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# Rapid Antimicrobial Susceptibility Testing of Isolates from Blood Cultures by Direct Inoculation and Early Reading of Disk Diffusion Tests

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**Disk diffusion tests, inoculated directly from positive blood cultures, were evaluated for accuracy of reading zone diameters after 4- and 6-h and overnight incubation. In comparisons with results from standard disk diffusion tests, the 4-h results were in agreement for 83% of tests with gram-positive organisms and 64% of tests with gram-negative organisms. When minor discrepancies were ignored, the 4-h readings were in agreement for 98% of the tests with gram-positive organisms and 95% of the tests with gram-negative organisms. After 6 h of incubation, 91% of the tests with gram-positive organisms and 86% of the tests with gram-negative organisms agreed with standard results. The agreement was 99% for tests with both gram-positive and gram-negative organisms when minor discrepancies were excluded. Very major discrepancies occurred in two tests (0.1%) with gram-positive organisms and were not observed in tests with gram-negative organisms. The frequencies of major discrepancies were 3.5% after 4 h, 0.6% after 6 h, and 0.7% after overnight incubation. Ampicillin and cephalothin tests with *Escherichia coli* and *Klebsiella* spp. accounted for 81% of the major discrepancies in tests with gram-negative organisms. Oxacillin tests accounted for more than half of the major discrepancies in tests with staphylococci. The results of this study, which did not include the newer antibiotics, indicate that direct susceptibility tests from blood cultures read after 6 h of incubation are more reliable than 4-h results and produce less than 1% major errors in comparisons with standard susceptibility tests.**

The results of direct antimicrobial susceptibility testing of positive blood cultures have been shown to correlate well with those of standardized tests. In most of the foregoing studies, blood culture samples were subcultured to broth to allow adjustment of the inocula before susceptibility testing (6, 7, 12, 15). However, even studies utilizing inocula taken directly from blood culture bottles have reported very good agreement with results from standardized tests (4, 5). Reller et al. recommend that the inoculum density should be adjusted to match a 0.5 McFarland turbidity standard for direct susceptibility tests from blood cultures (14).

When physicians request more rapid information to guide their selection of antimicrobial agents for patients with positive blood cultures, it is common practice in many laboratories to report presumptive susceptibilities after 4- to 6-h incubation of direct disk diffusion tests from unadjusted blood cultures. Although the reliability of early reading of zone diameters has been evaluated in studies that prepared standardized inocula from random clinical isolates (1, 2, 8, 10, 11), there has been no evaluation of rapid susceptibility results in which both direct inoculation and early reading were combined. This study was undertaken to test the accuracy of 4- and 6-h readings of disk diffusion tests that were inoculated directly from positive blood cultures.

## MATERIALS AND METHODS

**Blood cultures.** The data were collected in the clinical microbiology laboratories of the University of Washington teaching hospitals, including the University Hospital (UH), Harborview Medical Center (HMC), Seattle Veterans' Administration Medical Center (SVA) and Children's Orthopedic Hospital and Medical Center (COH). During the first phase of the study, between January 1976 and September 1978, direct susceptibility tests were only done on blood cultures that did not have colonies available for standardized inocula. In the interest of collecting more data, the study was resumed between February and September of 1979, and direct tests were done on all positive blood cultures. Each laboratory used its routine blood culture media: the aerobic media were (i) tryptic soy broth (Prepared Media, Tualatin, Ore.) plus 1% yeast extract at University Hospital and Harborview Medical Center, (ii) TSY plus 0.16% starch at Children's Orthopedic Hospital and Medical Center, and (iii) TSY supplemented with vitamin K, hemin, and 0.17% agar at Seattle Veteran's Administration Medical Center. The anaerobic medium was supplemented peptone broth (Becton Dickinson and Co.) at University Hospital and Harborview Medical Center. Neither the Children's Orthopedic Hospital and Medical Center nor the Seattle Veterans' Administration Medical Center utilized an anaerobic blood culture bottle during this study. The TSY bottle was used for direct susceptibility tests unless the supplemented peptone bottle alone was positive.

