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Collaborative Investigation of the Accuracy and Reproducibility of Sceptor Breakpoint Susceptibility Panels

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A combination Sceptor Breakpoint/ID panel (Johnston Laboratories, Inc., Towson, Md.), which determines interpretive susceptibility results (susceptible, moderately susceptible, and resistant) using two to three selected concentrations of antimicrobial agents, was tested in comparison with full-range Sceptor microdilution MIC panels. The inter- and intralaboratory interpretive reproducibilities for 24 control strains tested in three laboratories on three consecutive days were 97.0 and 95.7%, respectively. The equivalency of breakpoint results to category results obtained by the microdilution MIC procedure for 10,368 control organism-antimicrobial agent comparisons was 94.1%. The level of interpretive agreement between breakpoint and MIC category results using 101 fresh clinical isolates was 97.0% for 51 gram-negative and 50 gram-positive bacteria. Among the total 4,872 clinical organism-antimicrobial agent comparisons, major and very major discrepancies were seen in 0.2% of gram-negative bacteria and very major discrepancies were seen in 0.9% of gram-positive bacteria. All very major discrepancies with gram-positive organisms were associated with trailing endpoints using trimethoprim or sulfisoxazole and staphylococci. The breakpoint concept of testing selective antimicrobial agent concentrations was highly reproducible and accurate and allows for placement of more antimicrobial agents into a panel than is possible with full-dilution MIC testing.

One of the major roles of the clinical microbiology laboratory is to evaluate the effectiveness of different antimicrobial agents against bacteria as a guide to therapy. Most laboratories perform either the qualitative agar disk diffusion procedure or the quantitative broth microdilution procedure for antimicrobial susceptibility testing, although these may not be the sole methods used. Performance standards for agar disk diffusion and dilution susceptibility tests provide interpretive criteria which relate the zone size or MIC to antimicrobial activity in the form of susceptibility categories (susceptible, intermediate, moderately susceptible, or resistant [6, 7]). Interpretive results are on the basis of achievable levels in serum and, for a few specialized antimicrobial agents, in the urinary tract.

The utility of routine reporting of quantitative MIC results in situations other than systemic infections has been a subject of debate and source of confusion between laboratory workers and physicians (4). For this reason, some laboratories perform MIC tests but report only interpretive or both MIC and interpretive results. These laboratories frequently use commercial broth microdilution systems because the procedures can be semiautomated or automated and fit into their work flow better than does the agar disk diffusion method. If quantitative MIC results are not needed, then the number of test wells containing a particular antimicrobial agent might be limited to a few concentrations representing the susceptible and resistant breakpoints. The unused wells could be used for testing more antimicrobial agents than would be possible in full-microdilution plates. A number of manufacturers have recently applied the breakpoint concept to formulating microdilution antimicrobial agent susceptibility panels. In this report, we summarize the accuracy and reproducibility of Sceptor Gram-Positive and Gram-Negative Breakpoint/ID panels (Johnston Laboratories, Inc., Towson, Md.; Sceptor is a registered trademark of Becton Dickinson and Co.) evaluated in three laboratories. The interpretive results obtained with breakpoint and full-dilution MIC panels were compared.

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MATERIALS AND METHODS

Organisms. The bacterial strains selected for this study were tested in two phases. In phase 1, 24 quality control strains commonly used to evaluate Sceptor panels were processed. The organisms tested were *Acinetobacter antitratus* ATCC 33498, *Bacillus cereus* ATCC 11778, *Enterobacter aerogenes* ATCC 35028, *E. aerogenes* ATCC 35029, *Enterobacter cloacae* ATCC 35030, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 33495, *Proteus vulgaris* ATCC 8427, *Providencia stuartii* ATCC 33672, *P. stuartii* ATCC 35031, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 35032, *Serratia rubidaea* ATCC 33670, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* ATCC 33497, *S. aureus* 83PO279 and 83PO280 (methicillin-resistant), *Staphylococcus epidermidis* 80P8018, *Staphylococcus sciuri* ATCC 29062, *Staphylococcus xylosus* ATCC 35033, *Streptococcus bovis* ATCC 35034, *Streptococcus faecalis* ATCC 29212, and *Streptococcus pyogenes* ATCC 19615. These organisms were selected to provide on-scale results for each of the antimicrobial agents used. Each strain was tested on three different days. On each test day, three breakpoint panels and one of each reference panel were tested in three participating laboratories (BBL Microbiology Systems, Cockeysville, Md.; Sinai Hospital of Detroit, Detroit, Mich.; and Veterans Administration Medical Center, Baltimore, Md.).

