of veterinary clinical isolates. The results indicate that the Sceptor System may be used to test gram-positive and fastidious gram-negative bacteria.

In vitro antimicrobial susceptibility testing of clinical isolates is performed in order to assist medical personnel in proper drug selection. Traditionally, the disk agar diffusion method described by Bauer et al. (2) has been used in North American bacteriology laboratories because of its technical simplicity and reproducibility. The qualitative results derived from the disk diffusion technique attempt to correlate

ically entered into a computer which calculates the MICs and adds the National Committee for Clinical Laboratory Standards (NCCLS) (13) interpretation. Jones et al. (10) demonstrated greater than 96.9% agreement for 9,840 MIC determinations performed for stock culture strains and 95.0% agreement for 7,308 MICs obtained for clinical isolates. Sceptor breakpoint panels also demonstrated 94.1%

TABLE 1. Comparison of the Sceptor System and an agar dilution method for antimicrobial susceptibility testing of fastidious gram-negative bacteria of veterinary origin

Antimicrobial agent	Range of dilutions tested ($\mu\text{g/ml}$) ^a	No. (%) of results in complete agreement	No. of errors		
			Minor	Major	Very major
Amikacin	2-32	68 (90.7)	7	0	0
Ampicillin	2-16	67 (89.3)	2	0	6
Cephalothin	2-32	69 (92.0)	6	0	0
Chloramphenicol	8-32	73 (97.3)	2	0	0
Gentamicin	0.5-8	55 (73.3)	20	0	0
Neomycin	1-16	50 (66.7)	23	1	1
Tetracycline	1-16	74 (98.7)	1	0	0
Trimethoprim-sulfamethoxazole	1/19-16/304	74 (98.7)	0	0	1
Total (%)		530 (88.3)	61 (10.2)	1 (0.2)	8 (1.3)

^a Doubling dilutions.

inhibitory-zone sizes with achievable antimicrobial drug levels in human serum. However, these results may be inappropriate in veterinary medicine because of differences in animal drug metabolism. Libal (12) demonstrated that the inhibitory-zone sizes for some antimicrobial agents should be larger for animal pathogens than those zone sizes recommended for testing human pathogens. It has been suggested that MIC determination will result in a more rational and successful use of antimicrobial agents by veterinary clinicians (12).

The Sceptor System (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) is a broth microdilution system that uses plastic microtiter plates with doubling dilutions of desiccated antimicrobial agents. The results are automat-

agreement with 10,368 control organism-antimicrobial agent comparisons and 97.0% concordance with 4,872 clinical comparisons (5). Gram-positive MIC determinations by the Sceptor System compared favorably with those obtained by a reference agar dilution method (9). However, these studies involved human clinical isolates and commercially available Sceptor panels. In this report, we assessed the accuracy of the customized Sceptor panels for the antimicrobial susceptibility testing of gram-positive and fastidious gram-negative bacteria isolated from veterinary clinical cases.

The organisms tested were recovered from veterinary clinical specimens submitted to the Clinical Microbiology Laboratory of the Ontario Veterinary College or the Veterinary Laboratory Service of the Ontario Ministry of Agriculture and Food. The isolates were identified by conventional methods (3, 4, 6, 7, 11, 14, 15). A total of 136 gram-positive

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TABLE 2. Comparison of the Sceptor System and an agar dilution method for antimicrobial susceptibility testing of gram-positive bacteria of veterinary origin

Antimicrobial agent	Range of dilutions tested ($\mu\text{g/ml}$) ^a	No. (%) of results in complete agreement	No. of errors		
			Minor	Major	Very major
Amikacin	2-32	119 (87.5)	14	0	3
Ampicillin	2-16	133 (97.8)	3	0	0
Amoxicillin-clavulanic acid	4/2-64/32	135 (99.3)	0	1	0
Cephalothin	2-32	134 (98.5)	1	1	0
Chloramphenicol	2-32	131 (96.3)	4	1	0
Clindamycin	0.25-4	131 (96.3)	4	1	0
Erythromycin	0.5-8	133 (97.8)	3	0	0
Gentamicin	0.5-8	129 (94.9)	6	0	1
Tetracycline	1-16	128 (94.1)	6	1	1
Trimethoprim-sulfamethoxazole	1/19-16/304	129 (94.9)	5	1	1
Total (%)		1,302 (95.7)	46 (3.4)	6 (0.4)	6 (0.4)

