Direct Susceptibility Testing of Positive Blood Cultures by Using Sensititre Broth Microdilution Plates

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Traditional susceptibility testing of blood cultures requires overnight incubation in order to obtain isolated colonies. Susceptibility results can be reported up to 24 h sooner by using a bacterial pellet from the blood culture broth. This study evaluated the accuracy of direct susceptibility testing from positive ESP blood culture broths by using Sensititre broth microdilution plates compared to testing with isolated colonies. Practical inclusion criteria were applied to gram-positive organisms to avoid reporting susceptibilities for probable contaminants. All gram-negative organisms were tested directly. An aliquot of the blood culture was centrifuged, and the resulting pellet was used to make a 0.5 McFarland suspension. Microdilution plates were inoculated and interpreted according to the manufacturer’s instructions. Colony counts were performed to ensure proper colony density was achieved. A total of 199 patient and seeded blood cultures were evaluated for both essential (within ±1 twofold dilution) and categorical (sensitive, intermediate, or resistant) agreement.

Testing of 93 gram-positive isolates (1,214 antimicrobial agent-organism combinations) yielded 98% essential agreement and categorical error rates of 0.3% minor, no major (false resistance), and 1.7% very major (false susceptibility) errors. For 106 gram-negative isolates (1,828 antimicrobial agent-organism combinations), the essential agreement was 99%. Categorical error rates were 0.5, 0, and 2.0% for minor, major, and very major errors, respectively. Performance was comparable for both gram-positive and gram-negative isolates, as well as for both aerobic and anaerobic media. Using this direct testing methodology, reliable susceptibility results can be reported to physicians 24 h sooner, allowing earlier appropriate modification of antimicrobial therapy.

Timely intervention in the treatment of bloodstream infection is of paramount importance to increase the chances of a favorable outcome, since empirical therapy can be modified upon receipt of in vitro antimicrobial susceptibility testing (AST) results. AST results can assist in modifying antimicrobial therapy (13), and investigators have demonstrated decreased mortality with early treatment (10). Further data from outcome-based studies assessing the effect of rapid reporting of susceptibility results have shown a decrease in the number of laboratory tests and procedures ordered (4), decreased length of stay (1), and decreased health care costs and quicker modification of antimicrobial therapy (1, 4, 23).

The standard protocol for identification and susceptibility testing of positive blood cultures involves Gram stain and subculture of blood culture broth onto solid medium and overnight incubation to obtain isolated colonies. These colonies are then used to make a standardized inoculum used for AST. A direct susceptibility testing algorithm from positive ESP (TREK Diagnostic Systems, Cleveland, Ohio) broths were designed that circumvents the isolation process and expedites the reporting of AST results. A prospective study comparing the direct AST (DAST) results for gram-negative and gram-positive isolates to those obtained by standard AST (SAST) methods with Sensititre microdilution plates (TREK Diagnostic Systems) with the Automated Reading and Incubation System (ARIS; TREK Diagnostic Systems) was performed. Sensititre/ARIS has been previously compared to another automated AST system (2), and Sensititre/ARIS results have been compared to the results of Sensititre plates read manually (M. C. Musgnug and K. C. Chapin, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-243, 2003). To our knowledge, such a prospective study has not been performed with this blood culture medium and AST system combination.

MATERIALS AND METHODS

Specimens. A total of 81 patient and 118 seeded blood culture bottles that signaled positive in the ESP and appeared to be unimicrobial by Gram stain were evaluated. All species tested were encountered in ≥1 patient blood culture. Only aerobic and facultative organisms were included. Blood cultures yielding more than one organism on subculture were excluded from data analysis.

Since coagulase-negative staphylococci (CoNS) are common contaminants in blood cultures (20), criteria were developed to avoid reporting susceptibilities on isolates that were probable contaminants. For bottles with Gram stains showing gram-positive cocci in clusters (suggesting staphylococcus), a direct tube coagulase test (TCT) was performed. TCT-positive specimens, indicating Staphylococcus aureus, were tested by DAST. TCT-negative specimens were evaluated based on the following exclusion criterion: ≥1 set of blood cultures and/or a catheter tip culture had to be positive for CoNS to warrant direct testing. This screening criterion limited the direct testing of CoNS to those strains that were most likely clinically significant (12, 20).

Tube coagulase test. Five drops of blood culture broth were added to 0.5 ml of rabbit plasma with EDTA (BBL, Cockeysville, Md.), followed by incubation at 35°C for 4 h. Clotting of the plasma indicated a positive test and identified the organism as S. aureus. A negative TCT, no clotting of the plasma, was interpreted as gram-positive cocci in clusters, a finding consistent with staphylococcus species.

