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Comparison of Direct and Standardized Antimicrobial Susceptibility Testing of Positive Blood Cultures

JAMES E. JOHNSON AND JOHN A. WASHINGTON II*
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

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In an effort to determine the feasibility and accuracy of performing direct susceptibility tests from positive blood cultures, minimal inhibitory concentrations (MICs), determined by the agar dilution method, of direct and standardized tests with seven antibiotics were compared. Results were analyzed as to the number of very major (change in MIC from susceptible in preliminary direct testing to resistant in final standardized testing), major (change in MIC from resistant to susceptible), and minor (change in MIC without change in interpretation) discrepancies. The results for gram-positive cocci and for gram-negative bacilli were 5, 6, and 82 of 162 strains tested and 3, 12, and 79 of 90 strains tested, respectively. Of the total number of susceptibility tests compared, major and very major discrepancies occurred in only 1 and 2.4% of instances with gram-positive and -negative isolates, respectively. The majority of discrepancies were noted with *Staphylococcus epidermidis* (four very major, five major), *Klebsiella* (two very major, four major), and *Alcaligenes* (five major). The antibiotics most often exhibiting discrepancies were penicillin, ampicillin, and cephalothin. The results indicate that preliminary susceptibility testing directly from positive blood culture bottles is generally both feasible and accurate.

It has been stated that preliminary antimicrobial susceptibility testing may be done directly from a positive blood culture bottle (3; R. C. Bartlett, P. D. Ellner, and J. A. Washington II, *Cumitech I*, American Society for Microbiology, Washington, D.C.). Although many laboratories follow this practice with positive blood cultures, they almost always confirm their results by using a standardized procedure on the isolated microorganism (2).

This study was undertaken to determine the feasibility and accuracy of performing direct susceptibility tests from positive blood cultures by comparing results of direct and standardized susceptibility tests obtained by the agar dilution method.

MATERIALS AND METHODS

Standardized susceptibility tests were performed by the agar dilution test with expanded dilution steps (Table 1), according to procedures described elsewhere (8). In the direct tests, an attempt was made to adjust the turbidity of the broth removed from positive blood culture bottles (tryptic soy, Difco Laboratories) to match that of one-half of a McFarland no. 1 barium sulfate standard by adding a sample of the broth to 2 ml of Mueller-Hinton broth (Difco Laboratories) and incubating the mixture at 35°C for 2 to 4 h. Gram-positive organisms were tested with cephalothin, chloramphenicol, clindamycin, erythromycin, oxacillin, and penicillin. Gram-negative organisms were tested with ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, and tetracycline. Ampicillin was also tested against enterococci, and carbenicillin was tested against the pseudomonads. All antibiotics were mixed in Mueller-Hinton agar (BioQuest).

Interpretation. Discrepancies between the direct and standardized methods were classified as minor, major, and very major. A minor discrepancy was one in which a change in minimal inhibitory concentration (MIC) was noted without a corresponding change in interpretation of susceptibility or resistance, or, rarely, in which a change occurred from a susceptible or a resistant MIC to an intermediate MIC. A discrepancy was considered to be major if the organism was resistant by direct testing and susceptible by the standard method. Discrepancies were considered to be very major when organisms were found to be susceptible by direct testing and resistant by the standardized method. Clinically oriented MIC guidelines were used for defining levels of resistance and susceptibility (Table 2).

RESULTS

A summary of the results comparing direct and standardized susceptibility tests according to antibiotics is shown in Table 3. The overall correlation was 87.9%. Most of the discrepancies were in the minor category, and only 8 (0.5%) very major and 18 (1.2%) major discrepancies were observed. Agreement between the two methods was 98.3% if minor discrepancies

TABLE 1. *Concentrations of antimicrobials tested against bacterial isolates by agar dilution method*

Antimicrobial	Concn (μg/ml)									
	0.1	0.5	1.0	3.0	5.0	10.0	20.0	50.0	100	200
Ampicillin			X		X	X	X			
Carbenicillin								X	X	X
Cephalothin			X		X	X	X			
Chloramphenicol					X	X	X			
Clindamycin			X		X	X				
Erythromycin	X		X		X					
Gentamicin			X	X	X	X				
Kanamycin			X		X	X				
Oxacillin			X		X	X				
Penicillin	X	X	X		X					
Tetracycline			X		X	X				

TABLE 2. *Clinically oriented interpretative criteria of MICs*

Antimicrobial	MIC (μg/ml)	
	Resistant	Susceptible
Ampicillin		
Gram-negative and enterococci	>20	<10
Carbenicillin		
Pseudomonas aeruginosa	>200	<100
Cephalothin	>20	≦10
Chloramphenicol	>20	≦10
Clindamycin	>5	≦1
Erythromycin	>5	≦1
Gentamicin	>5	≦3
Kanamycin	>10	≦5
Oxacillin	>10	≦5
Penicillin G		
Staphylococci	>0.1	≦0.1
Tetracycline	>5	≦5

were regarded as insignificant. Changes in MIC and in interpretation of results were most frequently noted with penicillin and ampicillin.