^a Doubling dilutions.

bacteria including 6 *Bacillus cereus*, 5 *Bacillus licheniformis*, 5 *Corynebacterium pseudotuberculosis*, 3 *Enterococcus faecium*, 10 *Listeria monocytogenes*, 30 *Staphylococcus aureus*, 2 *Staphylococcus epidermidis*, 2 *Staphylococcus hyicus* subspecies *hyicus*, 6 *Staphylococcus intermedius*, 6 *Streptococcus agalactiae*, 3 *Streptococcus constellatus*, 6 *Streptococcus dysgalactiae*, 5 *Streptococcus equi*, 2 *Streptococcus equisimilis*, 7 *Streptococcus* group G, 5 *Streptococcus mutans*, 3 *Streptococcus sanguis*, 4 *Streptococcus suis*, 1 *Streptococcus uberis*, and 25 *Streptococcus zooepidemicus* isolates were tested with the Sceptor System and a reference agar dilution method (13). Similarly, the Sceptor System and agar dilution method were used to compare 10 *Actinobacillus equuli*, 10 *Actinobacillus suis*, 22 *Bordetella bronchiseptica*, 17 *Pasteurella haemolytica*, and 16 *Pasteurella multocida* isolates.

The Sceptor System was operated in accordance with the manufacturer's instructions. Organisms of the genera *Actinobacillus* and *Pasteurella* required Sceptor anaerobe broth for growth. The MICs were recorded after 18 to 24 h of incubation at 37°C, except for *C. pseudotuberculosis*, which required 48 h of incubation. Interpretation of the MICs was derived from the NCCLS standards (13).

Antimicrobial agents were incorporated into Mueller-Hinton agar plates as described by Barry (1). The range of antimicrobial dilutions tested was identical to those of the Sceptor gram-negative and gram-positive test panels (Tables 1 and 2). For trimethoprim-sulfamethoxazole, laked horse blood replaced calf blood (8). The MICs were recorded and interpreted according to the NCCLS standards (13). Categorical agreement classified the results as complete agreement, minor error (involving a moderately susceptible result), major error (resistant by the Sceptor System and susceptible by the reference method), and very major error (susceptible by the Sceptor System and resistant by the reference method).

The quality of the Sceptor panels and the agar dilution method was assessed by testing the following quality control strains: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213. The MICs were all within NCCLS limits (13).

There was 88.3% complete agreement between the Sceptor System and agar dilution method for fastidious gram-negative bacteria (Table 1). Gentamicin and neomycin resulted in only 73.3 and 66.7% complete agreement,

respectively. In both cases, the majority of errors were minor and involved isolates from all species tested. Although the therapeutic relevance of the high number of minor errors is questionable, Sceptor System results from testing fastidious gram-negative bacteria with gentamicin and neomycin should be interpreted with caution.

Six isolates of *P. haemolytica* were susceptible to ampicillin when tested by the Sceptor System and resistant when tested by the agar dilution method. These strains produced β -lactamase, which was not induced in the Sceptor System ampicillin test wells. The use of Sceptor anaerobe broth instead of gram-negative broth may have influenced the production of this enzyme.

Testing gram-positive bacteria with the Sceptor System and the agar dilution method resulted in 95.7% complete agreement. There was less than 90% complete agreement when amikacin was tested. The errors were mainly minor and involved streptococci.

Approximately 60% of the gram-negative and 77% of the gram-positive bacteria were "off scale" with respect to the MIC dilutions tested. The large number of off-scale MIC results was expected, since the bacteria were selected from random clinical specimens and not for their antimicrobial characteristics. This may be significant because differences in MIC results can be masked when one or both MICs are off scale.

Thornsberry (14) suggested that complete agreement of a new system be at least 90% and that the combined major and very major errors be less than 5%. The Sceptor System clearly met these guidelines when gram-positive bacteria were tested (Table 2) and may be used to routinely assess antimicrobial susceptibility of gram-positive veterinary isolates. Fastidious gram-negative bacteria resulted in only 88.3% complete agreement, but the combined major and very major errors were less than 5% (Table 1). If gentamicin and neomycin were not tested with fastidious gram-negative bacteria, there would be greater than 90% complete agreement. Therefore, gentamicin and neomycin susceptibility results should not be reported when fastidious gram-negative bacteria of veterinary origin are tested by the Sceptor System.

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