JOURNAL OF CLINICAL MICROBIOLOGY, July 1998, p. 2052–2056 Vol. 36, No. 7
0095-1137/98/$04.00+0

Direct Susceptibility Testing with Positive BacT/Alert Blood Cultures by Using MicroScan Overnight and Rapid Panels

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Received 4 December 1997/Returned for modification 16 March 1998/Accepted 24 April 1998

Studies were conducted on a method of direct inoculation of MicroScan dried overnight and of rapid panels with positive aerobic blood cultures obtained from the BacT/Alert to determine antimicrobial susceptibilities. Inocula were limited to specimens that appeared unimicrobic on Gram stain. Results were compared to those obtained from panels inoculated following subculture. For 133 gram-negative bacilli, there were 94.7 and 93.5% categorical agreements between direct and standard methods for all drugs tested with overnight and rapid panels, respectively. For 104 gram-positive cocci, there were 93.2 and 93.1% categorical agreements for overnight and rapid panels, respectively. The major error (false resistance) rate for gram negatives was 1.4% for overnight versus 0.7% for rapid panels. The very major error (false susceptibility) rate was 2.7% for overnight versus 8.1% for rapid panels. The total error rates were 1.6% for overnight panels and 1.5% for rapid panels. The major error rates for gram-positive direct susceptibility tests were 2.6% for overnight and 2.5% for rapid panels. The very major error rates were 8.8 and 7.2% for overnight and rapid panels, respectively. Total error rates were 3.6% for overnight and rapid gram-positive panels. These findings suggest that susceptibility results obtained from directly inoculated gram-negative overnight panels have the greatest correlation to those obtained by standard methods. When discrepant results occur with direct-susceptibility testing, they are more likely to show false susceptibility than false resistance.

Results of antimicrobial susceptibility tests are essential to guide clinicians in the selection of the most appropriate and cost-effective treatment for persons with bacteremia or other serious infections. Availability of these data as soon as possible after infection is confirmed may result in reduced costs for the pharmacy, laboratory, and other general charges. These data may also lead to timely changes to more effective therapy in the event the infecting organism is resistant to coverage provided by empiric antimicrobial choices (3, 14). Continuously monitoring automated blood culture systems such as the BacT/Alert (Organon Teknika Corporation, Durham, N.C.) minimize the length of time required for detection of positive blood cultures from bacteremic patients, often indicating a positive culture within 24 h following the initial incubation, and have emerged as the new standard in blood culture technology (16). Conventional methodology requires instrument-positive bottles to be subcultured to solid media and incubated overnight to produce bacterial colonies, which are then used to prepare standardized suspensions for species identification and susceptibility testing (8). Direct inoculation from positive blood culture bottles into MicroScan panels (Dade MicroScan, Inc., West Sacramento, Calif.) is a potential alternative to decrease further the length of time from initial inoculation of blood culture media to reporting of susceptibility results. We performed a prospective study in which fluid from positive blood culture bottles from the BacT/Alert were inoculated directly into MicroScan rapid and overnight panels for gram-negative and gram-positive bacteria and compared susceptibility results with those obtained in standard fashion with each panel type.

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MATERIALS AND METHODS

Specimens tested. Positive aerobic blood cultures collected between September 1995 and April 1996 from patients suspected of having bacteremia at the University of Alabama at Birmingham Hospital were examined by Gram stain. Specimens that appeared to contain a single organism were included in the study. Specimens that were unimicrobic on Gram stain but yielded more than one isolate after subculture were excluded from analysis. Only aerobic and facultative bacteria were included. *Haemophilus* spp., *Neisseria* spp., *Streptococcus pneumoniae*, yeasts, and anaerobes were excluded.

MicroScan instrumentation and panels. The WalkAway/40 instrument with Version 20.30 of the Data Management System was used for all reading and interpretation of panel results. MicroScan Rapid Neg Combo Type 2 panels and MicroScan Dried Overnight Neg Combo Type 15 panels were used for gram-negative isolates. MicroScan Rapid Pos Combo Type 1 panels and MicroScan Overnight Pos Combo Type 6 panels were used for gram-positive isolates.

