Standardization of Direct Susceptibility Test for Blood Cultures

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Insufficient data are available to establish the reliability of direct disk diffusion susceptibility tests performed utilizing positive blood culture broth as inoculum. When *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used, 0.03 ml of turbid overnight blood culture broth was found to produce zone diameters closely approximating the size of diameters obtained by a standardized method. Results of direct (0.03 ml of inoculum) and standardized susceptibility tests were then compared for 116 positive blood cultures (1,069 individual disk comparisons). There were 1,011 test agreements (94.6%). There were also 48 (4.5%) minor discrepancies (change between sensitive and intermediate or between intermediate and resistant) and 10 (0.9%) major discrepancies (change between sensitive and resistant). The major discrepancies were randomly distributed among several organisms and antibiotics. Discrepancies occurred most frequently in the more clinically acceptable direction; i.e., in 79.3% the direct test indicted greater resistance than the standardized test. These data establish that 0.03 ml of turbid overnight blood culture broth produces results which compare closely to those obtained with standard methods, and in practice yield direct susceptibility results with a clinically acceptable level of reliability.

The administration of appropriate antimicrobial therapy at the earliest time is essential in reducing the high incidence of mortality associated with bacteremia. In an attempt to shorten reporting time for susceptibility results, several authors (2, 3) have suggested that in urgent situations susceptibility plates may be inoculated directly with clinical material. The results of these direct or preliminary tests are then confirmed the following day by using one of the accepted standardized methods. However, detailed standardized methods for performing direct tests from positive blood cultures are unavailable, and little is known about the accuracy of such testing. We present below a simple, standardized method for performing direct susceptibility tests on positive blood culture broth and a clinical evaluation of the method.

MATERIALS AND METHODS

Organisms. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used in all experiments.

Antibiotics. Antibiotic disks were obtained from Pfizer, Inc. In the inoculum standardization experiments the susceptibility of *S. aureus* was tested to ampicillin, cephalothin, chloramphenicol, clindamycin,

erythromycin, gentamicin, kanamycin, methicillin, penicillin G, and tetracycline. The susceptibility of *E. coli* was tested to ampicillin, carbenicillin, cephalothin, chloramphenicol, colistin, gentamicin, kanamycin, streptomycin, and tetracycline. The susceptibility of *P. aeruginosa* was tested to carbenicillin, gentamicin, and tobramycin. In the clinical trials the susceptibility of gram-positive and gram-negative organisms was tested to the batteries of antibiotics described above for *S. aureus* and *E. coli*.

Controls. Controls, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (4), were performed by using the standardized test and the three organisms described above.

Inoculum standardization. Blood culture bottles containing tryptic soy broth (Baltimore Biological Laboratory) plus sodium polyanethiolsulfonate (0.03%) were lightly inoculated with a portion of five colonies of one of the test organisms. The bottles were incubated at 35°C for 24 h, after which the growth was evenly suspended in the broth. Various volumes of the suspension (0.01, 0.02, 0.03, 0.05, 0.10, 0.50, and 1.00 ml) were pipetted in duplicate onto the surfaces of 150-mm Mueller-Hinton agar plates. Dry sterile swabs were used to streak the inoculum in three planes. Antibiotic disks were then applied, and the plates were incubated at 35°C for 18 h. The zone diameters were measured to the nearest millimeter, and values of the duplicate trials for each antibiotic at each inoculum volume were averaged. The entire experiment was repeated 10 times for each of the three test organisms. A zone diameter equal to the midpoint of the accept-

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able range of individual test zone diameters for each antibiotic-organism combination, as suggested by the NCCLS (4), was considered to be the optimal zone diameter, and the volume of turbid broth producing this diameter was considered the optimal inoculum volume.

Clinical trial. Direct and standardized susceptibility tests were performed on 116 positive blood cultures processed in the clinical laboratory. Patient blood was cultured in tryptic soy broth (aerobic; 100 ml; Baltimore Biological Laboratory), tryptic soy broth (anaerobic; 50 ml; Pfizer), and Thiol broth (anaerobic; 50 ml; Difco). The direct test was performed from the aerobic tryptic soy broth if this bottle was positive. Alternatively, the anaerobic tryptic soy broth or the Thiol broth bottles were used. When visible growth occurred in any bottle, an initial check of purity was performed by Gram stain. If the culture appeared pure, the growth was then evenly suspended, and a portion of the suspension was removed with a syringe and needle. Two drops of the cell suspension (previously determined to equal 0.03 ml) was applied to the surface of a Mueller-Hinton plate and streaked in three planes with a dry sterile swab. All subsequent steps were performed as recommended by the NCCLS (4). Also at this time various culture media were inoculated and streaked for isolation as an additional check on purity. The standardized test was performed the following day from isolated colonies by using the method described by the NCCLS.

