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Comparison of Direct and Standard Antimicrobial Disk Susceptibility Testing for Bacteria Isolated from Blood

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To determine the reliability of early antimicrobial susceptibility testing, we compared the results of direct and standard single-disk diffusion methods for 581 positive blood cultures processed routinely by the clinical microbiology laboratory. The direct procedure differed from the standard one only in that the 0.5 McFarland inoculum was prepared from 1 ml of turbid broth rather than five isolated colonies from a subculture plate. A major discrepancy in results was defined as a change from susceptible to resistant or vice versa according to interpretive standards for zone diameters, whereas a minor discrepancy was defined as a shift to or from the intermediate category when paired direct and standard tests were compared. The overall agreement between the two methods was 94.6% of 2,308 comparisons. There were 119 minor (5.2%) and 6 major (0.3%) discrepancies. The major discrepancies were seen with three strains of *Staphylococcus epidermidis* and one strain each of *S. aureus, Escherichia coli*, and *Enterobacter* sp. Direct susceptibility testing of positive blood cultures that were pure by gram-stained smear provided reliable results 24 to 36 h earlier than conventional procedures; therefore, we recommend this procedure to guide early antimicrobial therapy in patients with bacterial sepsis.

Antimicrobial susceptibility testing of bacteria isolated from blood is used to guide treatment, but results usually are not available by most techniques until 48 to 72 h after blood cultures are taken. More rapid susceptibility results could enable a change from empirical to specific antimicrobial therapy earlier, with possible reductions in cost and toxicity. In our hospital, over two-thirds of septic patients have their antimicrobial therapy altered after results of susceptibility tests are reported. Therefore, we compared the reliability of direct disk diffusion testing of 581 blood isolates with the standardized single-disk method for antimicrobial susceptibility testing as done routinely by the clinical microbiology laboratory.

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MATERIALS AND METHODS

Blood cultures. The 581 strains of bacteria studied were isolated from the blood of patients at Colorado General Hospital during a series of controlled evaluations of blood culture media (L. B. Reller, J. H. Tenney, S. Mirrett, and W.-L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol., 1977, C184, p. 66; M. P. Weinstein, L. B. Reller, S. Mirrett, and W.-L. L. Wang, Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, C176, p. 306). The media used were: a 20-ml tube and 50-ml bottle of supplemented peptone broth (SPB) with 0.03% sodium polyanetholsulfonate, a 50-ml bottle of supplemented peptone broth-sodium polyanetholsulfonate with 10% added sucrose, and a 50-ml bottle of Trypticase soy broth with 0.05% sodium amylosulfate (BD Division, Becton, Dickinson & Co., Rutherford, N.J.). All blood culture bottles were examined for macroscopic growth twice daily for 7 days before terminal subcultures. Stained smears and subcultures were done on all bottles after 12 to 24 h of incubation at 35°C and at the first sign of possible growth, such as turbidity, hemolysis, or gas.

Criteria for direct susceptibility testing. All turbid blood culture bottles with growth confirmed by a Gram-stained smear were included in the study except for the following: (i) any bottle that showed mixed flora by Gram stain; (ii) any bottle that looked pure on Gram stain but grew mixed flora on subculture; (iii) any isolates with predictable antimicrobial susceptibility patterns such as beta-hemolytic streptococci and *Streptococcus pneumoniae*; and (iv) any isolate detected first by subculture.

Disk diffusion testing. Antimicrobial susceptibility testing was done by the standardized single-disk method (4) exactly as updated by the National Committee for Clinical Laboratory Standards (NCCLS) (16). *Haemophilus influenzae* was tested on chocolate agar prepared from GC medium base (Difco Laboratories, Detroit, Mich.) with 1% hemoglobin and 1%

IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.), and zone diameters were interpreted as recommended by Thornsberry and Kirven (20). Modification of the standard method to enable direct susceptibility testing involved only a different preparation of the inoculum as described below. Reference strains of *Escherichia coli* (ATCC 25922; American Type Culture Collection, Rockville, Md.), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used each day as controls. Antibiotic disks from BBL were used throughout the study period. Antimicrobial agents tested routinely against blood isolates in our laboratory included only those of greatest clinical usefulness as recommended by Kunin (12). Staphylococci were tested against penicillin, methicillin, cephalothin, and clindamycin; enterococci were tested against ampicillin, chloramphenicol, and tetracycline; suspected *H. influenzae* were tested against ampicillin and chloramphenicol; and gram-negative rods were tested against ampicillin, cephalothin, gentamicin, kanamycin, chloramphenicol, and carbenicillin (*P. aeruginosa* only). Pneumococci and group A streptococci usually were not tested. When the technologist felt it was possible to differentiate clusters of staphylococci from chains of streptococci on the basis of the Gram-stained smear (1), the appropriate disks were placed on Mueller-Hinton agar for staphylococci or Mueller-Hinton agar with 5% sheep blood for enterococci. If a decision could not be made with certainty, disks and media for both staphylococci and enterococci were used.

Standardization of inoculum. The inoculum for the standard test was prepared with isolated colonies picked from subculture plates exactly as described by the NCCLS (16). The turbidity of actively growing, pure broth cultures was adjusted to match a 0.5 McFarland $BaSO_4$ turbidity standard by visual comparison with the aid of a modified Rh-typing view box (18). The inoculum for the direct test was prepared as follows: (i) about 1 ml of turbid supernatant was removed from the blood culture bottle with a needle and syringe without disturbing the sedimented layer of erythrocytes; (ii) the aspirate was mixed 1:1 with Trypticase soy broth containing 1% yeast extract and incubated at 35°C for 1 h; and (iii) the turbidity of the broth mixture then was adjusted with 0.9% sterile saline to match the 0.5 McFarland $BaSO_4$ standard. Mueller-Hinton agar plates were inoculated by streaking a swab over the entire surface in three directions; this step was identical for both the direct and standard techniques (16).

Criteria for interpretation. The diameters of the zones of inhibition for the direct and standard tests were recorded to the nearest 0.1 mm as measured by sliding calipers. Interpretive standards for resistant (R), intermediate (I), and susceptible (S) zone diameters were those published by the NCCLS (16), except that we used an intermediate (13 to 14 mm) category for gentamicin as advocated by Minshew et al. (15). Comparisons of results with the direct and standard methods for antimicrobial disk susceptibility testing were classified as follows: (i) "same" meant no change in interpretation (R, I, or S) with direct versus standard testing; (ii) a "minor" discrepancy was a change from R or S to I or a change from I to R or S; and (iii) a "major" discrepancy was a change from R to S or S to R.

RESULTS

The 581 blood cultures studied and the antimicrobial disks used enabled 2,308 comparisons between the direct and standard methods for disk diffusion susceptibility testing. The same interpretation of results occurred in 2,184 (94.6%) of the comparisons. There were 6 (0.3%) major discrepancies and 119 (5.2%) minor discrepancies.

Table 1 summarizes the major and minor discrepancies that occurred with gram-positive cocci isolated from blood on more than 10 occasions. *Staphylococcus epidermidis* showed the most discrepancies among the gram-positive cocci and accounted for 3 of the 6 major and 31 of the 119 minor discrepancies found in this study. In our studies of bacteremia we have found that about 97% of our blood isolates of *S. epidermidis* were judged ultimately to be contaminants by strict clinical criteria (M. P. Weinstein, K. A. Lichtenstein, and L. B. Reller, unpublished data). *S. aureus* accounted for one major (a shift from R by direct to S by standard testing with penicillin) and six minor discrepancies. Figures 1A, B, and C show the ranges of zone sizes seen with all strains of *S. aureus* isolated from blood and tested against penicillin, methicillin, and clindamycin, respectively. The histograms for direct and standard zone diameters overlap almost perfectly. The mean zone diameters for all comparisons that resulted in the same interpretations were almost identical; differences between readings in the direct and standard tests averaged less than 1 mm, which is well within the precision of the disk diffusion technique (3, 19). We found only 1 minor discrepancy (a shift from R by direct to I by standard testing with ampicillin) among the 15 enterococci studied. Penicillin and methicillin accounted for 22 and 15, respectively, of the 38 minor discrepancies seen with gram-positive cocci (Table 1).