**Criteria for direct susceptibility testing.** Blood cultures found to be positive during daily examination for turbidity or by routine blind Gram stain were included in the study unless mixed flora was observed on Gram stain or on subculture.

**Disk diffusion tests.** For direct susceptibility tests, a sterile cotton swab was dipped into a sample from a well-mixed, unadjusted blood culture, and excess fluid was expressed before streaking onto a Mueller-Hinton agar plate. Blood

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TABLE 1. Percentage of isolates with direct tests read after 4 or 6 h

| Blood culture isolate | No. of isolates | % Read after: | |
|---|---|---|---|
| | | 4 h | 6 h[a] |
| Gram positive | | | |
| *S. aureus* | 60 | 22 | 63 |
| Coagulase-negative staphylococci | 87 | 3 | 21 |
| Beta-hemolytic streptococci | 30 | 37 | 87 |
| Enterococci | 21 | 19 | 52 |
| Pneumococci | 21 | 10 | 38 |
| Viridans streptococci | 14 | 0 | 0 |
| Total for gram positive | 233 | 14 | 44 |
| Gram negative | | | |
| *E. coli* | 84 | 52 | 85 |
| *Klebsiella spp.* | 38 | 40 | 76 |
| *Enterobacter spp.* | 12 | 42 | 92 |
| *P. aeruginosa* | 11 | 0 | 64 |
| Others[b] | 25 | 36 | 60 |
| Total for gram negative | 170 | 43 | 78 |

[a] Percentage of isolates read within 6 h includes those read at 4 h.
[b] Includes *Proteus* spp. (7), *Salmonella* spp. (6), *Serratia* spp. (4), *Acinetobacter* spp. (4), *Moraxella* spp. (2), *Providencia* spp. (1), and group IIk (1).

cultures that contained gram-positive cocci were tested on Mueller-Hinton agar supplemented with 5% sheep blood. These were the only deviations from the standardized inoculation procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for antimicrobic disk susceptibility tests (13). Standard disk diffusion tests were performed from subcultures on the following day by the method of the NCCLS and read after 16 to 20 h of incubation at 35°C. Gram-positive organisms were tested with penicillin, oxacillin, cephalothin, chloramphenicol, clindamycin, erythromycin, and tetracycline. Gram-negative organisms were tested with ampicillin, cephalothin, chloramphenicol, gentamicin, and tobramycin. Antibiotic disks (BBL Microbiology Systems) were used throughout the study. Quality control strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used for routine internal quality control according to each laboratory schedule (range, 1 to 7 days).

Disk diffusion plates were incubated aerobically at 35°C and examined after 4 h. If inhibition zones were not clearly visible, the plates were reincubated and examined once again after 6 h of incubation. The plates were then reincubated, and the zone sizes were recorded after overnight incubation.

To facilitate the reading of zones of inhibition in the thin lawns observed after incubation for 4 or 6 h, the agar surface of an open plate was examined with reflected light. After overnight incubation, zones were read by the method of the NCCLS: through the back of the petri dish held against a black background and illuminated with reflected light. Plates containing blood agar were read from the surface. Zones of inhibition for direct and standard tests were measured with calipers, and zone sizes were recorded in millimeters.

**Interpretation of zone sizes.** Susceptibility interpretations, i.e. susceptible, intermediate, or resistant, conformed to the criteria of the NCCLS (13). The results from direct susceptibility tests were compared with results from standard tests, and discrepancies were classified as very major, major, or minor. A very major discrepancy was a susceptible result by the direct method and a resistant result by the standard method (S→R). A major discrepancy was a resistant result by the direct method and a susceptible result by the standard method (R→S). A minor discrepancy was any change involving an intermediate result (R↔I↔S).