A positive culture was rejected from the study if it was found to be mixed on the basis of Gram stain or observation of the subsequent growth on either the isolation or susceptibility plates.

When results of both the direct and standardized tests were available, an individual antibiotic comparison was scored as an agreement if both tests produced the same reportable results, i.e., sensitive, intermediate, or resistant. An individual comparison was scored as a major discrepancy if a shift between sensitive and resistant occurred. An individual comparison was scored as a minor discrepancy if a shift between sensitive and intermediate or between intermediate and resistant occurred.

RESULTS

Results of the inoculum standardization experiments indicate that no one inoculum volume was optimal for each of the three control strains tested (Table 1). Three inoculum volumes (0.03, 0.05, and 0.10 ml) produced a similarly high total number of zone diameters which varied from the optimal zone diameters by 2 mm or less. Of the remaining zone diameters produced by these three inoculum volumes, however, only those produced by the 0.03-ml inoculum were all within the control limits established by the NCCLS.

In the clinical trial a comparison of the direct test (using a 0.03-ml inoculum volume) and the standardized test was performed on 116 positive blood cultures. A total of 1,069 individual disk

TABLE 1. Frequency with which various inoculum volumes of turbid overnight broth cultures of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 produced mean zone diameters within 2 mm of the optimal zone diameter

Inoculum vol (ml)	No. with diam within 2 mm/no. tested		
	<i>E. coli</i> ^a	<i>S. aureus</i> ^b	<i>P. aeruginosa</i> ^c
0.01	79/90	45/100	30/30
0.02	69/90	58/100	28/30
0.03	75/90	68/100	26/30
0.05	74/90	65/100	26/30
0.10	69/90	74/100	22/30
0.50	42/90	83/100	0/30
1.00	23/90	62/100	0/30

^a Ten repetitions with nine antibiotics at each of the indicated inoculum volumes.

^b Ten repetitions with 10 antibiotics at each of the indicated inoculum volumes.

^c Ten repetitions with three antibiotics at each of the indicated inoculum volumes.

comparisons were performed. There were 1,011 (94.6%) agreements and 58 (5.4%) discrepancies, of which 48 (4.5%) were minor and 10 (0.9%) were major. The organisms tested and the distribution of discrepancies by organism are shown in Table 2.

For gram-positive organisms, a total of 250 individual disk comparisons were performed. The direct and standardized tests agreed in 243 (97.2%) cases. Five minor (2.0%) and two major (0.8%) discrepancies occurred (Fig. 1). For gram-negative organisms, a total of 819 individual disk comparisons were performed. The direct and standardized tests agreed in 768 (93.8%) cases. A total of 43 minor (5.2%) and 8 major (1.0%) discrepancies occurred (Fig. 2).

The frequency with which major and minor discrepancies occurred for each antibiotic is shown in Table 3. The greatest number of discrepancies occurred with cephalothin, although it should be noted that only two were major. The 10 major discrepancies were distributed randomly among six different antibiotics.

DISCUSSION

No single volume of inoculum produced the optimal zone diameter for all antibiotic-organism combinations tested. However, since the best agreement was obtained when 0.03 ml was used, this volume was selected for the clinical trial.

