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Comparison of BIOGRAM and Commercial Microdilution Antimicrobial Susceptibility Test Systems

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The BIOGRAM (Difco Laboratories, Detroit, Mich.) system, which is designed to calculate MICs from disk diffusion zone diameters, was compared with two commercial microdilution antimicrobial susceptibility systems. A total of 111 clinical isolates were evaluated with each test system. Six additional isolates were tested in a comparison between BIOGRAM and Sceptor (Johnston Laboratories, Inc. Towson, Md.) systems. BIOGRAM demonstrated an overall correlation with the Sceptor microdilution method of 95.7% for 1,287 organism-antimicrobial susceptibility combinations. The BIOGRAM and UniScept (Analytab Products, Inc., Plainview, N.Y.) systems were in agreement in 90.3% of 1,048 organism-antimicrobial susceptibility combinations tested. All methicillin-resistant staphylococci were detected by the standard disk agar diffusion method used with the BIOGRAM system. The BIOGRAM system provides an acceptable alternative to these commercial systems for the determination of quantitative susceptibility.

The disk agar diffusion test is a highly standardized and acceptable qualitative test used for the determination of in vitro susceptibility of most clinical isolates (5, 12). It offers clinical microbiology laboratories the advantage of selecting their own batteries of routine antimicrobial agents for testing, with highly accurate and reproducible results (8, 11). In a recent survey (6), most respondents preferred to report susceptibility results qualitatively as susceptible, intermediate, or resistant category interpretations, although many institutions also report the quantitative MIC on request. In fact, many of those surveyed believed that quantitative reporting of MICs may be misleading. The ability to report MICs, however, is important in severe infections and in cases of multiple drug resistance.

The BIOGRAM (Difco Laboratories, Detroit, Mich.) system can accommodate qualitative reporting by disk testing and can calculate the MIC and interpret the inhibitory quotient from zone diameters without a delay in reporting or added expense to the laboratory. Zone diameters generated by the disk agar test are used to derive an MIC based on the inverse linear relationship between the zone size and drug concentration (micrograms per milliliter). Recently, D'Amato et al. (1) have shown the BIOGRAM system to be a reliable test method compared with the standard reference method for dilution susceptibility testing (13). In this report we summarize a comparison of the BIOGRAM system with the Sceptor (Johnston Laboratories, Inc., Towson, Md) and UniScept (Analytab Products, Inc., Plainview, N.Y.) microdilution antimicrobial susceptibility test systems. It is the first report in which the BIOGRAM system is compared with other commercial systems.

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MATERIALS AND METHODS

Organisms. A total of 111 isolates from individual patients were obtained from Harrisburg Hospital, Harrisburg, Pa. (75 isolates); Sinai Hospital of Detroit, Detroit, Mich. (19 isolates); and University of Chicago Medical Center, Chicago, Ill. (17 isolates). Test organisms were selected for resistant organism-antibiotic combinations and on-scale results. The distribution of bacteria were *Acinetobacter antitratus* (6 isolates), *Enterobacter aerogenes* (3 isolates), *Enterobacter cloacae* (6 isolates), *Escherichia coli* (4 isolates), *Klebsiella pneumoniae* (3 isolates), *Morganella morganii* (5 isolates), *Proteus mirabilis* (3 isolates), *Pseudomonas aeruginosa* (1 isolate), *Pseudomonas cepacia* (1 isolate), *Pseudomonas maltophilia* (9 isolates), *Pseudomonas putrefaciens* (1 isolate), *Serratia marcescens* (7 isolates), methicillin-resistant *Staphylococcus aureus* (48 isolates), and methicillin-susceptible *Staphylococcus aureus* (14 isolates). An additional six isolates were tested at Sinai Hospital of Detroit and included one isolate each of *Enterobacter cloacae*, *Escherichia coli*, *Proteus mirabilis*, and *Serratia marcescens* and two isolates of *Pseudomonas maltophilia*. All methicillin-resistant *Staphylococcus aureus* strains, which were obtained from three different geographical areas, were confirmed resistant by the oxacillin agar screen procedure (14). The organisms were isolated and identified in accordance with the approaches outlined in the *Manual of Clinical Microbiology* (7), and their antimicrobial susceptibility was determined in each test system.

Susceptibility test systems. The inoculum used for each test system was prepared from pure cultures of bacteria grown on Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) for 18 to 20 h at 35°C. The Sceptor system was used at Sinai Hospital of Detroit, the UniScept system was used at Harrisburg Hospital, and the disk agar diffusion method was used at both institutions.