## RESULTS

The data from direct disk diffusion tests of 403 positive blood cultures were compared with standard disk diffusion test results. As shown in Table 1, less than half of the tests with gram-positive isolates had readable zones during the early observation periods; only 14 and 44% could be read after 4 and 6 h, respectively. None of the 14 viridans streptococci lawns could be read after 6 h of incubation, but it was psssible to read zones of inhibition for more than 50% of the *S. aureus* and enterococcal isolates and for 87% of the beta-hemolytic streptococci. Forty-three percent of the direct disk diffusion tests with gram-negative isolates were readable after 4 h, and 78% were readable after 6 h.

The results from zone size interpretations of direct susceptibility tests read after incubation for 4 and 6 h and overnight were compared with the interpretations from standard diffusion tests incubated for 16 to 18 h (Table 2). There were two very major discrepancies in tests with gram-positive organisms: one was due to an erythromycin zone on a beta-hemolytic streptococcus culture read after 4 h, and the other was due to an oxacillin zone on a coagulase-negative staphylococcus culture read after overnight incubation. There were 14 major discrepancies; 8 occurred in oxacillin tests with staphylococci, including three *S. aureus* and five coagulase-negative isolates.

There was no very major discrepancy in direct tests with gram-negative organisms. Major discrepancies were found in 4.7% of the tests read after 4 h and in 0.7% of the 6-h readings. The frequency of minor discrepancies after 4 h was more than twice that found after 6 h of incubation: 31.6 and 13.5%, respectively. A comparison of the results from the standard and direct tests after overnight incubation yielded 6 major discrepancies (0.8%) and 73 minor discrepancies (9.6%). Of the 26 major discrepancies, 24 occurred with *E. coli* or *Klebsiella* spp., and 21 (88%) of these occurred in ampicillin or cephalothin tests.

Table 3 summarizes the percent agreement for tests with each antibiotic and gram-positive isolates read after 4 or 6 h or overnight incubation. The 4-h results from chloramphenicol and erythromycin tests were least reliable. Results from

TABLE 2. Discrepancies from direct tests compared with standardized tests

| Isolate type, time incubated | No. of tests | No. of discrepancies | | | Overall agreement (%) |
|---|---|---|---|---|---|
| | | Very major (%) | Major (%) | Minor (%) | |
| Gram positive | | | | | |
| 4 h | 216 | 1 (0.5) | 3 (1.4) | 32 (14.8) | 83.3 |
| 6 h | 494 | 0 | 3 (0.6) | 39 (7.9) | 91.4 |
| Overnight | 1,307 | 1 (0.07) | 8 (0.6) | 65 (5.0) | 94.3 |
| Gram negative | | | | | |
| 4 h | 361 | 0 | 17 (4.7) | 114 (31.6) | 63.7 |
| 6 h | 438 | 0 | 3 (0.7) | 59 (13.5) | 85.8 |
| Overnight | 762 | 0 | 6 (0.8) | 73 (9.6) | 89.6 |

overnight incubation of direct tests correlated with standard test results in more than 89% of the tests with every antibiotic. Table 3 also presents the percent agreement when minor discrepancies were omitted. Minor discrepancies accounted for the vast majority of disagreements for tests with all antibiotics except oxacillin, with which there were one very major and eight major discrepancies.

Table 4 summarizes the correlations between results of direct and standard tests with each antibiotic and gram-negative isolate. The overall correlations from 4-h readings were poor, particularly in tests with cephalothin (47.3%) and gentamicin (44%). The 6-h results were much better than those from the 4-h readings, and results after overnight incubation were better for tests with all drugs except cephalothin. Minor discrepancies accounted for almost all disagreements in tests with gram-negative isolates and chloramphenicol, gentamicin, and tobramycin.