Since approximately 95% overall agreement was obtained in the clinical trial when 0.03 ml of inoculum was used, this volume is recommended. In our opinion this represents a clinically acceptable level of reliability. The variation of zone diameters observed in the standardiza-

TABLE 2. *Organisms included in the clinical comparison of the direct and standardized susceptibility tests*

Organism	No. of strains tested	Discrepancies		Agreements
		Major	Minor	
<i>E. coli</i>	46	2	22	390
<i>Klebsiella</i>	16	2	12	130
<i>Proteus mirabilis</i>	8	2	3	67
<i>Providencia stuartii</i>	1	0	1	8
<i>Citrobacter diversus</i>	1	0	0	9
<i>Citrobacter freundii</i>	1	0	1	8
<i>Enterobacter aerogenes</i>	3	0	1	26
<i>Enterobacter cloacae</i>	3	2	1	24
<i>Enterobacter agglomerans</i>	1	0	0	9
<i>Serratia marcescens</i>	3	0	0	27
<i>P. aeruginosa</i>	4	0	0	36
<i>Pseudomonas</i> species	2	0	2	16
<i>Bordetella pertussis</i>	1	0	0	9
<i>Acinetobacter calcoaceticus</i>	1	0	0	9
<i>S. aureus</i>	12	0	0	120
<i>Staphylococcus epidermidis</i>	8	2	4	74
<i>Enterococcus</i>	3	0	1	29
Group D <i>Streptococcus</i> (not <i>Enterococcus</i>)	1	0	0	10
Viridans <i>Streptococcus</i>	1	0	0	10

		STANDARDIZED SUSCEPTIBILITY TEST		
		S	I	R
DIRECT SUSCEPTIBILITY TEST	S	190 (76%)	1 (0.4%)	0
	I	2 (0.8%)	6 (2.4%)	0
	R	2 (0.8%)	2 (0.8%)	47 (18.8%)

FIG. 1. Comparison of 250 direct and standardized susceptibility tests performed on 25 strains of gram-positive organisms. Test agreements are shown in the unshaded areas. Minor and major discrepancies are indicated by light and heavy shading, respectively. S, Sensitive; I, intermediate; R, resistant.

		STANDARDIZED SUSCEPTIBILITY TEST		
		S	I	R
DIRECT SUSCEPTIBILITY TEST	S	606 (74.0%)	6 (0.7%)	1 (0.1%)
	I	29 (3.5%)	21 (2.6%)	4 (0.5%)
	R	7 (0.9%)	4 (0.5%)	141 (17.2%)

FIG. 2. Comparison of 819 direct and standardized susceptibility tests performed on 91 strains of gram-negative bacilli. Test agreements are shown in the unshaded areas. Minor and major discrepancies are indicated by light and heavy shading, respectively. S, Sensitive; I, intermediate; R, resistant.

TABLE 3. *Distribution of discrepancies between direct and standardized susceptibility tests by antibiotic*

Antibiotic	No. of comparisons	Discrepancies		
		Total	Major	Minor
Ampicillin	116	4 (3.8) ^a	1	3
Carbenicillin	91	4 (4.3)	0	4
Cephalothin	116	16 (13.8)	2	14
Chloramphenicol	116	6 (5.2)	3	3
Clindamycin	25	0	0	0
Colistin	91	6 (6.6)	2	4
Erythromycin	25	0	0	0
Gentamicin	116	0	0	0
Kanamycin	116	1 (0.8)	0	1
Methicillin	25	1 (4.0)	0	1
Penicillin	25	3 (12.0)	1	2
Streptomycin	91	9 (9.9)	0	9
Tetracycline	116	8 (6.9)	1	7

^a Numbers in parentheses represent the total number of discrepancies expressed as a percentage of the number of comparisons.

tion experiments indicates that consistency of performance within and between laboratories can be achieved only if a standard inoculum volume is employed. However, the occurrence of discrepancies, although infrequent, reemphasizes the necessity, as noted by others (1, 5), of confirming all direct tests by standardized methods.

The two major sources of error in direct susceptibility testing are unstandardized inoculum and mixed cultures. We have shown that with a standardized inoculum of 0.03 ml the direct test produces reportable results equivalent to the standardized test in 94.6% of the cases. Minor

and major errors are made in 4.5 and 0.9% of the tests, respectively. Culture purity is checked three times (Gram stain of the original broth and examination of both the subculture and susceptibility plates). With these precautions the chance of failing to detect a mixed culture is virtually nil. If a mixed culture is detected, the direct results should not be reported. The lack of reliability of susceptibility tests performed on mixed cultures has been well established (1, 5).

It should further be noted that when discrepancies occurred, they did so in the most clinically acceptable direction; i.e., in 46 of 58 discrepancies (79.3%) the standardized test indicated greater sensitivity than did the direct test. In only 12 of the 58 cases (20.7%) did the standardized test indicate that the organism was more resistant.

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