Comparison of Direct and Standard Microtiter Broth Dilution Susceptibility Testing of Blood Culture Isolates

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Turbid broth (0.5 ml) from blood culture bottles was inoculated into 0.5 ml of brain heart infusion broth, incubated for 3 to 6 h, diluted 1:500 in distilled water, and then inoculated directly into microtiter broth dilution susceptibility trays to test for minimal inhibitory concentrations. The results were compared to the standard tests performed 24 h later on colonies from subculture plates. The minimal inhibitory concentrations measured by these two methods were compared in 1,875 organism-antibiotic tests. The two minimal inhibitory concentrations were identical in 86.0% and within one twofold dilution in 98.0% of the tests. An organism was judged to be susceptible by one method and resistant by the other in 13 tests (0.7%). These 13 discrepancies were distributed among several organism-antibiotic combinations; no more than two were seen for any one combination. Highly accurate susceptibility testing can be achieved by using direct inoculation of turbid blood culture broth.

In an effort to shorten the time required to obtain antibiotic susceptibility data on blood culture isolates, turbid blood broths have been directly inoculated into susceptibility testing systems. The direct inoculation procedure compared with the conventional method was shown to be accurate for disk diffusion (3, 6, 9) and agar dilution (5) methods. This study was undertaken to determine the accuracy of performing direct susceptibility tests from turbid blood culture bottles by using a microtiter broth dilution method. We compared minimum inhibitory concentration (MIC) tests of 271 turbid broths with the standard method by using a stationary-phase inoculum technique and a commercial microtiter system.


MATERIALS AND METHODS

Blood cultures. Blood cultures were performed by aseptically dispensing blood from patients in either of two methods depending on the time of collection. During the day blood was drawn into Vacutainer tubes (165 by 16 mm) containing 3.4 ml of 0.35% sodium polyanethol sulfonate (Becton, Dickinson & Co., Rutherford, N.J.). Blood from these tubes was inoculated in the laboratory into two 50-ml Columbia broth bottles supplemented with 1 g of cysteine per liter (Becton, Dickinson & Co.). During the evening, blood was directly inoculated into two 50-ml Columbia broth bottles supplemented with cysteine and 0.025% sodium polyanethol sulfonate. One of the two bottles was vented with a single-draw Vacutainer needle to aerate the media. All bottles were incubated at 35°C for 7 days and examined daily, and the broth was routinely subcultured onto chocolate agar between 6 and 24 h and at 5 days. When the broth was turbid, subcultures were performed on the appropriate media as dictated by the Gram stain results. Mixed cultures were detected by Gram stains of the turbid broth bottles or by morphology of the colonies on subculture plates; mixed cultures were rejected from the study.

Susceptibility testing. The MicroScan microdilution antimicrobial susceptibility system (MicroScan, Inc., Hillsdale, N.J.) was used for all MIC determinations. Kits consisted of antimicrobial test panels, tubes containing 0.5 ml of brain heart infusion broth, tubes containing 25 ml of sterile distilled water supplemented with 0.02% Tween 80, plastic seed trough trays, and transfer lids with prongs. The test panels were delivered frozen to the laboratory and were stored at −20°C. Once thawed, the panels were used within 2 h.

Antibiotics. The only antibiotic-organism combinations evaluated were those with clinical relevance. Staphylococci were tested against cephalothin, clindamycin, erythromycin, methicillin, penicillin, and vancomycin; enterococci were tested against ampicillin, erythromycin, penicillin, and vancomycin; members of the Enterobacteriaceae were tested against amikacin, ampicillin, carbencillin, cefamandole, cephalothin, chloramphenicol, gentamicin, tetracycline, and tobramycin; and Pseudomonas aeruginosa was tested against amikacin, carbencillin, gentamicin, and tobramycin.

Control organisms. E. coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella oxytoca.
MS101, Streptococcus faecalis ATCC 29219, and Staphylococcus aureus ATCC 29213 were used throughout the study. 

MIC inoculum preparation. For direct inoculum preparation, a 0.05-sample of turbid blood culture broth was aspirated into a 1.0-ml syringe and inoculated into 0.5 ml of brain heart infusion broth. The broth was incubated at 35°C for 3 to 6 h.

The manufacturer's recommendations were followed for the standard inoculum preparation. Four or five isolated colonies were chosen from the isolation plate, inoculated into 0.5 ml of brain heart infusion broth, and then incubated at 35°C for 4 to 6 h.

