



## DIRECT ANTIMICROBIAL SUSCEPTIBILITY TESTING OF GRAM NEGATIVE BACILLI FROM POSITIVE BLOOD CULTURES

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### ABSTRACT

Conventional blood culture and susceptibility pattern takes around 48 hours after blood culture bottles turns positive on Bact/ALERT system. Rapid initiation of antimicrobial therapy is required in management of sepsis patients to reduce its adverse effects on mortality and morbidity. Objective of this study was to execute rapid and accurate direct antimicrobial susceptibility testing (DST) of gram negative bacilli on positive blood culture bottles by the disk diffusion method and compare the results with those of standard antimicrobial susceptibility testing (AST). This prospective study was performed at the Saveetha Institute of Medical and Technical Science, Sriperumbudur, with mono-bacterial gram negative bacilli. 100-gram negative bacilli (GNB) samples were flagged from positive blood cultures with duration of 3 months. Both DST and AST results were interpreted, and the isolates were identified by vitek2. On 100 GNB isolates, the most common organism was *Escherichia coli*, followed by *Klebsiella pneumoniae* (27%), *Pseudomonas aeruginosa* (11%), *Acinetobacter baumannii* (8%), *Salmonella typhi* (7%), *Enterobacter cloacae* (3%), *Acinetobacter lwoffii* (3%) and *Serratia marsacens*. The DST and AST correlations were found to be in 99% categorical agreement, with 1% minor error, 0% major error, and 0% very major error. The concentration of bacterial inoculums impacts the results between DST and AST. DST reduces the crucial time from 24 to 48 hours for patients with bloodstream infections treated with empirical antibiotic therapy, which may or may not be resistant. DST initiates early antibiotic therapy that is susceptible to treating a bloodstream infection or sepsis. **Aims and Objective:** To evaluate categorical agreement of antimicrobials between DST and AST from positive blood culture bottles by Kirby-Bauer disk diffusion method. **Materials and methods:** A prospective study was conducted in tertiary care hospital, with sample size of 100 gram negative bacilli from positive blood culture bottles in patients suspected with blood stream infections during the period from October 2022 to December 2022. **RESULTS:** Out of 100 gram negative bacilli, *Escherichia coli* (37%) was the most common isolate, followed by *Klebsiella pneumoniae* (27%), *Pseudomonas aeruginosa* (11%), *Acinetobacter baumannii* (8%), *Salmonella typhi* (7%), *Enterobacter cloacae* (3%), *Acinetobacter lwoffii* (3%) and *Serratia marsacens* (2%). **Conclusion:** During this study, there was 99% categorical agreement between DST and AST. Earlier treatment with antimicrobials can reduce the mortality and morbidity in bacteraemia patients.

### KEYWORDS :

#### INTRODUCTION

Sepsis is the body's systemic immune reaction to an infectious process that can cause multi organ failure and death in its last stages. Bloodstream infection leads to sepsis and its complication due to ineffective empirical antibiotic therapy. Simultaneously, treatment with broad spectrum antibiotics increases the chance for development of resistance<sup>1,2</sup>.

For the most effective treatment and results of patient with sepsis, it is essential to learn the knowledge of blood culture isolate antimicrobial susceptibility pattern. This lets for appropriate de-escalation and escalation of antibiotics for conclusive therapy<sup>3</sup>.

Standard antimicrobial susceptibility testing involves gram staining, sub culturing to get isolated colonies and the turnaround time for testing is around 48-96 hours from the time blood culture flags positive<sup>4</sup>.

Direct susceptibility test shows result within 6-24 hours of time, with the data from this study the antibiotic therapy can be started earlier which is benefit to patient with sepsis and bacteraemia<sup>5</sup>.

#### MATERIALS AND METHODS:

**Study Design:** This is a hospital-based prospective study.

#### Sampling method:

100 Gram negative bacilli with single organism positive blood cultures from patients suspected of bloodstream infection and sepsis during the period from October 2022 to December 2022 were considered for this study.

#### Study setting:

Department of Microbiology, Saveetha Medical College and Hospital, a tertiary care teaching hospital located in Sriperumbudur.