Blood culture instrumentation and media. Blood culture testing was performed on the Organon Teknika BacT/Alert instrument with standard aerobic media.

Direct susceptibility testing. Preliminary studies were performed to determine the optimum method of direct inoculation of MicroScan panels by using seeded cultures (1) and actual patient specimens (18). Serum separator tubes (Becton-Dickinson Vacutainer Systems, Rutherford, N.J.) containing 0.2 ml of Triton X-100 (Sigma, St. Louis, Mo.) were inoculated with 9.5 ml of each blood specimen from positive BacT/Alert bottles with a 22-gauge needle. The tubes were centrifuged at 1,400 × *g* for 10 min at room temperature. Bacteria were harvested from the surface of the silicon layer by using a cotton swab to make an inoculum suspension equivalent to a 0.5 McFarland standard, confirmed by a MicroScan turbidity meter. This technique has been shown previously to be an acceptable means for preparation of bacterial suspensions for direct susceptibility testing (16). All blood culture specimens were tested the same day that the instrument flagged them as positive. MicroScan panels were inoculated and incubated in the MicroScan WalkAway/40 according to the manufacturer's instructions, by using the suspensions prepared as described above.

Standard susceptibility testing. A small amount of the blood culture fluid was inoculated onto Trypticase soy agar with 5% sheep blood (BBL, Cockeysville, Md.) and a MacConkey agar plate (BBL) with crystal violet and lactose. The blood agar plate was incubated in 5% CO_2, and the MacConkey agar plate was incubated in air for 18 to 24 h at 35°C to produce bacterial colonies for inoculation. Rapid gram-negative panels were inoculated from MacConkey agar. All other panels were inoculated from blood agar plates according to the manufacturer's instructions. Panels were then incubated in the same manner as in the direct susceptibility test panels.

Quality control. Appropriate identification and susceptibility quality control organisms for the MicroScan rapid and overnight panels as defined by the

TABLE 1. Isolates tested

Organism type	No. tested	No. providing overnight MIC results	No. providing rapid MIC results
Gram negative			
Acinetobacter spp.	6	6	5
Enterobacter spp.	13	13	12
Escherichia coli	48	48	48
Klebsiella spp.	30	30	30
Pseudomonas aeruginosa	13	13	1
Proteus and *Morganella* spp.	8	8	8
Salmonella spp.	2	2	2
Serratia spp.	3	3	3
Other	10	10	5
Total	133	133	114
Gram positive			
Beta-hemolytic *Streptococcus* spp.	8	8	8
Enterococcus spp.	7	7	7
Micrococcus spp.	2	2	0
Staphylococcus aureus	37	36	35
Coagulase-negative *Staphylococcus* spp.	50	49	31
Total	104	102	81

manufacturer were tested weekly, and all results were acceptable. All other daily maintenance for the MicroScan WalkAway/40 and the BacT/Alert was performed in accordance with each manufacturer's instructions.

Data analysis. Direct testing on MicroScan rapid panels was compared to standardized testing on rapid panels. Direct testing on overnight panels was compared to standardized testing on overnight panels. Drug-organism combinations which have not been approved by the Food and Drug Administration for testing with MicroScan were not included in data analysis. Only antimicrobial agents considered appropriate by the National Committee for Clinical Laboratory Standards guidelines were evaluated by using their published susceptibility breakpoints (11). Gram-positive isolates resistant to oxacillin were considered resistant to other beta-lactam antimicrobials. All drug-organism contraindications were based on the direct test method identification. Organism identification used for summary purposes was that obtained with the standard method.