Microtiter tray inoculation. After incubation, the direct and standard bacterial suspensions were diluted by pipetting 0.05 ml of each stationary-phase suspension into a sterile screw-capped tube containing 25 ml of distilled water with 0.02% Tween 80. The tube was inverted 8 to 10 times, and the liquid was poured into a seed trough. The transfer lid was used to inoculate the antimicrobial panels. The microtiter trays were incubated for 15 to 18 h at 35°C without supplemented CO₂.

Interpretation. The lowest concentration of antibiotic showing complete inhibition of growth was recorded as the MIC of each antibiotic. The criteria for reporting an isolate as susceptible, intermediate, or resistant were derived from the National Committee for Clinical Laboratory Standards standards (7).

Differences in results between the direct and standard methods were expressed as the MIC ratio (direct/standard method). If the MIC values of the two methods were identical, the ratio was 1; if the MIC was higher with the direct method, the ratio was 2, 4, or 8. If the MIC was lower with the direct method, the ratio was 0.5, 0.25, or 0.125.

RESULTS

Direct and standard MIC methods were performed on 271 organisms and 1,988 organism-antibiotic combinations. Results from the two methods differed by at least one dilution in 375 tests. In 113 of these 375 tests, one of the MIC values was beyond the concentrations tested.

These results were considered not valid for analysis and were excluded from the abovementioned comparison. If the off-scale MIC was read as the concentration of the antibiotic at the last dilution tested, 91 of the 113 discrepancies would have varied by only one dilution.

Comparisons of the remaining direct and standard MIC results for the individual genera and the 14 antibiotics are shown in Tables 1 and 2. Of the 1,875 valid comparisons between the two methods, 86% of the tests yielded identical MIC results. In 98%, the MIC's differed by one twofold dilution or less. The direct method did not consistently give higher or lower MIC values than did the standard method (Table 1). For each genus, 96.1% or more of the paired tests gave MIC results within one twofold dilution. The ratios of the direct and standard method comparisons listed by antibiotic also showed a high degree of correlation (Table 2). The MIC values were within one twofold dilution at least 90.3% of the time for each antibiotic.

Of the 375 differences between the MIC values measured by the direct and standard methods, 13 were sufficient to be interpreted as indicating that an organism was susceptible by one method and resistant by the other. There was no clustering of these discrepancies by antibiotic or by organism. There were no more than two discrepancies for any single antibiotic and no more than three for any single species. The six discrepancies of most concern, where the direct MIC indicated that the organism was susceptible and the standard MIC indicated resistance, were observed with five different genera of organisms and six different antibiotics.

In 23 of the paired tests, one test indicated that the organism was susceptible or resistant, whereas the other indicated an intermediate susceptibility. There was also no clustering of organisms or antibiotics for these discrepancies.
DISCUSSION

Accurate and timely reporting of antimicrobial susceptibility results are an important function of the clinical microbiology laboratory. Although some authors have cautioned against testing for susceptibility directly from material obtained from wound exudates (2), urine (4), and mixed cultures (8), other data indicate that direct testing of blood culture broths provides acceptable results (3, 5, 6, 9). These authors have stressed the importance of standardizing the inoculum for the direct susceptibility tests.

Our comparison of direct and standard MIC susceptibility tests confirms the accuracy of direct blood culture broth susceptibility testing. Identical values were obtained with 86% of the valid comparisons, and 98% were within one twofold dilution. Even if all of the 113 nonidentical results, which involved off-scale antibiotic concentrations and were not analyzed, were assumed to have differed by more than one dilution, 92.5% of the overall comparisons would still be within one twofold dilution. No single antibiotic or organism gave MIC values that were systematically higher or lower by either method.

The overall agreement for interpretive values (susceptible, intermediate, and resistant) was 98.2%, and the discrepancies were randomly distributed. The very high overall agreement between the direct and standard MIC tests was probably due in part to the method used for inoculum preparation. We used the stationary-phase technique described in the manufacturer's literature that was originally reported by Barry et al. (1). Bacteria were allowed to reach the stationary phase rapidly in a small volume of medium. The suspension was diluted and used immediately for susceptibility testing. A standard inoculum was thus achieved.

In conclusion, the results of this study indicate that MIC susceptibility testing performed directly from blood culture broths is accurate.

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LITERATURE CITED