A total of 101 fresh clinical isolates were tested in phase 2. The distribution of bacteria were *Citrobacter diversus* (8 isolates), *E. aerogenes* (4 isolates), *E. cloacae* (8 isolates), *E.*

coli (8 isolates), *Klebsiella ozaenae* (1 isolate), *K. pneumoniae* (6 isolates), *Morganella morganii* (1 iso late), *Proteus mirabilis* (6 isolates), *P. vulgaris* (1 isolate), *Serratia marcescens* (8 isolates), methicillin-resistant (heteroresistant) *S. aureus* (15 isolates), methicillin-susceptible *S. aureus* (15 isolates), methicillin-resistant, coagulase-negativestaphylococci (10 isolates), and methicillin-susceptible, coagulase-negative staphylococci (10 isolates). All methicillin-resistant staphylococcal strains were confirmed resistant by the oxacillin agar screen procedure (5). The organisms were identified in accordance with the approaches outlined in the *Manual of Clinical Microbiology* (1). Each clinical isolate was tested on one day in triplicate with breakpoint panels and once with each of the full-dilution MIC panels. The Veterans Administration Medical Center tested gram-negative bacilli (51 isolates), and Sinai Hospital of Detroit tested gram-positive strains (50 isolates).

Susceptibility test panels. Each laboratory was provided with common lots of commercially prepared Sceptor panels and broth. Only the susceptibility portion of the combination susceptibility/identification panel was evaluated.

(i) Breakpoint/ID. The Sceptor Gram-Negative Breakpoint/ID panel is an 84-well plastic plate containing 24 antimicrobial agents and 24 biochemical substrates in dehydrated form. The antimicrobial agents and concentrations (micrograms per milliliter) include: amikacin, 16 and 32; ampicillin, 8, 16, and 32; azlocillin, 16, 64, and 128; cefamandole, 8, 16, and 32; cefazolin, 8, 16, and 32; cefoperazone, 16, 32, and 64; cefotaxime, 8 and 32; cefoxitin, 8, 16, and 32; ceftazidime, 8 and 16; ceftriaxone, 8 and 32; cefuroxime, 8, 16, and 32; cephalothin, 8, 16, and 32; chloramphenicol, 8 and 16; gentamicin, 4 and 8; mezlocillin, 16, 64, and 128; moxalactam, 8 and 32; nalidixic acid, 16 and 32; netilmicin, 4 and 16; nitrofurantoin, 64 and 128; piperacillin, 16, 64, and 128; trimethoprim-sulfamethoxazole, 2/38, 8/152, and 16/304; tetracycline, 4 and 8; ticarcillin, 16, 64, and 128; and tobramycin, 4 and 8.

The Sceptor Gram-Positive Breakpoint/ID panel contains 16 dried antimicrobial agents both in full-range and limited concentrations and 24 biochemical substrates. The antimicrobial agents tested and concentrations (micrograms per milliliter) were: amikacin, 16, 32, and 64; chloramphenicol, 8, 16, and 32; clindamycin, 0.5, 1, and 4; nitrofurantoin, 64 and 128; oxacillin, 2 and 4; sulfisoxazole, 256 and 512; tetracycline, 4, 8, and 16; and trimethoprim, 8 and 16. The remaining antimicrobial agents with full-range concentrations were not evaluated. In both panel designs, the antimicrobial agent concentrations were selected to correlate with MIC interpretive standards for categories of susceptibility recommended by the National Committee for Clinical Laboratory Standards (8).

(ii) Reference. The Sceptor full-dilution MIC microdilution procedure was used as the reference method. Selective antimicrobial agents from several conventional panels were chosen for evaluation on the Breakpoint/ID panels.