Categorical agreement for the susceptibility interpretations by direct versus standard methods was determined for each antimicrobial agent. Categorical agreement was defined as complete agreement of the direct and standard interpretive results. Since several of the antimicrobials were present in three or fewer dilutions on some panels, no attempt was made to compare directly the actual MICs obtained with direct and standard methods. Major errors (false resistance) occurred when the test method categorized the isolate as resistant when the standard method result was susceptible. Very major errors (false susceptibility) occurred when the direct method characterized the isolate as susceptible while the standard method characterized it as resistant. Minor errors occurred when the direct method characterized the isolate as intermediate while the standard method characterized is as susceptible or resistant, or if the direct method characterized the isolate as susceptible or resistant while the standard method characterized it as intermediate. The standard method was repeated on isolates with five or more very major or major errors in comparison with the direct method to control for problems which may have occurred with performance of the standardized tests. The repeated standard results were then substituted for original testing results for analytical purposes. Isolates showing only minor errors were not considered in data analysis. The study design involved testing sequential bacterial isolates without regard for duplicate isolates of the same organism in multiple blood culture bottles.

RESULTS

A total of 253 specimens appeared unimicrobic based on initial Gram-stained smears. However, 16 (6.3%) specimens were eventually shown to be polymicrobic when subcultured and excluded from analysis, leaving 237 evaluable specimens. Among these, there were 133 gram-negative bacilli and 104 gram-positive cocci, broken down into species in Table 1.

Standardized susceptibility results were available for all 133 gram-negative bacteria tested on overnight panels. For 114 of 133 (85.7%) gram-negative isolates there were rapid panel MIC results. For 102 of 104 (98.1%) gram-positive cocci there were standardized MIC results on overnight panels, whereas for 81 of 104 (77.9%) there were rapid panel results. Two gram-positive cocci tested by overnight panels were excluded from analysis because of contamination and erroneous readings by the standard method, which were not detected until after the samples had been evaluated. All gram-negative and gram-positive isolates that did not give rapid panel results were due to insufficient growth. Six of 133 (4.5%) gram-negative isolates were retested due to five or more very major or major errors on initial testing. Four were due to errors in the overnight panel, and one was due to errors in the rapid panel. Five of 104 (4.8%) of the gram-positive cocci were retested due to five or more major or very major errors on initial testing. Four were retested due to errors in the overnight panel and one was retested because of errors in the rapid panel. After repeat testing, one coagulase-negative *Staphylococcus* sp. (CNS), two *Escherichia coli* spp., and one *Pseudomonas aeruginosa* sp. still had five major errors.

One hundred fifteen of 133 (86.4%) gram-negative isolates showed no major or very major errors for any drugs on the overnight panel, compared to 98 of 114 (85.9%) on the rapid panel. Complete categorical agreement, i.e., no very major, major, or minor errors for any drug tested, was observed with 94.7 and 93.5% of drugs tested on overnight and rapid gram-negative panels, respectively (Table 2). Seventy-seven of 102 (75.5%) gram-positive cocci showed no major or very major

TABLE 2. Categorical agreement of individual susceptibility results for direct versus standard methods

Interpretive category	Overnight panels				Rapid panels			
	No. of drugs tested	No. of concordant drugs (%)	No. of major errors (%)[a]	No. of very major errors (%)[a]	No. of drugs tested	No. of concordant drugs (%)	No. of major errors (%)[a]	No. of very major errors (%)[a]
Gram negative								
Susceptible	1,770	1,723 (97.3)			1,656	1,620 (97.8)		
Intermediate	106	63 (59.4)			100	50 (50.0)		
Resistant	374	344 (92.0)			221	179 (81.0)		
Total	2,250	2,130 (94.7)	25 (1.4)	10 (2.7)	1,977	1,849 (93.5)	11 (0.7)	18 (8.1)
Gram positive								
Susceptible	867	833 (96.1)			558	535 (95.9)		
Intermediate	20	4 (20.0)			15	6 (40.0)		
Resistant	231	205 (88.7)			165	146 (88.5)		
Total	1,118	1,042 (93.2)	22 (2.6)	18 (8.8)	738	687 (93.1)	14 (2.5)	12 (7.2)

[a] Denominator for major error rate is total number of MICs indicating bacterium susceptibility by standard method, while that for very major errors is total number of MICs indicating bacterium resistance by standard method.