There was a general trend for zone sizes to increase in diameter when tests with susceptible organisms were incubated beyond 4 h. The data in Tables 5 and 6 summarize the average zone sizes from 4- and 6-h and overnight measurements for each organism-antimicrobial comparison. Relatively large increases were seen in tests with susceptible staphylococci; the smallest difference between 4-h and overnight mean zone sizes was 3 mm for tests with *S. aureus* and tetracycline, and the largest was 8 mm for tests with coagulase-negative staphylococci and chloramphenicol. The only test with staphylococci that did not show a marked increase in zone diameters with increasing incubation time was the test with *S. aureus* and penicillin; 91% of the isolates were resistant. In tests with streptococci, substantial increases only occurred in tests with enterococci and ampicillin and tests with beta-hemolytic streptococci and penicillin and cephalothin. The mean zone diameters after overnight incubation of susceptible members of the family *Enterobacteriaceae* were usually 2 to 4 mm larger than the corresponding 4-h mean zone sizes. Zone diameters did not increase in organism-drug combinations which are usually resistant, i.e., ampicillin tests of *Klebsiella* spp. and ampicillin and cephalothin tests of *Enterobacter* spp.

For every organism-antibiotic combination, the mean zone diameters of standardized tests were larger than mean zones from overnight readings of direct tests (Tables 5 and 6).

The effect of added penicillinase on direct susceptibility tests was observed in one laboratory as a result of a routine protocol in which a blood culture bottle from a patient receiving penicillin was injected with a drop of penicillinase sufficient to inactivate $3 \times 10^5$ U of penicillin. The ampicillin zone diameters in the direct test read after 4- and 6-h and overnight incubation were only 6 to 8 mm, whereas the ampicillin zone size in the standard test was 21 mm. The data from this blood culture were excluded from the study.

TABLE 3. Percent agreement between interpretations of direct and standard tests of gram-positive isolates

| Antibiotic | Agreement (%) between standard tests and direct tests incubated[a]: | | |
|---|---|---|---|
| | 4 h | 6 h | Overnight |
| Penicillin | 90.3 (96.7) | 85.7 (98.6) | 89.8 (99.5) |
| Oxacillin | 83.9 (93.5) | 92.4 (98.5) | 92.2 (96.7) |
| Cephalothin | 80.6 (100) | 94.4 (100) | 95.2 (99.5) |
| Chloramphenicol | 67.6 (100) | 90.3 (100) | 94.2 (100) |
| Clindamycin | 93.3 (100) | 97.2 (100) | 98.4 (100) |
| Erythromycin | 74.2 (96.7) | 88.7 (100) | 94.1 (100) |
| Tetracycline | 93.5 (96.7) | 93.1 (98.6) | 96.8 (99.5) |

[a] Numbers in parentheses refer to percent agreement with minor discrepancies excluded.

TABLE 4. Percent agreement between interpretations of direct and standard tests of gram-negative isolates

| Antibiotic | Agreement between standard tests and direct tests incubated[a]: | | |
|---|---|---|---|
| | 4 h | 6 h | Overnight |
| Ampicillin | 73.0 (91.8) | 87.8 (97.8) | 93.6 (98.1) |
| Cephalothin | 47.3 (89.2) | 81.4 (98.8) | 74.0 (98.7) |
| Chloramphenicol | 74.3 (100) | 94.4 (100) | 94.2 (100) |
| Gentamicin | 44.0 (98.7) | 70.0 (100) | 90.3 (100) |
| Tobramycin | 82.8 (96.9) | 96.4 (100) | 96.5 (99.3) |

[a] Numbers in parentheses refer to percent agreement with minor discrepancies excluded.