Susceptibility test method. The inoculum was prepared from a pure culture of bacteria grown on Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) for 18 to 20 h at 35°C. Common bacterial suspensions prepared in Trypticase soy broth (BBL) served as inocula for reference and breakpoint panels. The Sceptor system was inoculated, incubated, and read according to the directions of the manufacturer. Breakpoint and reference susceptibility panels were read for the presence or absence of visible turbidity. A susceptibility category result was assigned to the corresponding growth patterns for two and three concentrations of antimicrobial agents. For example, an organism growing in all or two of three wells was considered resistant, an organism growing in the well containing the lowest concentration was considered moderately susceptible, and no visible growth in any well indicated susceptibility.

Analysis of data. The interpretive results obtained with the breakpoint and full-dilution MIC panels were compared for each organism-antimicrobial agent combination. In phase 1, the modal reference MIC was converted into an interpretive result for this comparison. The data were then analyzed for intra- and interlaboratory reproducibility and equivalency. Intralaboratory data evaluated represent modal results obtained in nine replicate tests performed by one laboratory. Interlaboratory data evaluated represent variations in the modal results obtained in 9 replicate MIC tests performed by each laboratory compared with the grand modal results obtained in 27 replicate tests performed by all three laboratories.

For phase 2, the level of comparability between the breakpoint and MIC test systems was determined in two clinical laboratories. Disagreements were categorized into minor, major, or very major discrepancies. Minor discrepancies were defined as a susceptible or resistant test result with the breakpoint procedure and a moderately susceptible result with the MIC procedure, and vice versa. Major discrepancies were defined as a resistant test result with the breakpoint procedure and a susceptible result with the MIC procedure. Very major discrepancies were defined as a susceptible test result with the breakpoint procedure and a resistant result with the MIC procedure.

RESULTS

Summaries of the reproducibility and equivalency results for breakpoint susceptibility testing from the first phase of this study are shown in Tables 1 and 2. The overall levels of agreement for intra- and interlaboratory reproducibility were 97.0 and 95.7%, respectively (Table 1). Most errors were caused by minor discrepancies ranging from 2.0 to 3.9%. The frequencies of major and very major discrepancies were less than 1%.

Comparisons between modal reference MIC and breakpoint interpretive results for each laboratory (Table 2) varied from 92.6% at BBL Microbiology Systems to 94.8% at Sinai Hospital and Veterans Administration laboratories. The overall level of agreement for 10,368 organism-antimicrobial agent comparisons was 94.1%. When minor discrepancies

TABLE 1. Reproducibility of Sceptor Breakpoint/ID panels using 24 control bacterial strains[a]

Laboratory	% Intralaboratory/interlaboratory interpretive			
	Agreement[b]	Disagreement[c]		
		Minor	Major	Very major
BBL	96.1/95.0	3.1/3.9	0.3/0.5	0.5/0.6
Sinai	97.4/96.3	2.4/3.2	0.1/0.4	0.1/0.1
Veterans	97.6/95.9	2.0/3.3	0.2/0.1	0.2/0.7

[a] A total of 12 gram-negative and 12 gram-positive control strains were tested in three laboratories, representing 10,368 organism-antimicrobial agent susceptibility comparisons. Each laboratory tested 3,456 organism-antinicrobial agent combinations.

[b] The overall intralaboratory/interlaboratory percents agreement were 97.0 and 95.7%, respectively.

[c] The overall intralaboratory/interlaboratory percents minor, major, and very major discrepancies were 2.5/3.4, 0.2/0.4, and 0.3/0.5%, respectively. For explanation of terms, see the text.

TABLE 2. Comparison of Sceptor breakpoint and MIC interpretive results obtained for 24 control bacterial strains[a]

Laboratory	% Interpretive[b]			
	Agreement	Agreement including minor discrepancies	Disagreement	
			Major	Very major
BBL	92.6	98.9	0.7	0.4
Sinai	94.8	98.8	0.9	0.3
Veterans	94.8	98.9	0.3	0.8

[a] A total of 12 gram-negative and 12 gram-positive control strains were tested in three laboratories, representing 10,368 organism-antimicrobial agent susceptibility comparisons. Each laboratory tested 3,456 organism-antimicrobial agent combinations.