TABLE 3. Analysis of errors by species and antimicrobial

Organism (no. different isolates)	Overnight panel		Rapid panel	
	No. of major errors (drug[s])	No. of very major errors (drug[s])	No. of major errors (drug[s])	No. of very major errors (drug[s])
Gram negative				
Acinetobacter spp. (3)	0	0	0	3 (Azt, Cfp, To)
Enterobacter spp. (6)	2 (Am, Crm)	3 (Azt, T-S [2])	4 (Am, Ctn, Mz, Pi)	0
Escherichia coli (7)	12 (Am, Azt [3], Caz, Cf, Cfz [2], Crm [2], Imp, To)	2 (A-S, Azt)	4 (A-S, Mz, Pi, T-S)	1 (Cfp)
Klebsiella spp. (7)	5 (A-S, Azt, Cf, Cfz, Crm)	1 (T-S)	3 (Am, Mz, T-S)	3 (Am, Cfz, T-S)
Pseudomonas aeruginosa (2)	5 (Caz, Cp, Mz, Pi, Tim)	1 (Mz)	0	0
Proteus-Morganella spp. (1)	0	0	0	3 (A-S, Am, Pi)
Salmonella-Shigella spp. (1)	0	0	0	3 (A-S, Mz, Pi)
Serratia marcescens (2)	0	3 (Caz, Cft, Crm)	0	4 (Cfz, Crm, Imp [2])
Other (1)	1 (Azt)	0	0	1 (Mz)
Gram positive				
Beta-hemolytic *Streptococcus* spp.	0	0	0	0
Enterococcus spp. (1)	2 (Am, P)	0	0	1 (Sts)
Micrococcus spp. (1)	0	1 (P)	0	0
Staphylococcus aureus (14)	9 (Aug [2], Cd, Ery, Gm [2], Ox, P, Va)	4 (E, Ox, P, Rif[a])	2 (Cd, Imp)	5 (Cd [4], Ox)
Coagulase-negative *Staphylococcus* spp. (21)	11 (Cd [2], Cp, Gm, Ery [2], P [2], Ox, T-S, Te)	13 (Cd, Gm [2], Ox, T/S [8], Te)	12 (Cd, E [3], Ox [2], P, Te [2], Va [3])	6 (Cd [2], Ox [4])

[a] Rifampin was not present on rapid gram-positive panels. Am, ampicillin; A-S, ampicillin-sulbactam; Azt, aztreonam; Cd, clindamycin; Cfz, cefazolin; Cfp, cefoperazone; Cft, cefotaxime; Ctn, cefotetan; Caz, ceftazidime; Crm, cefuroxime; Cf, cephalothin; Cp, ciprofloxacin; Ery, erythromycin; Gm, gentamicin; Imp, imipenem; Mz, mezlocillin; Ox, oxacillin; P, penicillin; Pi, piperacillin; Rif, rifampin; Sts, streptomycin synergy; Tim, ticarcillin-clavulanate; To, tobramycin; T-S, trimethoprim-sulfamethoxazole; Va, vancomycin.

errors on overnight panels, versus 64 of 81 (79.0%) on rapid panels. Complete concordance was observed with 93.2 and 93.1% of drugs tested on overnight and rapid gram-positive panels, respectively.

The major error rate for all gram-negative direct susceptibility tests was 1.4% for overnight panels versus 0.7% for rapid panels. The very major error rate was 2.7% for overnight versus 8.1% for rapid panels. The total error rates for gram-negative bacteria, obtained by combining very major and major errors and dividing by the total number of those bacteria for which MICs indicated susceptibility and resistance, were 1.6% for overnight panels and 1.5% for rapid panels.

The major error rates for gram-positive direct susceptibility tests were 2.6% for overnight panels and 2.5% for rapid panels. The very major error rates were 8.8 and 7.2% for overnight and rapid panels, respectively. Total error rates were 3.6% for both overnight and rapid gram-positive panels.