#### Inclusion criteria:

100 gram negative bacilli, with single organism gram-negative bacilli from positive blood culture bottles.

#### Exclusion criteria:

Blood culture with two or more organisms.  
Blood culture with yeast or gram positive cocci.

#### Brief Procedure:

Gram staining: Gram staining is done on Bact/ALERT positive blood culture bottles to identify gram negative bacilli.

#### Direct susceptibility testing

- Positive blood culture bottles were thoroughly mixed 5–10 times.
- A 20 gauge needle was inserted into the top of the sterile bottle.
- Four drops of blood were dispensed on Muller-Hinton agar; using a sterile cotton swab, the blood culture broth was spread around the entire MHA plate, and two more streaking were done with the plate rotated at 60 degrees each time<sup>6</sup>.
- After 15 minutes, antimicrobial discs such as Meropenam 10 mcg, Piperacillin Tazobactam 100 mcg, Ciprofloxacin 5 mcg, Cefotaxime 30 mcg, Ceftazidime 30 mcg, and Cefoperazone Sulbactam 75/30 mcg were placed intact on the agar surface and incubated for 12 hours.

#### Antimicrobial susceptibility testing

- Bact/ALERT positive bottles were subcultured onto blood agar, chocolate agar, and MacConkey agar, incubated at 37 degrees Celsius.
- Subculture plates were read after 18 hours of incubation, Isolated colonies of gram-stained, gram-negative bacilli were streaked onto a Muller-Hinton agar plate with a 0.5 McFarland standard.
- After 15 minutes, antimicrobial discs such as Meropenam 10 mcg, Piperacillin Tazobactam 100/10 mcg, Ciprofloxacin 5 mcg, Cefotaxime 30 mcg, Ceftazidime 30 mcg, Cefoperazone Sulbactam 75/30 mcg were placed intact on the agar surface and incubated for 12 hours.

- Routine bacterial identification and antimicrobial susceptibility testing were carried out in the Vitek2 system. Bacteriological profile data was collected<sup>6,7</sup>.

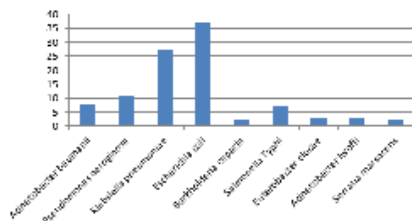
#### Matching of DST with AST

- Zone size of DST MHA plate and AST MHA plate measured and susceptibility data was recorded.
- Minor Discrepancy - AST is Resistant (R) or Susceptible (S), DST is intermediate (I); AST is Intermediate, DST is S or R.
- Major Discrepancy - AST is S and DST is R
- Very Major Discrepancy - AST is R and DST is S.
- Correlation between DST and AST analyzed and percentage was calculated for categorical variables. Bacteriology data profile collected from Vitek-2 system for further analysis<sup>8</sup>.

#### RESULTS:

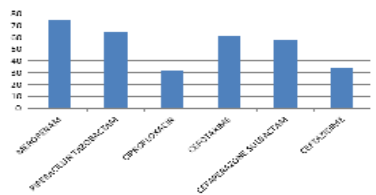
- Out of 1407 suspected blood stream infection patients from in and out patient of hospital, 100(7.10%) were gram negative blood cultures positive for the duration period of three months.
- Among the Gram negative bacilli, *Escherichia coli* (37%) was the most common isolate, followed by *Klebsiella pneumoniae* (27%), *Pseudomonas aeruginosa* (11%), *Acinetobacter baumannii* (8%), *Salmonella typhi* (7%), *Enterobacter cloacae* (3%), *Acinetobacter lwoffii* (3%) and *Serratia marsacens* (2%).

