INTRODUCTION

Sepsis is one of the major causes of mortality and morbidity in hospitalized patients. Blood culture is the gold standard method for the diagnosis of sepsis, and it is included in the early investigation to be sent for sepsis according to the Surviving Sepsis Campaign guidelines.1

The availability of culture and sensitivity results in patients with infections is important for clinicians in guiding them to select the most appropriate antimicrobial for treatment, thereby increasing the chances of maximal therapeutic effect.1 Microbiology laboratory provides such information promptly, especially regarding cases of bloodstream infections (BSI).1 With the advent of automated blood culture methods, the time taken for detection of the micro-organism has been reduced from 3–4 days to 2–3 days.1 However, the advent of automation in place of subculture is required to obtain pure growth so that antimicrobial susceptibility testing (AST) can
be carried out either by the Kirby–Bauer method or an automated method.1,2

The empirical therapy started initially with broad-spectrum antimicrobials perforce continues until the sensitivity results are made available.3 However, it is to be emphasized that about 20–50% of all the prescribed antimicrobials are inappropriate. Patients getting these inappropriate antimicrobials get no extra clinical benefits while being at risk of suffering from adverse effects.3 The most serious and ever-increasing public health problem is the emergence of antimicrobial resistance due to the misuse of antimicrobials.4 These drug-resistant pathogens pose a threat to the health of patients in a health-care setup.5

One of the useful inputs in the implementation of antimicrobial stewardship is the early availability of AST, which can help the clinician to de-escalate the antimicrobial, thereby reducing the chances of the emergence of resistant organisms. The disk diffusion method for AST takes 48 h for the result to be generated. This includes the 24-h time taken for subculture from the positively flagged culture bottle onto solid culture media to obtain pure growth, in addition to AST, which takes another day to complete. Even the automated methods for AST take another half to 1 day for the results to be available.6

Sepsis is one of the major causes of mortality and morbidity in hospitals. BSIs affect approximately 2% of all hospitalized patients and 70% of patients admitted to the Intensive Care Units. Detection of BSI is one of the most important tasks performed in the microbiology laboratory. Rapid identification of isolates and their antimicrobial susceptibility is essential for patients with BSIs. Timely initiation of appropriate antimicrobials can improve the outcome of the patients. The availability of an early preliminary AST report will be useful in direct antimicrobial therapy. This study aims to evaluate the usefulness of the direct AST method from a positive blood cultures broth, thereby helping to reduce the turnaround time (TAT) and early initiation of antibiotics in critically ill patients.5

Aims and objectives
This study aims to evaluate the usefulness of direct antimicrobial sensitivity testing from positive blood culture broth in suspected bacteremia and also to compare the direct AST with the conventional disc diffusion method.

MATERIALS AND METHODS

This is a descriptive cross-sectional study conducted in a tertiary care hospital, in Chennai, Tamil Nadu, for 1 year. With the approval from the Institutional Ethical Committee and informed consent, patients were included in the study by simple random sampling method. A total of 842 blood culture samples were collected from patients with clinical suspicion of sepsis.

Inclusion criteria
The following criteria were included in the study:
• Blood culture samples were received in the laboratory for culture and sensitivity from adult patients with suspected sepsis
• Blood cultures with positive signal flagging off from the automated blood culture system show only one type of organism (Gram positive or Gram negative) by direct Gram-film.

Exclusion criteria
The following criteria were excluded from the study:
Positive blood cultures with more than one type of bacteria or skin commensals seen in direct Gram film, also negative alert signaling blood culture bottles were excluded.

Collection of blood samples for blood culture
Under utmost sterile precautions, 10 mL blood samples were collected by doing venipuncture and injected immediately into blood culture bottles. The bottles were loaded into the BACT/ALERT 3D automated system. The blood culture bottles were monitored for flag off signal from the automated system.