Analysis of errors detected by direct susceptibility testing with overnight and rapid panels according to individual antimicrobials and bacterial species is shown in Table 3. Among drugs on the gram-negative panels, only amikacin and gentamicin had no major or very major errors. Among those on gram-positive panels, narrow-spectrum cephalosporins and gentamicin synergy had no major or very major errors. Aztreonam had the most errors (seven on overnight and one on rapid panels), followed by mezlocillin (two on overnight and five on rapid panels), and cefuroxime (five on overnight and one on rapid panels) among agents tested on gram-negative panels. Drugs with the most errors on gram-positive panels were clindamycin (four on overnight and eight on rapid panels), oxacillin (four on overnight and seven on rapid panels), and trimethoprim-sulfamethoxazole (nine on overnight panels).

One *P. aeruginosa* and two *E. coli* isolates produced 15 of 25 major errors on overnight gram-negative panels. One CNS isolate gave 5 of 12 major errors on rapid panels. Overall, CNS gave 42 of 66 errors on both panel types combined.

DISCUSSION

Direct susceptibility testing on positive blood cultures has been recommended to facilitate early confirmation of antimicrobial susceptibility (2, 4, 6–10, 12, 13). Automated blood culture systems such as BacT/Alert and rapid MIC panels provided by automated bacterial identification and susceptibility testing systems such as MicroScan can potentially make direct susceptibility testing even more clinically valuable, allowing preliminary results in a matter of hours after growth is first detected in the blood culture bottle. Although the concept of direct inoculation of fluid from positive blood culture bottles into MIC panels was investigated in the early 1980s (6), no prospective evaluations by using the current MicroScan instrumentation, panels, software, and database, newer antimicrobials, or with more recently developed continuously monitoring blood culture instruments such as the BacT/Alert have been published.

This study demonstrated that MicroScan overnight and rapid panels can be used to provide direct susceptibility test results for unimicrobic gram-negative blood culture isolates detected by the BacT/Alert with 94.7 and 93.1% overall categorical agreement for MICs in comparison to standardized methodology by using the same instrumentation. For gram-positive bacteria, direct and standard testing with overnight and rapid panels provided 93.2 and 93.1% categorical agreement.

The definitive evaluation of direct susceptibility testing would involve a third reference test method for arbitration of discrepancies between the standardized result and the direct result. However, this was not attempted in the present evaluation, mainly because the objective of the study was to determine whether direct susceptibility testing was an acceptable alternative for use with an automated instrument already pres-

ent in our laboratory. MicroScan susceptibility test capability has already been compared with reference methods numerous times (7, 17).

Only 6.3% of the specimens tested that appeared unimicrobic were later found to be polymicrobic and unsuitable for direct susceptibility testing by this method, numbers which are in agreement with previously published rates for polymicrobic bacteremia (10). Verification of direct inoculum purity could be made following incubation of overnight panels and prior to reporting results if a subculture to agar is performed at the time of direct inoculation of susceptibility panels. This would not be possible for rapid panels for which MIC data would be available prior to verification of culture purity if rapid direct susceptibility data are to be reported as soon as results are available.

False susceptibility occurred with direct susceptibility tests with a greater frequency than false resistance in overnight and rapid panels for gram-negative as well as gram-positive bacteria. When considering the total error rates, there was minimal difference between overnight versus rapid gram-negative panels and none between overnight versus rapid gram-positive panels. Total error rates for overnight gram-negative panels were less than the corresponding values for overnight gram-positive panels.

Among gram-positive cocci, CNS were responsible for the most errors. Of major concern, however, was false susceptibility in directly inoculated panels for oxacillin in two isolates of *Staphylococcus aureus*, one with an overnight panel and the other with a rapid panel. This finding was not surprising since oxacillin-resistant *S. aureus* is often present in heterogeneous populations and tends to grow more slowly.

In comparative evaluations of susceptibility testing procedures, very major errors should occur in $<1.5\%$ of all tests, and the overall agreement between tests and the reference method should be $\geq 95\%$ (12). Rigorous application of these criteria to data from the present evaluation would mean that direct susceptibility testing with either overnight or rapid panels would be inappropriate, mainly because of the high false-susceptibility rates. However, it has been suggested that these criteria may be too restrictive (12).