(i) BIOGRAM. The disk agar diffusion procedure was performed by using the performance standard described by the National Committee for Clinical Laboratory Standards (12). Disk zone sizes obtained with Mueller-Hinton agar (BBL) were recorded with a standard caliper at 18 to 20 h for

TABLE 1. Comparison of BIOGRAM and Sceptor MIC ratios for staphylococci[a]

Antimicrobial agent	% of tests with the following MIC ratio[b]:					% Agreement[c]
	≤0.25	0.5	1	2	≥4	
Cefamandole	1.5	7.6	65.2	12.1	13.6	84.8
Cephalothin	1.5	4.5	65.2	21.2	7.6	90.9
Clindamycin	0.0	1.5	98.5	0.0	0.0	100.0
Erythromycin	1.5	1.5	95.5	0.0	1.5	97.0
Gentamicin	0.0	1.5	47.0	33.3	18.2	81.8
Methicillin-NaCl	9.4	25.0	65.6	0.0	0.0	90.6
Nitrofurantoin	0.0	0.0	100.0	0.0	0.0	100.0
Oxacillin-NaCl	1.6	0.0	89.1	7.8	1.6	96.9
Penicillin G	6.3	7.9	79.4	4.8	1.6	92.1
Trimethoprim-sulfamethoxazole	4.7	0.0	93.8	1.6	0.0	95.3
Vancomycin	0.0	1.5	98.5	0.0	0.0	100.0

[a] A total of 62 strains of staphylococci, including 48 methicillin-resistant *S. aureus* isolates, were evaluated representing 682 organism-antibiotic susceptibility comparisons.
[b] BIOGRAM MIC/Sceptor MIC (1).
[c] The on-scale (ratio of 1) results and overall percent agreement (ratios of 0.5 to 2) were 81.6 and 93.6%, respectively.

gram-negative bacilli and at 24 h for staphylococci. Log sheets containing test results from both institutions were sent to Giles Scientific, Inc., New York, N.Y., for data analysis. The zone diameters were manually entered into the BIOGRAM computer and converted into an MIC based on regression line analysis (1).

(ii) Commercial microdilution methods. The Sceptor and UniScept systems were inoculated, incubated, and read by directions of the manufacturers. Routine commercial panels and panels modified by supplementation of the methicillin and oxacillin wells with 2% NaCl, as recommended by Thornsberry and McDougal (14), were tested by both systems. The antimicrobial agents tested in each commercial system are listed in Tables 1 to 4. Common bacterial suspensions prepared in Trypticase soy broth (BBL) for disk testing also served as inocula in the Sceptor system (diluted 1:100 in 10 ml of Sceptor broth). In the UniScept system, inoculum was prepared in 0.85% NaCl and diluted to give a final concentration of approximately 10^5 CFU/ml.

Quality control. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, and two known methicillin-resistant (heteroresistant) *Staphylococcus aureus* strains (College of American Pathologists 1984 Survey no. B12 and patient clinical isolate) were used to control the performance of each test system (3). Quality control strains were tested at least weekly in accordance with the National Committee for Clinical Laboratory Standards.

Analysis of data. The comparative results were expressed as ratios of the BIOGRAM MIC divided by the commercial microdilution MIC; this ratio is similar to that used by D'Amato et al. (1). Results were considered in agreement (± 1 $\log_2$ dilution) when the calculated BIOGRAM MIC and microdilution MIC ratios ranged from 0.5 to 2.0.

RESULTS

The MIC agreement, as determined by calculating the ratio between the MICs determined with BIOGRAM and Sceptor systems, is shown in Tables 1 and 2. Overall agreement between the BIOGRAM and Sceptor MICs for staphylococci was 93.6% (Table 1). For cefamandole and gentamicin (less than 90% agreement), the trend was toward higher MICs with the BIOGRAM system. All 48 strains of methicillin-resistant *S. aureus* were detected with the BIOGRAM system, whereas the Sceptor system failed to detect two methicillin-resistant and one oxacillin-resistant *S. aureus* strains. For methicillin the trend was toward lower MIC ratios with the Sceptor system, with 9.4% having a ratio of less than 0.5.

Table 2 summarizes the results for 55 enteric and nonenteric aerobic, gram-negative bacilli. The MIC ratios between the BIOGRAM and Sceptor systems demonstrated excellent correlations for all antimicrobial agents. The overall agreement between the two methods was 97.0%. Although no appreciable differences between methods were seen for the *Pseudomonas* isolates tested (92.8% agreement), discrepancies with gentamicin were noted predominantly with these organisms (72.7% agreement). The trend was toward higher MICs with the Sceptor system.