Processing of the positive blood culture broth
Direct Gram film was done for positively flagged blood culture bottles from the BACT/ALERT 3D system within 8 h and then all positive blood culture broths were subcultured on blood agar and MacConkey’s agar. Those bottles with a single type of organism under Gram film were subjected to direct antimicrobial susceptibility test (DAST) and were performed by disk diffusion method as per CLSI guidelines for Enterobacterales and Pseudomonas and the European Committee on AST (EUCAST) rapid AST (RAST) guidelines for Gram-positive organisms and read as per the breakpoints.8,10

Procedure for RAST
The inoculum was mixed thoroughly by inverting the blood culture bottle 5–10 times, the 20-gauge venting needle was injected into the blood culture bottle after an alcohol wipe, and blood culture broth was withdrawn, four drops were dispensed on Mueller–Hinton agar (MHA) plate. Then, using a sterile cotton swab blood culture broth was spread across the entire surface of the MHA plate, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. After that leave the lid ajar for 3–5 min, antimicrobial disks were placed and pressed onto
the surface of the inoculated MHA plate. The plates were inverted and placed in the incubator at 37°C for 16–18 h for Gram-negative bacilli. Gram-positive cocci readings were taken after incubation for 8 h. Another blood agar plate was inoculated with the same inoculum to check the purity of the inoculum.¹⁰

Results were interpreted as follows; before starting interpretation, the blood agar plates were examined to ensure pure growth, and preliminary identification was done to confirm Gram-negative bacillus tested was Enterobacterales or Pseudomonas aeruginosa and Gram-positive cocci. Then, the test plates were examined to ensure confluent lawn of growth which was present. The zone diameters were measured and reported using the interpretive categories and zone diameter breakpoints.⁸,⁹

Conventional identification and susceptibility testing

The bacterial pathogens were identified with colony characters and biochemical parameters from the subcultured media and proceeded with AST.

Conventional AST (CAST)

AST was performed by the Kirby–Bauer disk diffusion method for the isolated organisms.

Procedure

The test organisms grown on culture media were inoculated into peptone water and incubated at 37°C for 2–4 h. The turbidity is matched with 0.5 McFarland. A lawn culture was made and the antibiotic disk was placed on MHA, according to the growth (Gram-positive or Gram-negative organisms) Fig. 1. The plates were incubated for 18–24 h at 37°C. The zone diameters were recorded and interpreted as sensitive, intermediate, or resistant according to CLSI standards 2022.⁸

Statistical analysis

The results were tabulated into Microsoft Excel format and means and percentages were calculated for the susceptibility of pathogens for the given antimicrobials as per CLSI/EUCAST guidelines and analyzed in SPSS software.

Based on the categorization of the strains into different susceptibility classes for the different tests, very major error (VME), major error (ME), and minor errors (mE) were calculated using proportions (percent). VME, ME, and mE are defined as a false susceptible result, a false resistant result, and a result involving an intermediate category, respectively. The AST by direct and conventional methods were compared using Cohen's kappa correlation coefficient statistics. The Kappa coefficient values were calculated and based on the kappa value that the results were graded as no agreement, slight agreement, fair agreement, moderate agreement, substantial agreement, near-perfect agreement, and perfect agreement.

RESULTS

Direct AST was performed as per CLSI guidelines for Gram-negative pathogens and Gram-positive cocci as per EUCAST RAST guidelines. Then, the AST results were compared with the conventional disk diffusion. Out of 842 blood culture samples received from patients with suspected BSIs, 112 were flagged for positive culture in the automated system as shown in Fig. 2. All the positive culture bottles were subjected to direct Gram film, and 28 were found to be polymicrobial, skin commensals and in the remaining, 24 were Gram-positive cocci and 60 were Gram-negative bacilli.

Among the 60 pathogens detected Gram-negative bacilli such as Klebsiella pneumoniae were 28 (46.6%), Klebsiella oxytoca 16 (26.6%), Escherichia coli 12 (20%), and P. aeruginosa 4 (6.6%) as shown in Fig. 3. Of the 24 Gram-positive cocci Enterococcus faecalis, Enterococcus faecium, Methicillin sensitive Staphylococcus aureus, and Coagulase negative Staphylococci contributed to 8 (33.3%), 2 (8.3%), 6 (25%) and 8 (33.3%) respectively.