## DISCUSSION

In this study of 3,578 comparisons of direct and standardized susceptibility tests, there was an overall agreement of 88.2%. As expected, the best correlations were obtained with direct tests that had been incubated overnight. Of 2,069 overnight tests, we found 1 very major (0.05%), 14 major (0.7%), and 138 minor (6.7%) discrepancies that resulted in a 92.7% overall agreement. These results are very similar to those from two of the three previous studies in which positive blood culture broths were used as unadjusted inocula for overnight disk susceptibility tests (4, 5, 15). Wegner et al. (15) found erratic results and abandoned this practice in favor of an intermediate subculture followed by a turbidity adjustment. Very good reliability was obtained by the other two laboratories that controlled the inoculum by counting the drops of blood culture broth used to streak the Mueller-Hinton plates (4, 5). Fay and Oldfather, using a 0.03-ml inoculum of blood culture, obtained an overall agreement of 94.6% with the results from standardized inocula (5). Doern et al. used a similar inoculum of 0.05 ml for 4,234 organism-antimicrobial comparisons, and found only 5 very major (0.1%), 64 major (1.5%), and 332 minor (7.8%) discrepancies (4). When discrepancies of 2 mm or less were disregarded, the overall agreement between their direct and standardized tests was 96.8%.

The overall agreement of tests with both gram-positive and gram-negative isolates read at 4 h in this study was only 71.2%. However, the vast majority of the discrepancies were in the minor category, which should not have a significant negative impact on the choice of therapy. It was interesting to find that the direct susceptibility tests with gram-negative isolates were more likely than those with gram-positive isolates to be readable after 4 to 6 h of incubation but the frequency of both major and minor errors was two to three times greater in gram-negative organisms.

All of the previous studies of early readings of disk diffusion tests were done with random clinical isolates from various body sites tested according to the NCCLS protocol (2, 10) or with some modifications of the NCCLS protocol that included a standardized inoculum (1, 8, 11). Testing only gram-negative organisms, Barry et al. found an overall agreement of 89.1% after 5 to 6 h of incubation (1), and Liberman and Robertson found a very similar agreement (90.1%) after 7 to 8 h of incubation (10). Although our early readings of direct tests of gram-negative isolates had only 75.8% overall agreement, our frequency of major discrepancies (2.5%) was very similar to the 1.0 and 3.4% found in these two studies. Data from the other studies of early

TABLE 5. Mean zone size of gram-positive blood culture isolates in direct and standard tests

| Antibiotic, test type | Mean zone size of isolate[a]: | | | |
|---|---|---|---|---|
| | *S. aureus* | Coagulase-negative staphylococci | Beta-hemolytic streptococci | Enterococci |
| Penicillin | | | | |
| Direct | | | | |
| 4 h | 10.5 | 16.0 | 22.7 | 14.8 |
| 6 h | 13.1 | 14.4 | 23.7 | 15.6 |
| Overnight | 11.1 | 19.0 | 26.9 | 16.7 |
| Standard | 15.5 (91.1) | 22.6 (57.6) | 29.7 (0) | 18.9 (5.3) |
| Oxacillin | | | | |
| Direct | | | | |
| 4 h | 11.5 | 13.0 | | |
| 6 h | 15.3 | 11.9 | | |
| Overnight | 16.7 | 17.0 | | |
| Standard | 19.1 (0) | 18.2 (12.3) | | |
| Cephalothin | | | | |
| Direct | | | | |
| 4 h | 20.1 | 28.7 | 23.6 | 12.0 |
| 6 h | 24.5 | 25.7 | 24.3 | 13.1 |
| Overnight | 26.7 | 32.1 | 27.1 | 13.5 |
| Standard | 29.1 (0) | 34.3 (1.4) | 29.7 (0) | 16.2 (30) |
| Chloramphenicol | | | | |
| Direct | | | | |
| 4 h | 18.1 | 14.3 | 19.1 | 17.8 |
| 6 h | 21.3 | 17.9 | 19.3 | 18.9 |
| Overnight | 21.7 | 22.4 | 20.5 | 17.8 |
| Standard | 24.4 (1.8) | 24.2 (15.3) | 22.4 (0) | 20.9 (0) |
| Clindamycin | | | | |
| Direct | | | | |
| 4 h | 18.5 | 15.3 | 19.2 | 6.0 |
| 6 h | 21.3 | 15.4 | 18.2 | 6.0 |
| Overnight | 23.1 | 20.8 | 20.3 | 7.8 |
| Standard | 24.8 (0) | 22.3 (25.9) | 22.7 (0) | 8.2 (85) |
| Erythromycin | | | | |
| Direct | | | | |
| 4 h | 17.3 | 13.3 | 21.5 | 17.5 |
| 6 h | 21.2 | 14.7 | 20.3 | 14.8 |
| Overnight | 23.2 | 20.4 | 21.6 | 14.4 |
| Standard | 24.9 (5.4) | 22.5 (32.4) | 24.6 (3.8) | 17.0 (35) |
| Tetracycline | | | | |
| Direct | | | | |
| 4 h | 19.2 | 14.3 | 16.5 | 11.3 |
| 6 h | 21.5 | 15.8 | 15.3 | 14.2 |
| Overnight | 22.5 | 19.6 | 15.7 | 12.7 |
| Standard | 25.0 (10.5) | 24.3 (36.2) | 16.7 (46.2) | 14.4 (55) |
| Ampicillin | | | | |
| Direct | | | | |
| 4 h | | | 23.5 | 17.3 |
| 6 h | | | 22.3 | 19.5 |
| Overnight | | | 24.6 | 21.2 |
| Standard | | | 27.6 (0) | 23.8 (0) |