Comparison between MIC ratios determined with the BIOGRAM and UniScept systems are shown in Tables 3 and 4. The overall agreement between the two methods for staphylococci was 87.7% (Table 3). Several antimicrobial agents, including cephalothin (77.0%), erythromycin (85.5%), methicillin (80.8%), nitrofurantoin (86.9%), oxacillin (75.4%), and penicillin (88.7%), demonstrated less than 90% agreement. With the exception of cephalothin and nitrofurantoin, the trend was toward lower MICs with the UniScept system. The UniScept system failed to detect seven methicillin-resistant and two oxicillin-resistant *Staphylococcus aureus* strains. Vancomycin, however, which is often used for the treatment of methicillin-resistant *Staphylococcus aureus* infections, showed 100% agreement. For methicillin and oxacillin, the trends were toward higher ratios, with 19.2 and 24.6%, respectively having MIC ratios of greater than 2.

The overall MIC agreement ratio between the BIOGRAM and UniScept systems for 49 enteric and nonenteric gram-negative bacilli was 93.0% (Table 4). Carbenicillin (86.0%), cefoperazone (86.0%), and cefotaxime (88.0%) were the only antimicrobial agents that demonstrated less than 90% agreement. The BIOGRAM system generally gave higher MICs than the UniScept system for this group of organisms.

TABLE 2. Comparison of BIOGRAM and Sceptor MIC ratios for aerobic gram-negative bacteria[a]

Antimicrobial agent	% of tests with the following MIC ratio[b]:					% Agreement[c]
	≤0.25	0.5	1	2	≥4	
Ampicillin	0.0	5.6	90.7	3.7	0.0	100.0
Carbenicillin	1.8	1.8	89.1	5.5	1.8	96.4
Cefamandole	6.8	6.8	86.4	0.0	0.0	93.2
Cefotaxime	3.6	1.8	90.9	3.6	0.0	96.4
Cefoxitin	2.3	4.5	93.2	0.0	0.0	97.7
Cephalothin	0.0	1.8	98.2	0.0	0.0	100.0
Gentamicin	5.5	0.0	94.5	0.0	0.0	94.5
Nitrofurantoin	0.0	3.8	96.2	0.0	0.0	100.0
Piperacillin	1.8	0.0	83.6	10.9	3.6	94.5
Tobramycin	1.8	1.8	85.5	10.9	0.0	98.2
Trimethoprim-sulfamethoxazole	4.0	0.0	94.0	2.0	0.0	96.0

[a] A total of 55 enteric and nonenteric gram-negative bacilli, including 11 multiply resistant *Pseudomonas* species, were evaluated representing 605 organism-antibiotic susceptibility comparisons.
[b] BIOGRAM MIC/Sceptor MIC (1).
[c] The on-scale (ratio of 1) results and overall percent agreement (ratios of 0.5 to 2) were 91.1 and 97.0, respectively.

Among the *Pseudomonas* isolates tested, 91.6% agreement between methods was observed, and there were similar discrepancies between the two systems. Carbenicillin and cefoperazone demonstrated less than 80% agreement, with 18.2 and 25.0%, respectively, having MIC ratios of less than 0.5.

A total of 1,287 organism-antimicrobial susceptibility combinations were evaluated between the BIOGRAM and Sceptor systems. The on-scale (ratio of 1) results and overall percent agreement (ratio of 0.5 to 2) were 86.7 and 95.7%, respectively. In comparison, the on-scale and overall percent agreement for 1,048 organism-antimicrobial susceptibility combinations between the BIOGRAM and UniScept systems were 79.5 and 90.3%, respectively.

DISCUSSION

The Sceptor and UniScept microdilution MIC systems have been shown in previous studies to be reliable methods for the determination of quantitative antimicrobial susceptibility (4, 5). Both systems incorporate panels containing dried antimicrobial agents which are convenient for laboratories that routinely perform disk testing to maintain for quantitative testing because of their long shelf life. The alternative of deriving an MIC in the BIOGRAM system from zone diameters would be appealing to those laboratories considering cost-containment measures.

The results of this evaluation indicate that the MIC determinations with the BIOGRAM system correlated very well with those with the Sceptor system. Significantly lower agreement, however, was noted between the MICs determined with the BIOGRAM and UniScept systems. Although UniScept has generally been found to be a reliable and accurate method (4), it is our experience that it is more inoculum dependent than other commercial MIC systems (unpublished data). Variations in inoculum density have been demonstrated with the use of disposable inoculators (2). Complete agreement between the BIOGRAM and commercial systems was lower than the reference MIC method evaluated previously (1). These differences are presumably due to the resistant organisms that were selected for use in this study and the test methodologies that were compared.