Even though automated systems have reduced the time required to detect microorganisms in bloodstream infections, the relatively low number of bacteria in culture bottles may compromise the inoculum concentration, if not standardized, for susceptibility tests. This may partially account for the relatively high error rates for direct susceptibility testing with MIC methods reported by some studies evaluating blood cultures performed in automated instruments (5, 12), but others (2, 6, 13) yielded more favorable comparisons.

Kiehn et al. (6) found only 0.7% categorical discrepancies between MicroScan MIC panels inoculated directly and by standard methodology from nonautomated blood culture systems. More recently, Pettigrew et al. (13) compared results of direct inoculation from positive BacT/Alert bottles to MicroScan gram-positive and gram-negative rapid panels. Complete agreements for 1,403 antimicrobial agent-organism combinations were 96.9% for gram-negative bacilli and 96.8% for 1,083 gram-positive coccus combinations, slightly more favorable results than obtained in our investigation, which was performed in a similar manner.

Zimmer et al. (18) also evaluated direct susceptibility testing by using MicroScan rapid and dried overnight panels, testing three different inoculum preparation methods and employing multiple media from three different blood culture systems. Due to a small number of positive cultures, results from all media and blood culture systems were combined in data analysis. Overall categorical agreement rates similar to those reported in the present study were described. They also detected false susceptibility by using direct testing more often than they detected false resistance, consistent with our findings.

Direct susceptibility testing of bacteria from positive blood culture bottles by agar disk diffusion is a relatively inexpensive procedure, not tied to bacterial identification, in contrast to direct testing with an automated system such as MicroScan. Agar disk diffusion has been the most widely studied technique of direct susceptibility testing and has the greatest correlation with standardized methods, according to most studies (9).

Most laboratories which invest in MicroScan technology use the instrument for both bacterial identification and susceptibility testing, typically employing the same biochemical-antimicrobial panel for each organism tested. Due to the costs of panels, it is not practical to perform direct susceptibility testing with this technology if results must be repeated with a standardized inoculum. Likewise, it would be impractical to perform direct susceptibility testing unless direct bacterial identification also proves to be accurate so that confirmation following subculture is unnecessary.

Organism identification evaluated as part of this investigation has been preliminarily described (15). Ninety-six percent of overnight and 72% of rapid panels showed complete agreement between direct and standard methods for identification of gram-negative bacteria, with the highest concordance (99%) occurring with *Enterobacteriaceae*. As might be expected, nonfermentative gram-negative bacilli proved more difficult to identify by direct inoculation. Significant problems occurred with gram-positive cocci, for both overnight and rapid panels inoculated directly. Only 82% concordance with standard identification occurred for overnight and 52% for rapid panels directly inoculated with gram-positive cocci. These discrepancies were predominantly due to the inability to distinguish among CNS, but problems also occurred with identification of *S. aureus* in directly inoculated panels. Even though Pettigrew and colleagues (13) had a better correlation in their study with respect to direct versus standardized susceptibility testing by using MicroScan rapid panels, their identification agreement was only 89% for gram-negative bacilli and 70% for gram-positive cocci.

Our practice has been to perform direct susceptibility testing on all blood culture isolates by agar disk diffusion and confirm results for reporting purposes by using MicroScan overnight panels which provide both susceptibility and organism identification. We did not change this practice after performance of this evaluation. If MicroScan panels are to be used for direct susceptibility testing, results should be considered preliminary, mainly because of potential false susceptibility. However, direct susceptibility results obtained from gram-negative overnight panels indicating resistance are likely to be in agreement with standard methods and could be considered for immediate reporting if laboratories choose to use such a direct method to improve turnaround time.

Given the many potential advantages of rapid turnaround times for antimicrobial susceptibility results, periodic evaluations of automated microbiological instrumentation are extremely important. It is particularly relevant to the study of emerging resistant organisms and newer antimicrobials. Reliability of direct susceptibility testing by using any automated method should be tested by individual laboratories before the method is considered for routine use.

ACKNOWLEDGMENTS

Financial support for this project was provided by Dade MicroScan, Inc., West Sacramento, Calif., and Organon Teknika Corporation, Durham, N.C.

The technical assistance of Linda Van Pelt and Mary McKinnon of Dade Microscan, Inc., is appreciated.

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