[a] Numbers in parentheses indicate the percentage of isolates that were found to be resistant in standard susceptibility tests.

readings cannot be readily compared with ours, since they did not include minor discrepancies.

The results of tests with certain combinations of organisms and antimicrobials were found to be less reliable than others. Of the 14 major discrepancies (57%) in gram-positive organisms, 8 were due to oxacillin tests of both coagulase-positive and -negative staphylococci. In tests with gram-negative isolates, 22 of the 26 major discrepancies were in ampicillin and cephalothin tests, and all but one of these occurred with either *E. coli* or *Klebsiella* spp. Ampicillin and cephalothin tests with four *E. coli* strains had major discrepancies. None of the previous studies of either direct susceptibility tests of blood cultures or of early readings from random clinical isolates noted similar problems with these organism-drug combinations. Barry noted that discrepancies in *E. coli* and *Klebsiella* spp. were uncommon but that very major errors were likely to occur in *Enterobacter* spp. tested with ampicillin and cephalothin, due to the late development of inner colonies (1). This late development was presumably due to the mutational enzymatic resistance recently characterized by Lampe et al. (9).

TABLE 6. Mean zone sizes of gram-negative blood isolates in direct and standard tests

| Antibiotic, test type | Mean zone size of isolate[a]: | | |
|---|---|---|---|
| | *E. coli* | *Klebsiella* spp. | *Enterobacter* spp. |
| Ampicillin | | | |
| Direct | | | |
| 4 h | 12.3 | 7.9 | 9.2 |
| 6 h | 14.0 | 8.8 | 8.2 |
| Overnight | 16.6 | 7.3 | 8.4 |
| Standard | 18.6 (12.7) | 9.1 (78.1) | 10.0 (83.3) |
| Cephalothin | | | |
| Direct | | | |
| 4 h | 15.2 | 15.8 | 12.0 |
| 6 h | 17.2 | 16.1 | 9.3 |
| Overnight | 17.3 | 18.1 | 8.1 |
| Standard | 20.2 (3.8) | 21.2 (9.4) | 9.1 (83.3) |
| Chloramphenicol | | | |
| Direct | | | |
| 4 h | 18.9 | 15.0 | 19.2 |
| 6 h | 18.9 | 17.1 | 20.4 |
| Overnight | 22.7 | 18.7 | 23.4 |
| Standard | 23.9 (3.8) | 21.0 (21.2) | 24.7 (0) |
| Gentamicin | | | |
| Direct | | | |
| 4 h | 16.3 | 15.3 | 14.2 |
| 6 h | 17.2 | 15.9 | 15.6 |
| Overnight | 19.1 | 17.7 | 17.6 |
| Standard | 21.4 (0) | 20.0 (0) | 19.6 (8.3) |
| Tobramycin | | | |
| Direct | | | |
| 4 h | 15.6 | 15.9 | 14.0 |
| 6 h | 16.8 | 15.4 | 15.1 |
| Overnight | 18.7 | 16.2 | 17.1 |
| Standard | 20.7 (0) | 18.2 (16.1) | 18.7 (25.0) |