Table 2 summarizes the results of comparisons of direct and standard susceptibility tests with all strains of *Enterobacteriaceae* that were isolated more than 10 times from blood cultures. *E. coli* was the most frequent blood isolate and accounted for 40 of the 50 minor discrepancies and 1 (a shift from R by direct to S by standard testing with chloramphenicol) of the 2 major discrepancies seen with the *Enterobacteriaceae*. Figures 2A, B, and C show the frequency distributions of zone diameters measured by direct and standard testing of *E. coli* against ampicillin, gentamicin, and kanamycin, respectively. The mean zone diameters measured by direct and

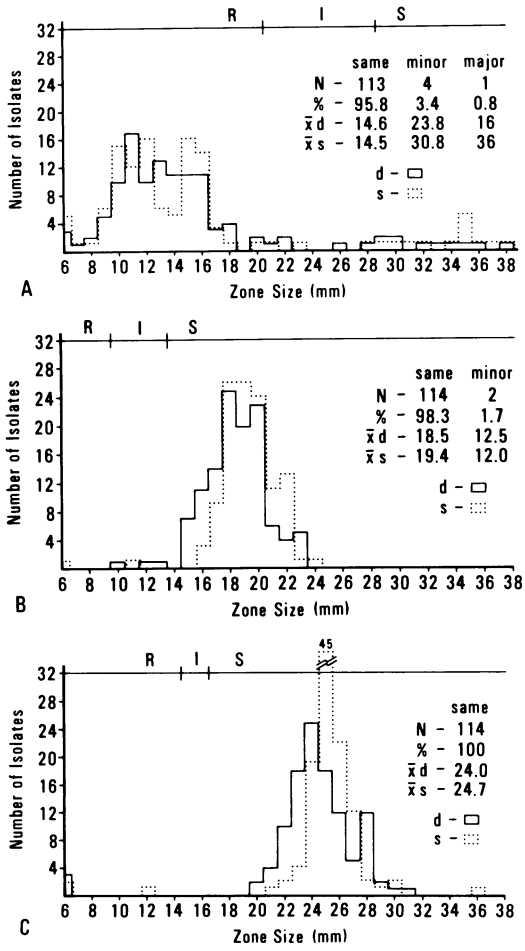

FIG. 1. *Comparisons of zone diameters by direct (d) and standard (s) methods for antimicrobial disk susceptibility testing of S. aureus versus penicillin (A), methicillin (B), and clindamycin (C). R, Resistant; I, intermediate; S, susceptible; N, number of strains tested; and x̄, mean zone diameter (millimeters).*

standard techniques differed by <2 mm, regardless of interpretive category, with strains of *E. coli* tested against ampicillin, gentamicin, and kanamycin. There were no major but 22 minor discrepancies with the 56 isolates of *Klebsiella pneumoniae*, when tested against all antibiotics (Table 2). Detailed results of *K. pneumoniae* versus cephalothin are shown in Fig. 3. The second and last major discrepancy seen among the *Enterobacteriaceae* was with an *Enterobacter cloacae* that was susceptible to ampicillin by direct testing but resistant in the standard test.

Not shown in the tables or figures are results from 19 strains of *H. influenzae* isolated from blood; none showed any discrepancy. It should

TABLE 1. *Comparison of interpretive results with direct and standard methods for antimicrobial disk susceptibility testing of gram-positive cocci cultured from blood*[a]

Blood culture isolates	Penicillin			Methicillin			Cephalothin			Clindamycin			Ampicillin			Tetracycline			Total		
	n	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor
S. aureus	118	1	4	116	0	2	119	0	0	114	0	0	—	—	—	—	—	—	467	1 (0.2)	6 (1.3)
S. epidermidis	180	0	18	178	2	13	180	0	0	177	1	0	—	—	—	—	—	—	715	3 (0.4)	31 (4.3)
Enterococci	11	0	0	—	—	—	—	—	—	—	—	—	15	0	1	13	0	0	39	0	1 (2.6)
Total		(0.3)	(7.1)		(0.7)	(5.1)					(0.3)				(6.7)					(0.3)	(3.1)

[a] Values in parentheses incidate percent. *n*, Number of bacterial strains compared. Major discrepancy is a change from susceptible to resistant or vice versa. Minor discrepancy is a change to or from the intermediate category. —, Isolate not tested.