**Figure 1: PREVALANCE OF GRAM NEGATIVE BACILLI IN POSITIVE BLOOD CULTURES.**



- Overall drug susceptibility pattern for both DST and AST is as follows, The most susceptible drug is Meropenam 75%, Piperacillin tazobactam 64%, Cefotaxime 61%, Cefaperazone sulbactam 58%, Ceftazidime 34%, Ciprofloxacin 32% as shown in Figure 2.
- Direct susceptibility testing is compared to Antimicrobial susceptibility testing and the interpretation result of this study is 6 minor errors, 0 major errors, 0 very major errors with overall 99% categorical agreement.
- Piperacillin tazobactam had highest errors with 3 minor error, followed Ciprofloxacin 2 minor error and Meropenam 1 minor error. Cefotaxime, Cefaperazone Sulbactam, Ceftazidime had 100% categorical agreement followed by Meropenam 99%, Ciprofloxacin 98%, Piperacillin tazobactam 97% as shown in Table 1

**FIG 2: DRUG SUSCEPTIBILITY PATTERN OF GNB**



**TABLE 1 INTERPRETATION RESULT BETWEEN DST AND AST**

ANTIMICROBIAL	Minor Error	Major error	Very major error	Categorical aggrement
MEROPENAM	1	0	0	99%
PIPERACILLIN TAZOBACTAM	3	0	0	97%
CIPROFLOXACIN	2	0	0	98%
CEFOTAXIME	0	0	0	100%
CEFAPERAZONE SULBACTAM	0	0	0	100%
CEFTAZIDIME	0	0	0	100%

#### DISCUSSION

Patients with bacteraemia or sepsis desperately need precise and timely antibiotic medications. At present, it takes around three days to carry out antimicrobial susceptibility testing. To shorten the time, DST was used to cut short the subculture process and could show results within 6–24 hours<sup>9</sup>.

This study followed CLSI guidelines for lawning on an MHA plate<sup>6</sup>, whereas Rahila Yakoob et al<sup>10</sup> followed BSAC guidelines with 20µl of blood in 5ml sterile water, and Soo YT et al<sup>11</sup> followed EUCAST guidelines with 150µl of blood cultured lawned into an MHA plate. Difference in inoculum concentration can affect results of categorical agreement between DST and AST<sup>9</sup>.

The most common gram negative bacilli were *Escherichia coli* (37), followed by *Klebsiella pneumoniae* (27) and *Pseudomonas aeruginosa* (11) which is similar to Rahila Yakoob et al and Soo YT et al and the prevalence of other GNB is as follows *Acinetobacter baumannii* (8), *Salmonella typhi* (7), *Enterobacter cloacae* (3), *Acinetobacter lwoffii* (3) and *Serratia marsacens* (2).

Meropenem was the least resistant in this study, which is similar to Rahila Yakoob et al. and Soo YT et al. Ciprofloxacin (68%) was the most drug-resistant in the study, followed by ceftazidime (66%), Cefaperazone sulbactam (42%), Cefotaxime (39%), and Piperacillin tazobactam (36%). Ceftazidime was included for anti-pseudomonal activity and the prevalence of pseudomonas species is 11% in the study.

The categorical agreement of the study was 99%, which was similar to Kumar M et al, whereas Rahila Yakoob et al showed 94%. Piperacillin tazobactam had the highest amount of discordance (3% minor error), which was similar to Soo YT et al, discordance of other antimicrobials are ciprofloxacin (2% minor error), and meropenam (1% minor error). Cefotaxime, cefaperazone sulbactam and ceftazidime had 100% categorical agreement.

This study demonstrated that disk diffusion DST can be used for quicker and highly accurate susceptibility testing. Further advantage of disk diffusion DST is that, there is no need for advanced equipment and facilities. A decrease in hands on time and lower cost is also an additional advantage. Since the categorical agreement of DST and AST in the study was 99%, DST can be implemented in hospital laboratories for aiding in sepsis. The Earlier the treatment of Bacteraemia patients with antimicrobials begins, the better outcome.

#### CONCLUSION:

This study yields reliable and faster DST which is 99% equivalent to conventional AST, which is lifesaving procedure, reduction of morbidity and mortality in blood stream infections. The Earlier administration of antimicrobial therapy also reduces emergence of resistant-antimicrobial isolates. The limitation of this study is only six antibiotics pattern were studied, Further studies can be done with more number of antibiotics. Implementation of DST would lead to better patient care and reduce their financial burden as well.

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