[b] The overall percents interpretive agreements, agreements including minor discrepancies, major discrepancies, and very major discrepancies were 94.1, 98.9, 0.6, and 0.5%, respectively.

were included, the level of agreement was greater than 98.0% for all laboratories. Again, major and very major discrepancies were less than 1%.

Comparative results from phase 2 between full-range MIC and breakpoint interpretive data are shown in Tables 3 and 4. The overall agreement for members of the family *Enterobacteriaceae* was 97.2% (Table 3). The level of agreement when minor discrepancies were included was greater than 98.0%. Among 10 major discrepancies observed (amikacin-*S. marcescens*, three; nitrofurantoin-*E. cloacae*, three; nitrofurantoin-*P. mirabilis*, three; and piperacillin-*S. marcescens*, one), nitrofurantoin was responsible for more than half the errors. Among 10 very major discrepancies noted (cephalothin-*S. marcescens*, one; chloramphenicol-*E. coli*, one; chloramphenicol-*S. marcescens*, one; nalidixic acid-*P. mirabilis*, three; nalidixic acid-*S. marcescens*, three; and nitrofurantoin-*P. mirabilis*, one), nalidixic acid accounted for more than half the errors. No significant antimicrobial agent-organism combination was responsible for major and very major discrepancies.

TABLE 3. Comparison of Sceptor breakpoint and MIC interpretive results for 51 gram-negative bacteria[a]

Antimicrobial agent	No. (%) of interpretive[b]			
	Agreements	Agreements including minor discrepancies	Disagreements	
			Major	Very major
Amikacin	147 (96.1)	3 (98.0)	3 (2.0)	
Ampicillin	150 (98.0)	3 (100)		
Azlocillin	150 (98.0)	3 (100)		
Cefamandole	149 (97.4)	4 (100)		
Cefazolin	153 (100)	0 (100)		
Cefoperazone	153 (100)	0 (100)		
Cefotaxime	152 (99.3)	1 (100)		
Cefoxitin	148 (96.7)	5 (100)		
Ceftazidime	149 (97.4)	4 (100)		
Ceftriaxone	152 (99.3)	1 (100)		
Cefuroxime	143 (93.5)	10 (100)		
Cephalothin	145 (94.8)	7 (99.4)		1 (0.6)
Chloramphenicol	137 (89.6)	14 (98.7)		2 (1.3)
Gentamicin	153 (100)	0 (100)		
Mezlocillin	150 (98.0)	3 (100)		
Moxalactam	151 (98.0)	2 (100)		
Nalidixic acid	147 (96.1)	0 (96.1)		6 (3.9)
Netilmicin	151 (98.6)	2 (100)		
Nitrofurantoin	146 (95.4)	0 (95.4)	6 (3.9)	1 (0.6)
Piperacillin	149 (97.4)	3 (99.3)	1 (0.6)	
Trimethoprim-sulfamethoxazole	153 (100)	0 (100)		
Tetracycline	149 (97.4)	4 (100)		
Ticarcillin	144 (94.1)	9 (100)		
Tobramycin	150 (98.0)	3 (100)		

[a] A total of 51 strains from the family *Enterobacteriaceae* were evaluated, representing 3,672 organism-antimicrobial agent susceptibility comparisons. For each antimicrobial agent, 153 organism combinations were tested.

[b] The overall numbers (percents) of interpretive agreements, agreements including minor discrepancies, major discrepancies, and very major discrepancies were 3,571 (97.2), 3,652 (99.4), 10 (0.2), and 10 (0.2), respectively.

The overall agreement for the staphylococci was 96.8% (Table 4). The level of agreement when minor discrepancies were included was greater than 99.0%. No major discrepancies were observed. All of the very major discrepancies occurred with trimethoprim (methicillin-resistant, coagulase-negative staphylococci, six; methicillin-susceptible, coagulase-negative staphylococci, three) and sulfisoxazole (methicillin-resistant *S. aureus*, three) and were random in distribution.