Among the 60 Gram-negative bacilli belonging to Enterobacterales (Klebsiella species and E. coli), AST results were analyzed as per antimicrobial agents for Enterobacterales, out of 392 antimicrobial agent

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of isolates tested (n=56) (%)</th>
<th>Agreement</th>
<th>VME</th>
<th>ME</th>
<th>mE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>56 (100)</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>55 (98.2)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>53 (94.6)</td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>53 (94.6)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>52 (92.8)</td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>Aztreonem</td>
<td>55 (98.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>53 (94.6)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>377 (96.1)</td>
<td>4 (1)</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>392</td>
</tr>
</tbody>
</table>

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error
combinations, 377 (96.1%) combinations showed
categorical agreement, whereas 15 combinations showed
disagreement of which 4 (1%) were VME. Two (0.5%) were
ME and 9 (2.2%) were mE and 100% categorical agreement
to antimicrobials such as ampicillin, meropenem,
and aztreonam with conventional disk diffusion method.
However, fair agreement was found to ceftazidime (63.7%),
ciprofloxacin (77%), and cotrimoxazole (53.8%). The
antimicrobial tobramycin alone showed 53.8% agreement
with the conventional method (Tables 1 and 2).

For *P. aeruginosa*, out of 14 (87.5%) combinations showed
categorical agreement whereas two combinations showed
disagreement of which 1 (6%) were ME and 1 (6%) mE.
The categorical agreement for tobramycin, ceftazidime, and
meropenem showed 100%, and fair agreement was found
ciprofloxacin (70%) with the conventional method, as
shown in Tables 3 and 4.

Among 24 Gram-positive organisms, *Staphylococcus* species
51 (91%) combinations showed categorical agreement
whereas five combinations showed disagreement of which
3 (5.3%) were ME, 2 (3.5%) mE, and fair agreement to
cefoxitin (72%), slight agreement to norfloxacin (42%),
clindamycin (53.5%), and gentamicin (53%) and no VME
for all antibiotic combinations (Tables 5 and 6).

For *Enterococcus* species, 36 (90%) combinations showed
categorical agreement whereas four combinations showed
disagreement of which 2 (5%) were ME, 2 (5%) mE, and
100% agreement was found to vancomycin and linezolid,
a fair agreement was found to ampicillin (43%) high-level
gentamicin (66.6%), as shown in Tables 6 and 7.

**DISCUSSION**

Blood cultures remain the central component to determining
the etiology of BSI as they are highly sensitive and easy
to perform. An expeditious and appropriate diagnosis
of the etiological agents along with their antimicrobial
sensitivity pattern is of utmost essential. In this present
era with practically a limited number of antimicrobials in
the development pipeline, optimum use of the existing
antimicrobials is crucial. This misuse or abuse of
antimicrobials has a direct relationship with the emergence
and dissemination of resistant strains in health-care setups.
The appropriate antibiotic treatment within the shortest
time can be initiated in BSIs with accurate and timely bacterial
identification, and determination of antibiotic susceptibility
in the microbiology laboratory and thus, accelerates the
time of selection of appropriate antibiotics, shortens the
time of stay in the hospital/intensive care unit, and reduces
mortality. This study proposes to compare the direct
Table 3: Agreement between direct AST and conventional AST for *Pseudomonas* spp.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of isolates tested (n=4)</th>
<th>Agreement (%)</th>
<th>VME</th>
<th>ME</th>
<th>mE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>3 (75)</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4 (100)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3 (75)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4 (100)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>14 (87.5)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

Table 4: Agreement between direct AST and conventional disk diffusion method AST among non-fermenters

<table>
<thead>
<tr>
<th>Drug</th>
<th>Direct (%)</th>
<th>Conventional (%)</th>
<th>Agreement (%)</th>
<th>Cohen’s K score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S I R</td>
<td>S I R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>100</td>
<td>3 1 0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 0 2</td>
<td></td>
<td>0.3478</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1 3 2</td>
<td>2 0 2</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>2 0 2</td>
<td>2 0 2</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

AST: Antimicrobial susceptibility test

Table 5: Agreement between direct AST and conventional AST among *Staphylococcus* species

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of isolates tested (n=14)</th>
<th>Agreement (%)</th>
<th>VME</th>
<th>ME</th>
<th>mE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>13</td>
<td>1</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13</td>
<td>1</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>13</td>
<td>1</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>51 (91%)</td>
<td>3 (5.3%)</td>
<td>2 (3.5%)</td>
<td>56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

**Figure 1:** Flow chart showing standard AST and direct AST. BAP: Blood agar, MAC: MacConkey agar, MHA: Mueller-Hinton agar, AST: Antimicrobial susceptibility test. Images were photographed by the authors
antimicrobial sensitivity testing from the positive blood culture broth with the conventional disk diffusion testing for the blood culture bottle flagged off from the automated Bact/Alert3D blood culture system.