When the results comparing the two test methods were analyzed according to the microorganism tested, some differences were noted (Tables 4 and 5). In tests of the gram-positive organisms, most discrepancies were noted with penicillin. The overall agreement of results for penicillin was 69.1%, with 4.3% of the discrepancies being due to major or very major changes. *Staphylococcus epidermidis* was the organism most commonly associated with discrepant results, 4 very major, 5 major, and 57 minor discrepancies being observed among the 93 strains tested. Of 30 strains of *Staphylococcus aureus* tested, 1 very major, 1 major, and 8 minor discrepancies were noted. No major or very major discrepancies were noted with group D streptococci or other gram-positive organisms. One hundred and sixty-two strains of gram-positive cocci were tested against six antibiotics, and only 11 major or very major discrepancies were observed. Eleven group D streptococci were also tested against ampicillin. No discrepancies of any type were observed.

The results obtained with 90 strains of gram-negative organisms were also compared against six antibiotics. A total of 15 major or very major discrepancies occurred in this group. The organisms most commonly involved were *Klebsiella*, which had two very major and four major discrepancies of 14 strains tested, and *Alcaligenes* spp., in which five major discrepancies were observed with four strains tested. The antibiotics most often involved in the discrepancies with gram-negative organisms were ampicillin and cephalothin. Twelve isolates of the *Pseudomonas* group of organisms were also tested with carbenicillin, with only one minor discrepancy noted.

DISCUSSION

In this study of 1,536 antibiotic comparisons, there was an overall agreement of 87.9% in results. The vast majority of the discrepancies observed, however, were minor and might have been attributed to test variability, as they generally reflected a one-dilution difference and did not significantly alter the interpretation of susceptibility or resistance applied to the MIC. There were only 26 major or very major discrepancies of 1,536 antibiotic test comparisons, resulting in 98.3% agreement in results when only these two significant groups of discrepancies are considered.

On the other hand, if one examines the data according to the strains tested, a slight difference in percent agreement is seen. Of a total of 252 strains compared, 128 (50.8%) strains provided results that were in complete agreement in both test systems. If minor discrepancies are disregarded, the results obtained with 233 (92.5%) of the strains agreed. Nineteen (7.5%) strains demonstrated the 26 major and very

TABLE 3. *Summary of results comparing direct and standardized susceptibility testing according to antimicrobial agent*[a]

Antimicrobial	No. of tests	No. (%) in agreement	No. (%) with discrepancies		
			VM	MA	MI
Ampicillin	102	80 (78.5)	3 (2.9)	4 (3.9)	15 (14.7)
Carbenicillin	12	11 (91.7)	0 (0)	0 (0)	1 (8.3)
Cephalothin	252	230 (91.3)	0 (0)	3 (1.2)	19 (7.5)
Chloramphenicol	252	219 (86.9)	0 (0)	0 (0)	33 (13.1)
Clindamycin	162	159 (98.1)	0 (0)	0 (0)	3 (1.9)
Erythromycin	162	145 (89.5)	1 (0.6)	1 (0.6)	15 (9.3)
Gentamicin	90	83 (92.2)	0 (0)	1 (1.1)	6 (6.7)
Kanamycin	90	80 (88.9)	0 (0)	0 (0)	10 (11.1)
Oxacillin	162	156 (96.3)	0 (0)	2 (1.2)	4 (2.5)
Penicillin	162	112 (69.1)	4 (2.5)	3 (1.9)	43 (26.5)
Tetracycline	90	74 (82.2)	0 (0)	4 (4.5)	12 (13.3)

[a] VM, Very major = susceptible by direct, resistant by standardized method; MA, major = resistant by direct, susceptible by standardized method; MI, minor = change in MIC without change in interpretation.

TABLE 4. *Discrepancies between direct and standardized susceptibility tests for gram-positive organisms*[a]

Antibiotic	*S. aureus*	*S. epidermidis*	Streptococci, group D	Other	% Agreement[b]	
					Overall	VM, MA
Ampicillin			0,0,0/11		100	100
Cephalothin	0,0,0/30[c]	0,0,0/93	0,0,1/11	0,0,0/28	99.4	100
Chloramphenicol	0,0,5/30	0,0,6/93	0,0,3/11	0,0,2/28	90.1	100
Clindamycin	0,0,0/30	0,0,0/93	0,0,3/11	0,0,0/28	98.1	100
Erythromycin	1,0,1/30	0,1,12/93	0,0,1/11	0,0,1/28	89.5	98.8
Oxacillin	0,0,0/30	0,2,2/93	0,0,2/11	0,0,0/28	96.3	98.8
Penicillin	0,1,2/30	4,2,37/93	0,0,1/11	0,0,3/28	69.1	95.7

[a] VM, Very major = susceptible by direct, resistant by standardized method; MA, major = resistant by direct, susceptible by standardized method. Minor change, Change in MIC without change in interpretation.

[b] Average: Overall = 91.8%; VM, MA = 99.0%.

[c] Each entry represents the number of very major, major, and minor discrepancies/total number of strains tested.