Direct susceptibility inoculum preparation. Serum separator tubes (BD Vacutainer Systems, Franklin Lakes, N.J.) were inoculated with 10 ml of positive blood culture broth by using a 21-gauge needle. Tubes were centrifuged at 1,300 × g for 10 min at room temperature. Bacteria were collected from the gel surface by using a cotton swab and added to demineralized water to make a suspension equivalent to a 0.5 McFarland standard adjusted by using a Sensititre nephelometer.

Standard susceptibility inoculum preparation. A drop of the blood culture broth was plated onto a Trypticase soy agar plate with 5% sheep blood (BBL) and incubated in 5% CO2 at 35°C overnight to obtain isolated colonies. These
colonies were added to demineralized water to make a suspension equivalent to a 0.5 McFarland standard, adjusted by using a Sensititre nephelometer.

**Susceptibility system.** Sensititre MH and MG plates (for gram-negative and gram-positive organisms, respectively) were used. Antimicrobials and dilutions are shown in Table 1. A 10-μl aliquot of the inoculum was aseptically transferred to 10 ml of Sensititre cation-adjusted Mueller-Hinton broth, and plates were inoculated according to manufacturer instructions. Plates were read at 18 to 24 h by the ARIS instrument, a fully automated susceptibility system that uses fluorescence to detect organism growth. Plates were also read manually by visualization of a growth button on the bottom of the microtiter well by using a SensiTouch light box. Both instruments were interfaced to a personal computer.

**Data analysis.** DAST results were compared to SAST results. Interpretations of MICs were determined by NCCLS standards (16), and only those antimicrobial agent-organism combinations suggested by NCCLS as appropriate for routine use were used for analysis. DAST and SAST susceptibility results were analyzed for both essential and categorical agreement. Essential agreement meant both MIC results were within 2 dilutions of each other. Categorical agreement. Essential agreement meant both MIC results were within 2 dilutions of each other. For categorical agreement, results were identified as minor, major, or very major errors. Minor errors represent an intermediate interpretation versus a sensitive or resistant result. Major errors result from a falsely resistant or sensitive result. Very major errors result from a falsely susceptible or resistant result. The number of antimicrobial agent-organism combinations tested per National Committee for Clinical Laboratory Standards (NCCLS) recommendations each day testing was conducted (14).

**RESULTS**

A total of 199 blood cultures (81 patient bottles and 118 seeded bottles) were evaluated by DAST and SAST. Of the 199 isolates, 106 were gram-negative bacilli and 93 were gram-positive cocci. A complete list of the organisms is found in Table 2. All staphylococci included were deemed clinically significant by the TCT or CoNS screening criteria. DAST results were typically available 19 to 24 h after a blood culture was signaled positive by the ESP compared to 35 to 48 h for SAST results, secondary to overnight incubation required to produce isolated colonies. Thus, the DAST results were available 16 to 24 h sooner than the SAST results.

For 106 gram-negative isolates (1,828 antimicrobial-organism combinations), we observed 99.0% essential agreement between DAST and SAST. Eighteen antimicrobial-organism combinations did not show essential agreement. DAST yielded.
9 (0.5%) minor, 0 (0%) major, and 6 (2.0%) very major errors. A total of 93 gram-positive isolates (1,214 antimicrobial-organism combinations) yielded 98.0% essential agreement, with 4 (0.3%) minor errors, 0 (0%) major errors, and 6 (1.7%) very major errors (Table 3). Automated results did not yield major or very major errors compared to manually determined results. Errors were not associated with seeded bottles more often than patient bottles.

Details of antimicrobial-organism combinations yielding very major errors are found in Table 4. No major errors were observed, and minor errors were not detailed. Gram-negative isolates yielded the following very major errors: mezlocillin with Escherichia coli (three errors), piperacillin with E. coli (two errors), and ampicillin with Klebsiella pneumoniae (one error). Very major errors occurring with gram-positive isolates were as follows: cefazolin with S. aureus (five errors) and clarithromycin with S. aureus (one error).

### DISCUSSION

DAST of positive blood cultures can allow a laboratory to report susceptibility results to physicians up to 24 h sooner than SAST. DAST is not a novel concept and was described with the disk diffusion method in the late 1970s with favorable results (3, 5, 6, 11) with manual blood culture systems with simple broth medium. Two major changes in microbiology have altered the interpretations of these early studies. First, disk diffusion is infrequently used as a routine susceptibility method in microbiology laboratories today, having been replaced by automated broth microdilution methodologies. These automated systems use fluorescence, red/ox indicators, and/or turbidity as indicators for interpretations. Second, standard blood culture systems are now continuously monitoring instruments that use high-volume blood culture vials. Two systems (Bactec [BD] and BacT/Alert [bioMerieux, Durham, N.C.] offer a multitude of medium options and recommend media with additives for optimal performance. These additives (i.e., charcoal resin and diatomaceous earth, respectively) have been shown to interfere with fluorescence and enzyme-based reactions and require procedural modification (17, 22) for direct testing of positive blood cultures. Thus, it is appropriate to consider both of these components in the performance and discussion of DAST results from blood cultures.