[a] Numbers in parentheses indicate the percentage of isolates that were found to be resistant in standard susceptibility tests.

Since two laboratories in the present study routinely used an anaerobic supplemented peptone broth that contained a sufficient amount of penicillinase to inactivate 4 U of penicillin, the data were examined for the possibility that penicillinase was responsible for the major discrepancies involving ampicillin and cephalothin tests with the gram-negative organisms; however, only 2 of these 22 major errors involved the broth medium containing penicillinase.

In this study, major discrepancies were 20 times more frequent than were very major discrepancies. In all of the previous studies of early readings, the two categories of discrepancies were very similar in frequency (1, 2, 8, 10, 11). However, results similar to ours were found in the two overnight studies of unadjusted blood cultures in which major discrepancies occurred 9 to 12 times more frequently than did very major errors (4, 5). The difference between the results of the two groups of studies might be explained by the fact that most of the inocula for direct susceptibility tests were much heavier than those for standard susceptibility tests. The expected effect of a very heavy inoculum is smaller zone diameters, which may result in major discrepancies when compared with the zones from standardized inocula. This is consistent with our observation that the mean overnight zone sizes from direct tests were smaller than those from standardized tests with every organism-antibiotic combination in this study.

The observation that zone sizes increased with longer incubation times has previously been reported by Liberman and Robertson (10) and Lorian (11). These results contrast with those of Cooper, who found that zone sizes are established during the first 3 h of incubation (3). One could attribute the apparent increase in zone sizes after 6 h of incubation to the fact that the early readings, taken from the surface of the plates, revealed thin growth that could not be seen from the reverse side that was read after overnight incubation. This does not explain the similar results with gram-positive organisms that were tested on blood-Mueller-Hinton plates and therefore could only be read from the surface. Since Liberman and Robertson used a Fisher-Lilly antibiotic zone reader throughout their study, and Lorian presumably read all plates from the surface, it is difficult to identify a technical explanation for increases in zone sizes after early readings in these three studies.

Although we found a fairly low incidence of major discrepancies in 4-h readings of direct blood culture susceptibility tests, the high frequency of minor discrepancies at 4 h indicates that zone diameters should not be read this early. On the other hand, the accuracy of results of direct susceptibility tests after 6 h of incubation suggests that this information could be offered on a routine basis for the drugs included in this study. Since most of our data were collected before our laboratories were routinely testing the newer aminoglycosides and second- or third-generation cephalosporins, we cannot predict the reliability of direct susceptibility testing with these drugs. Methicillin-resistant *S. aureus* was not encountered in this study, but we would expect that early reading would be less sensitive than the standard test for detection of methicillin resistance in some strains.

Laboratories that add penicillinase to blood cultures of patients receiving penicillin-related drugs should not perform direct susceptibility tests on these cultures, since carryover of the penicillinase might inactivate penicillin, ampicillin, and to a lesser extent, cephalothin, thus producing false resistance. Direct disk susceptibility tests should not be attempted with any type of specimen other than blood cultures, since either low inoculum densities or mixed cultures are likely to yield totally unreliable results. Even though overnight direct results closely agree with standardized test results, confirmation by standardized tests should be done in such clinically significant situations as positive blood cultures.

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