DISCUSSION

The Sceptor microdilution MIC system has been shown to be a reliable method for the determination of quantitative antimicrobial susceptibility (3). In this study, the interpretive agreement obtained using a limited number of antimicrobial agent concentrations (two to three) was shown to be highly reproducible and accurate when compared with full-dilution MIC results. The frequency of minor discrepancies was probably caused by differences between the actual MIC and breakpoint concentrations used to define susceptibility categories. Since most antimicrobial agents tested have a moderately susceptible category, the one doubling dilution variation considered an acceptable range for MIC testing results most often in minor discrepancies. Therefore, the inclusion of minor discrepancies may represent a more realistic level of equivalency when determining interpretive agreement.

In phase 2 of this study, very major discrepancies between members of the family *Enterobacteriaceae* and nalidixic acid were attributed to false-susceptible results with the Sceptor Breakpoint/ID panels. The problem was identified as a manufacturer's error in the actual concentration of antimicrobial agent in the wells and has been corrected. Among the staphylococci tested, all very major discrepancies were

TABLE 4. Comparison of Sceptor breakpoint and MIC interpretive results for 50 staphylococci[a]

Antimicrobial agent	No. (%) of interpretive[b]		
	Agreements	Agreements including minor discrepancies	Very major disagreements
Amikacin	136 (90.6)	14 (100)	
Chloramphenicol	141 (94.0)	9 (100)	
Clindamycin	150 (100)	0 (100)	
Nitrofurantoin	150 (100)	0 (100)	
Oxacillin	147 (98.0)	3 (100)	
Sulfisoxazole	147 (98.0)	0 (98.0)	3 (2.0)
Tetracycline	148 (98.7)	2 (100)	
Trimethoprim	142 (94.7)	0 (94.7)	8 (5.3)

[a] A total of 50 strains of staphylococci, including 15 methicillin-resistant (heteroresistant) *S. aureus* and 15 methicillin-resistant coagulase-negative isolates, were evaluated, representing 1,200 organism-antimicrobial susceptibility comparisons. For each antimicrobial agent tested, 150 organism combinations were tested.

[b] The overall numbers (percents) of interpretive agreements, agreements including minor discrepancies, major discrepancies, and very major discrepancies were 1,161 (96.8), 1,189 (99.1), 0 (0), and 11 (0.9), respectively. There were no major discrepancies.

associated with trimethoprim or sulfisoxazole. Trailing endpoints by the reference MIC method and absence of growth in the breakpoint panels accounted for these discrepancies.

The breakpoint broth microdilution concept was first described by Witebsky et al. (9), who tested clinically relevant concentrations of antimicrobial agents. Their scheme of selected antimicrobial agent concentrations was designed to simplify the interpretation and reporting of susceptibility data without eliminating a quantitative answer. In a comparison study by Doern et al. (2), the breakpoint broth microdilution test was reported to be at least as accurate as the standard disk diffusion procedure (88.1% concordance) for common aerobic and facultatively anaerobic bacteria. Our results indicate a greater level of interpretive agreement between full-range MIC correlates and breakpoint category results. Inherent differences between the disk diffusion and microdilution MIC procedures for comparison with breakpoint results may account for this disparity.

The addition of Breakpoint/ID panels to the Sceptor system offers several advantages. Each panel allows for up to 24 antimicrobial agents with simultaneous susceptibility and identification in a single setup. Because of the selective concentrations of antimicrobial agents present in the wells of the panel, endpoint determinations are distinct and easy to interpret. Breakpoint panels can be more cost effective and labor saving than the limited full-range MIC panels in determining a broader spectrum of in vitro activity to a variety of antimicrobial agents. This may be helpful to the pharmacy and medical staff that assess antimicrobial agent usage at their own institution. Even with restrictive antimicrobial agent reporting, the microbiology laboratory can select the most appropriate drug on the basis of antimicrobial activity, cost, and pharmacokinetics. Additional quality control testing will be required, however, to assure the performance of breakpoint panels. Although performed less frequently, full-dilution MIC and minimal bactericidal data are needed in certain situations, such as for patients with infective endocarditis.

In summary, the Sceptor Breakpoint/ID panel was found to be as reliable as the full-range microdilution MIC panel for determining the qualitative antimicrobial agent susceptibility of members of the family *Enterobacteriaceae* and staphylococci. The versatility of breakpoint panels makes it applicable to a variety of hospital formularies.

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