Growth of pathogenic microorganisms was found to be 9.8% in our study and this is by a study by Sarode et al., which showed 10.6%. However, in our study, blood culture contamination rate was 3.3% compared to the study by Sarode et al., where the contamination rate was in 3.5%.

The pathogens detected by the direct Gram stain result from the positive bottles were similar to those by Gram stain from the subsequent subcultures from the bottles obtained after overnight culture. Hence, the direct Gram film is very helpful to start empirical antibiotics in BSIs in the Golden hour.

Early detection of pathogens along with analysis of their antibiotic susceptibility patterns is always the main goals of any diagnostic microbiology laboratory. Compared to CAST, performing DAST on positive blood culture broth provides a clinical team with information on the identity of the pathogen and its antibiotic susceptibility 24 h earlier, which can accelerate switching from empirical therapy to definitive treatment of the disease. Some of the studies have also proposed methods for DAST on clinical specimens. Blood culture tests are critical investigations for any microbiology department, and a delay in reporting the results can significantly affect morbidity and mortality in patients.

In our study, the DAST showed 96.1% categorical agreement for Enterobacterales and 87.5% categorical agreement for Pseudomonas species with CAST. Similar
findings were reported by Desai et al.,18 and Rajshekar et al.,19 who found the categorical agreement to be 90.4% and 96%, respectively. Good categorical agreement for gram-negative organisms has been reported by Kumar et al.20

In the case of GPC, DAST showed 91% categorical agreement for Staphylococcus species and 90% categorical agreement for Enterococcus species. Staphylococcus species 51 (91%) combinations showed categorical agreement whereas five combinations showed disagreement of which 3 (5.3%) were ME, 2 (3.5%) mE, and fair agreement to Cefoxitin (72%), slight agreement to norfloxacain (42%), clindamycin (53.5%), and gentamicin (53%). The study by Rajshekar et al., reported among Gram-positive cocci, both Staphylococcus species and Enterococcus species had CA of >95% for all the antibiotics tested and VME was unsatisfactory in Staphylococcus species for cefoxitin (4.9%) and for HLG (4.4%) in Enterococcus species. ME and mE were satisfactory among both groups. A similar observation was seen in a study conducted by Bennett and Sharp,21 well correlating with our studies.

Various studies have compared the direct AST with the standard AST from blood culture bottles using different automated culture systems.22,23 Most of these studies have found very good categorical agreement for the Gram-negative organisms and not so good agreement for Gram-positive organisms.23,25 Good categorical agreement for Gram-positive organisms has been reported by Lupetti et al.22 Nevertheless, our study shows very good categorical agreement for most of the Gram-positive organisms and good agreement for Gram-negative organisms. Moreover, as the correlation analysis shows minimal VME, both methods can be considered to have good concordance.

Limitations of the study
Some of the limitations of this study are: 1) There is a lack of definitive identification of the infecting bacteria. 2) The exclusion of yeasts and polymicrobial organisms on Gram stain are not specified.

**CONCLUSION**

This study has demonstrated good concordance between the direct AST and CAST results. Hence, direct AST can be implemented in a routine diagnostic laboratory. Direct AST will be helpful to decrease the TAT and to start early antimicrobial therapy in critically ill patients. This direct AST and reporting will be helpful for the implementation of the antimicrobial stewardship program. Although the causative organism cannot be definitively identified by our method, it still enables preliminary AST testing, offering a chance for early institution of appropriate antimicrobial therapy.

**REFERENCES**

9. The European Committee on Antimicrobial Susceptibility Testing. Zone Diameter Breakpoints for Rapid Antimicrobial Susceptibility


Authors’ Contributions:
KR- Concept design, work protocol, preparation of manuscript, implementation of study protocol, literature survey, and preparation first draft of manuscript; RGP- Editing literature survey; SN- Study design, statistical analysis and interpretation literature survey, preparation of figures, manuscript correction and submission of article; RS- Data collection.

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