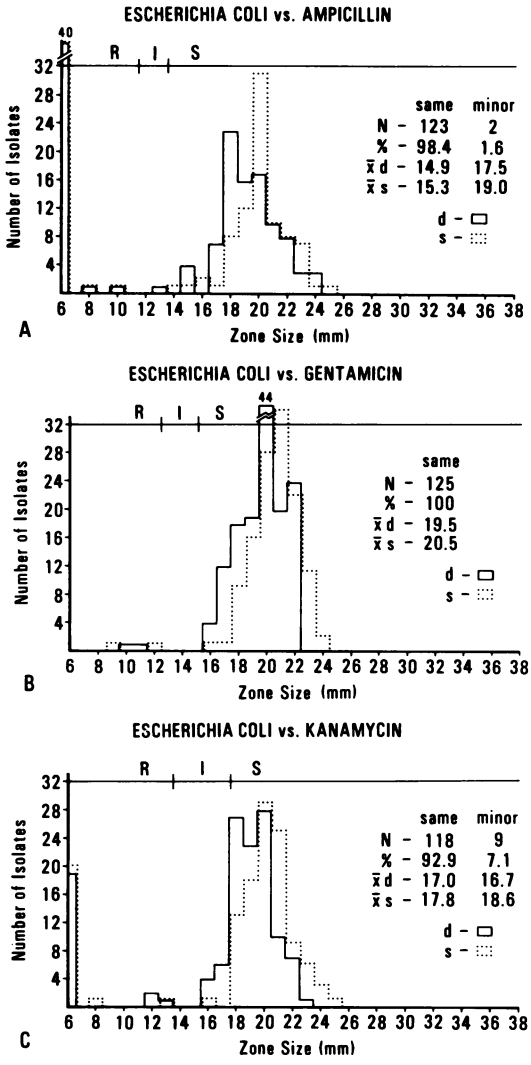

FIG. 2. *Comparisons of zone diameters by direct (d) and standard (s) methods for antimicrobial susceptibility testing of E. coli versus ampicillin (A), gentamicin (B), and kanamycin (C). Abbreviations as in legend to Fig. 1.*

be noted that, in all cases, *H. influenzae* was suspected by Gram stain, by known cerebrospinal fluid culture results, or because the patient was a child. Under these circumstances the technologist was alerted to include a chocolate agar plate with ampicillin and chloramphenicol disks. Also, 22 isolates of *P. aeruginosa* were tested against gentamicin with no major but 2 minor discrepancies (both were shifts from R by direct to I by standard testing). Of these *P. aeruginosa* strains, 11 were tested against carbenicillin with no major and 2 minor discrepancies.

TABLE 2. *Comparison of interpretive results with direct and standard methods for antimicrobial disk susceptibility testing of Enterobacteriaceae cultured from blood*[a]

Blood culture isolates	Ampicillin			Cephalothin			Gentamicin			Kanamycin			Chloramphenicol			Tetracycline			Total		
	n	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor	*n*	Major	Minor
E. coli	125	0	2	121	0	17	125	0	0	127	0	9	17	1	0	68	0	12	583	1 (0.2)	40 (6.9)
K. pneumoniae	54	0	7	56	0	5	56	0	1	56	0	7	23	0	0	33	0	2	278	0	22 (7.9)
Serratia marcescens	14	0	1	14	0	0	14	0	0	14	0	2	—	—	—	10	0	1	66	0	4 (6.1)
Salmonella spp.	13	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13	0	1 (7.7)
Enterobacter spp.	16	1	3	16	0	2	16	0	0	16	0	1	—	—	—	12	0	4	76	1 (1.3)	10 (13.2)
Total		(0.4)	(6.3)			(11.6)			(0.5)			(8.9)			(2.5)			(15.4)		(0.2)	(7.6)

[a] Values in parentheses indicate percent. *n*, Number of bacterial strains compared. Major discrepancy is a change from susceptible to resistant or vice versa. Minor discrepancy is a change to or from the intermediate category. —, Isolate not tested.