Oxacillin-resistant staphylococci were detected more frequently by the BIOGRAM system than by the commercial broth dilution systems, despite the addition of 2% NaCl to the panels. Discrepant MIC results for β-lactam antimicrobial agents between the dilution and BIOGRAM methods may be explained by staphylococcal β-lactamase production. β-Lactamase-positive strains have been shown to have smaller zones than β-lactamase-negative strains at the same MIC (9). The difficulty in detecting methicillin-resistant strains of *S. aureus* appears to be due to the fact that methicillin is more resistant to the action of β-lactamase than is oxacillin (10). The disk agar diffusion method (12) incorporated in the BIOGRAM system is more reliable for the detection of methicillin-resistant (heteroresistant) staphylococci. Erythromycin discrepancies between the MIC ratios determined with the BIOGRAM and UniScept systems cannot be explained because they were not observed with either the Sceptor or reference methods.

The BIOGRAM system offers several advantages to clinical laboratories that currently perform disk agar diffusion testing. It is designed to automate and further standardize this established procedure. In addition to reporting MICs, inhibitory quotients, and drug cost factors, the BIOGRAM data management center is equipped to perform epidemiological analysis and to plot antimicrobial agent distributions by zone diameter or MIC. The BIOGRAM system eliminates the need for laboratories to maintain additional quantitative MIC panels and quality control testing. Also, it does not restrict the selection and timely reporting of antimicrobial agents tested both qualitatively and quantitatively. We conclude that the BIOGRAM system is an acceptable alternative method to the commercial broth microdilution MIC systems tested and that disk agar diffusion is preferred for the detection of methicillin-resistant staphylococci.

TABLE 3. Comparison of BIOGRAM and UniScept MIC ratios for staphylococci[a]

Antimicrobial agent[b]	% of tests with the following MIC ratio[c]:					% Agreement[d]
	≤0.25	0.5	1	2	≥4	
Cephalothin-NaCl	18.0	6.6	65.6	4.9	4.9	77.0
Clindamycin	1.8	0.0	98.2	0.0	0.0	98.2
Erythromycin	1.8	1.8	81.8	1.8	12.7	85.5
Methicillin-NaCl	0.0	1.9	67.3	11.5	19.2	80.8
Nitrofurantoin	13.1	36.1	50.8	0.0	0.0	86.9
Oxacillin-NaCl	0.0	0.0	70.5	4.9	24.6	75.4
Penicillin-NaCl	4.8	1.6	83.9	3.2	6.5	88.7
Trimethoprim-sulfamethoxazole	0.0	0.0	100.0	0.0	0.0	100.0
Vancomycin	0.0	1.6	96.7	1.6	0.0	100.0

[a] A total of 62 strains of staphylococci, including 48 methicillin-resistant *S. aureus* isolates, were evaluated representing 558 organism-antibiotic susceptibility comparisons.
[b] All β-lactam antibiotics were supplemented with 2% NaCl.
[c] BIOGRAM MIC/UniScept MIC (1).
[d] The on-scale (ratio of 1) results and overall percent agreement (ratios of 0.5 to 2) were 78.8 and 87.7%, respectively.

TABLE 4. Comparison of BIOGRAM and UniScept MIC ratios for aerobic, gram-negative bacteria[a]

Antimicrobial agent	% of tests with the following MIC ratio[b]:					% Agreement[c]
	≤0.25	0.5	1	2	≥4	
Carbenicillin	10.0	2.0	76.0	8.0	4.0	86.0
Cefamandole	7.8	3.9	84.3	3.9	0.0	92.2
Cefoperazone	14.0	8.0	72.0	6.0	0.0	86.0
Cefotaxime	8.0	2.0	84.0	2.0	4.0	88.0
Cefoxitin	5.9	2.0	88.2	3.9	0.0	94.1
Cephalothin	2.0	2.0	96.0	0.0	0.0	98.0
Gentamicin	5.9	3.9	68.6	21.6	0.0	94.1
Nitrofurantoin	2.2	6.7	86.7	4.4	0.0	97.8
Tobramycin	2.0	4.0	52.0	40.0	2.0	96.0
Trimethoprim-sulfamethoxazole	2.0	2.0	96.0	0.0	0.0	98.0

[a] A total of 49 enteric and nonenteric gram-negative bacilli, including 9 *Pseudomonas* species, were evaluated representing 490 organism-antibiotic susceptibility comparisons.
[b] BIOGRAM MIC/UniScept MIC (1).
[c] The on-scale (ratio of 1) results and overall percent agreement (ratios of 0.5 to 2) were 80.3 and 93.0%, respectively.

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