TABLE 5. *Discrepancies between direct and standardized susceptibility tests for gram-negative organisms*[a]

Antibiotic	*E. coli*	*Klebsiella*	*Enterobacter*	*Serratia*	*Proteus*	*Pseudomonas*	*P. aeruginosa*	*Alcaligenes*	% Agreement[b]	
									Overall	VM, MA
Ampicillin	0,0,2/44[c]	2,1,7/14	1,0,1/6	0,0,1/5	0,0,2/5	0,0,2/7	0,0,0/5	0,3,0/4	75.6	92.2
Carbenicillin						0,0,1/7	0,0,0/5		91.7	100
Cephalothin	0,1,9/44	0,1,7/14	0,0,1/6	0,0,0/5	0,0,0/5	0,0,0/7	0,0,0/5	0,1,1/4	76.7	96.7
Chloramphenicol	0,0,3/44	0,0,6/14	0,0,1/6	0,0,0/5	0,0,2/5	0,0,3/7	0,0,0/5	0,0,2/4	81.1	100
Gentamicin	0,0,2/44	0,0,0/14	0,0,0/6	0,0,0/5	0,0,0/5	0,1,1/7	0,0,1/5	0,0,2/4	92.2	98.9
Kanamycin	0,0,3/44	0,0,5/14	0,0,1/6	0,0,0/5	0,0,1/5	0,0,0/7	0,0,0/5	0,0,0/4	88.9	100
Tetracycline	0,0,6/44	0,2,5/14	0,0,0/6	0,0,0/5	0,0,0/5	0,1,1/7	0,0,0/5	0,1,0/4	82.3	95.6

[a] VM, Very major = susceptible by direct, resistant by standardized method; MA, major = resistant by direct, susceptible by standardized method. Minor change, Change in MIC without change in interpretation.

[b] Average: Overall = 84.1%; VM, MA = 97.6%.

[c] Each entry represents the number of very major, major, and minor discrepancies/total number of strains tested.

major discrepancies noted. Of these, six strains of *S. epidermidis* and three strains of *Alcaligenes* were involved. Both of these organisms are generally considered to be contaminants of blood cultures, indicating that only 10 strains (4%) representing "significant" isolates demonstrated major or very major shifts in susceptibility. In like manner, if one were to remove

the 15 major discrepancies observed with these nine organisms, an overall agreement of 99.3% is noted for antibiotic test comparisons.

A number of positive blood cultures were not included in this study, either because the organisms were initially detected on subculture or because of polymicrobial bacteremia. Recently, our laboratory has instituted a procedure for the early subculture of blood cultures on the day they are collected (4). This has resulted in there being fewer gram-negative isolates that could be subjected to direct testing in this study, as these organisms tended to be detected initially on the chocolate blood agar plate used for subcultures. Polymicrobial bacteremia was infrequent during the course of this study and occurred in fewer than 2% of all positive blood cultures. Nevertheless, it is important to reiterate that susceptibility testing data are valid only when they are derived from pure cultures, as has been emphasized by Shahidi and Ellner (6) and by Barry et al. (1).

Fourteen of the 26 major and very major discrepancies were contained in the penicillin-related group of antimicrobial agents. Penicillin G and ampicillin were involved in seven instances each. Therefore, if direct susceptibility testing is done, one must exercise caution in interpreting the results with this group of antibiotics. Even so, there was approximately 95% agreement between direct and standardized susceptibility tests for each of these antibiotics.

In analyzing our data, it was quite evident that multiple discrepancies tended to occur that may well have been related to differences in inoculum density (1; A. W. Bauer, *in* H. P. Kuemmerle and P. Preziosi (ed.), *Third International Congress of Chemotherapy*, vol. 1, p. 466–479). Although we attempted to standardize the inoculum in the direct tests, it was not infrequently difficult to do so because of the presence of erythrocytes in the blood culture broth. Centrifugation of the broth at 700 rpm to eliminate the erythrocytes failed to improve the degree of agreement between the direct and standard tests, and this practice was discontinued after a brief trial. The organisms most commonly causing difficulty with adjustment of the inoculum density were the gram-positive cocci, because they tended to grow as colonies on the surface of the blood-broth interface in the bottle. This problem may well have been responsible for most of the discrepancies observed with *S. aureus* and *S. epidermidis*.

It is possible that some of the discrepancies noted between direct and standard testing could be related to the reproducibility of the agar dilution test itself. Because of experimental error and biological variation in antimicrobial susceptibility tests, their reproducibility might, even under ideal circumstances, not be expected to exceed 98% (7). Duplicate standardized susceptibility tests were not included in this study; however, internal quality control specimens submitted to our susceptibility testing laboratory as unknowns have never demonstrated a major or very major discrepancy in more than 5 years of testing. In like manner, the daily control organisms used in our laboratory have shown only one minor discrepancy in the past 2 months, indicating the high degree of reproducibility of the agar dilution test.

The degree of reproducibility and comparability observed with agar dilution tests may differ from that noted for disk diffusion methods. However, a study in our laboratory comparing direct and standardized disk diffusion susceptibility tests on urine specimens produced results similar to those obtained in this study (5).

In conclusion, the results of this study indicate that preliminary antimicrobial susceptibility testing directly from positive blood culture bottles is generally both feasible and accurate.

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