Direct susceptibility testing via broth microdilution plates was first described in 1982 (9). Subsequently, the most frequently investigated combination of the blood culture and AST systems has been Bactec with Vitek. Acceptable performance with this combination was shown for gram-negative isolates in several studies (7, 18, 21). Similarly, Buck et al. showed acceptable performance for gram-negative isolates with the newer Vitek 2 (L. L. Buck, A. Hermann, and S. Young, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-1, 2002). DAST on gram-positive isolates, using the Bactec and Vitek combination, ranged from acceptable (21) to poor (yielding many very major errors) (8). One study directed inoculated MicroScan plates with Bactec blood culture broth and lacked a centrifugation step. Not surprisingly, that study reported poor results (19).

Waites et al. tested BacT/Alert in conjunction with MicroScan plates and found reasonable overall agreement but very high categorical errors (24). These authors reported high major (2.6 and 14%) and very major (8.8 and 2.7%) error rates for gram-positive and gram-negative isolates, respectively.


Evaluation of direct susceptibility testing from positive ESP blood cultures using Sensititre broth microdilution plates has not been previously described. The ESP continuously monitoring instrument employs blood culture broth media free of additives and lends itself well to direct testing methods. The present study demonstrated that DAST of gram-negative isolates from ESP broths by using Sensititre standard microdilution plates performed well since 99.0% essential agreement and categorical error rates of 0.5, 0, and 2.0% for minor, major, and very major, respectively, were noted. Performance was equally adequate for gram-positive isolates based on the screening criteria for CoNS, with 98% essential agreement. There were 0.3% minor errors and 1.7% very major errors, with no major errors observed.

Problem antimicrobial-organism combinations in this evaluation included cefazolin with S. aureus (13 combinations) and mezlocillin with E. coli (3 combinations). In these cases, 12% of S. aureus isolates and 13% of E. coli isolates resulted in very major errors with the respective antimicrobial agent. No explanation for this elevated incidence of false susceptibility is evident. Although the cefazolin result would normally be
changed as a result of the oxacillin result, the present study looked at the performance of the susceptibility plates directly from blood cultures, and cefazolin was considered as an independent result.

Waites et al. attributed most gram-positive DAST errors with the BacT/Alert and MicroScan panels to CoNS (24). Preliminary work in the present study also showed that direct susceptibility testing of CoNS yielded less agreement between DAST and SAST (data not shown). However, when CoNS that were probable contaminants were eliminated, marked improved performance of DAST results was noted. This prompted the development of the algorithm for CoNS testing and is currently used in the laboratory when we are assessing whether AST should be performed on CoNS to prevent reporting AST results for probable contaminants.

False susceptibility with oxacillin and S. aureus (failure to detect methicillin-resistant S. aureus) was shown to be problematic for other researchers (24). In the present evaluation, however, 14 of 14 blood cultures found to be positive for methicillin-resistant S. aureus were detected by DAST.

We report here the favorable performance of direct testing by using Sensititre plates with ESP blood cultures. ESP broths yielded bacterial pellets sufficient to make a 0.5 McFarland suspension. We performed colony counts on the final inocula to confirm that they were within the NCCLS range of $3 \times 10^5$ to $7 \times 10^8$ CFU/ml (15). Inocula were consistently within the acceptable range. Less-favorable performance reported by some investigators may be due in part to a final inoculum density less than the NCCLS range. If the pellet contains materials other than bacteria, the suspension equivalent to a 0.5 McFarland verified by a nephelometer may contain less than $1.5 \times 10^8$ CFU/ml. Other studies fail to mention verifying the final inoculum density.

DAST of blood cultures by using Sensititre microdilution plates can be easily incorporated into the clinical laboratory workflow. Positive blood cultures can be “batched,” and susceptibility plates can be set up several times daily. The results are available in 18 to 24 h and, at that time, in contrast to rapid AST systems, the purity of the inoculum can be verified before the direct susceptibility results are reported. Given the results of the present study, replacement of traditional AST with DAST is feasible, whereas any suspicious results can be confirmed manually by visual inspection.

The benefits of timely reporting of susceptibility results have been highlighted. DAST of ESP blood culture isolates via Sensititre performed well compared to standard testing. Due to reports of variable results and the numerous combinations of blood culture and AST systems, laboratories must evaluate the performance of individual blood culture and susceptibility systems before such a method is adopted.

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**REFERENCES**