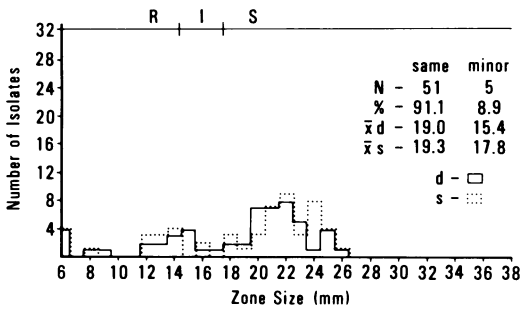

FIG. 3. *Comparison of zone diameters by direct (d) and standard (s) methods for antimicrobial susceptibility testing of K. pneumoniae versus cephalothin. Abbreviations as in legend to Fig. 1.*

DISCUSSION

There is general agreement that accurate in vitro susceptibility testing provides clinically useful, and often essential, information for specific antimicrobial therapy. Patients with suspected bacterial sepsis are frequently given potent combination chemotherapy until culture and susceptibility data are available. Although warranted, such empirical therapy is both expensive and potentially toxic. Earlier results of antimicrobial susceptibility testing could benefit patients. Moreover, studies at our medical center have shown that physicians did change from initial empirical therapy to more appropriate antimicrobial therapy in 71% of the episodes of confirmed bacteremia after susceptibility results were reported (M. P. Weinstein, L. B. Reller, J. R. Murphy, and K. A. Lichtenstein, unpublished data).

How then can susceptibility results be obtained faster, and what are the pitfalls? Barry et al. (2) have thoroughly discussed these issues and have warned against direct susceptibility testing of clinical specimens unless there is a single microorganism and the inoculum density can be controlled. There is ample support for these recommendations. Shahidi and Ellner showed that artificial mixtures of bacteria gave unreliable and unpredictable results by disk diffusion testing (17). Direct susceptibility testing of wound exudates (6) and mixed urine cultures (9) also have been shown to provide clinically misleading information. Conversely, pure urine cultures (9, 22) and positive blood cultures (7, 10, 21) have yielded accurate results by direct testing when care has been taken to provide an adequate inoculum density.

Our results in 581 positive blood cultures both confirm and extend observations in three published reports about the reliability of direct susceptibility testing with 74 (21), 252 (10), and 116 (7) blood isolates. Two of these reports used interpretive criteria comparable to our own. Johnson and Washington found an overall correlation of 87.9% between direct and standard susceptibility tests by the agar dilution method; they had 10.4% minor and 1.7% major discrepancies (10). Fay and Oldfather found 94.6% test agreements and 4.5% minor and 0.9% major discrepancies between direct and standard disk diffusion tests (7), whereas we report herein total agreement for 94.6% of all comparisons and 5.2% minor and 0.3% major discrepancies in 2,308 paired tests. Our results are well within the precision and accuracy expected within and between laboratories for the disk diffusion test (3, 19). Indeed, the intralaboratory reproducibility reported for the standard single-disk test itself (91.5% agreement, and 7.1% minor and 1.2% major discrepancies) (19) is remarkably similar to our results and those of others (7).

Standard susceptibility testing requires three time-consuming steps: (i) 16 to 20 h to obtain isolated colonies after subculture of blood, (ii) 2 to 8 h for growth of the broth inoculum, and (iii) 16 to 20 h before zones of inhibition or endpoints are measured (2, 16). Early reading of zone sizes can be done reliably in most cases after 6 to 10 h of incubation (2, 11, 13); this applies to both standard and direct tests. What direct testing provides is an additional saving of 18 to 24 h by eliminating steps 1 and 2. Yet the direct technique we describe differs from the standard method only in that the inoculum is prepared from 1 ml of blood culture broth rather than four or five well-isolated colonies on a subculture plate (16).

Moreover, the majority of our positive blood cultures met our criteria outlined for direct testing. Most of our positive blood cultures were detected initially by macroscopic examination of early routine-stained smears; this experience is similar to that of Blazevic et al. (5), who reported that only 12% of their positive blood cultures were found first by subculture. Also, about 90% of our true bacteremias were caused by a single organism, which is in accord with the finding by Hermans and Washington of a 6% incidence of polymicrobial bacteria in patients with bacteriologically confirmed bacteremia (8).

In conclusion, we have shown that direct antimicrobial susceptibility testing of bacteria isolated from blood is often feasible, useful, and most importantly, highly reliable provided that the criteria outlined are followed. Results can be available as early as 24 to 36 h after blood for culture is drawn from a patient. Confirmation of the purity of the culture and use of standardized inoculum are essential. This latter point can

be neither overemphasized nor stated better than McFarland did in 1907: "... if the density of the suspensions used are [sic] not measured by some fixed standards, exact repetition either by the experimenter himself or by those who try to repeat his work will be impossible" (14).

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