Original Research Paper

Volume - 14 | Issue - 06 | June - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

RAPID ANTIBIOTIC SUSCEPTIBILITY TESTING DIRECTLY FROM POSITIVE BLOOD CULTURE BOTTLE CAN BE A TOOL FOR ANTIBIOTIC STEWARDSHIP

Dr. Sumi Thakuria*

Demonstrator, Department of Microbiology, Fakhruddin Ali Ahmed Medical College and Hospital, Barpeta- 781301, Assam, India *Corresponding Author

ABSTRACT **Background:** Blood stream infection (BSI) with resistant bacteria is a major contributing factor of mortality and morbidity in intensive care units. Reducing the turnaround time of blood cultures sensitivity report will help in early de-escalation of antibiotic therapy as well as help in targeted treatment. **Aims:** To reduce the microbiological reporting hours by using antimicrobial susceptibility test (AST) directly from positively flagged blood culture bottles and compare with minimum inhibitory concentration results obtained from automated Vitek-2 system. **Material and methods:** A hospital based study was done for three months duration. During study period, 300 aerobic blood culture bottles showed positivity by automated BacT/ALERT system of which 167 showing monobacterium on Gram stain were processed further following European Committee on Antimicrobial Susceptibility Testing - rapid antimicrobial susceptibility test (EUCAST-RAST) guidelines. Simultaneously, as a routine procedure subculture was done from the broth followed by identification and AST of the isolates by automated system. **Results and conclusions:** The prevalence of BSI was found to be 32%. Out of total 167 isolates, 86 (51.5%) were Gram positive and 81 (48.5%) were Gram negative. Among the Gram positive isolates 100% concordance was found and among Gram negative isolates 96.3% concordance was seen between the two methods. Overall, this approach with high degree of concordance is promising and contributed to reduce the turnaround time of blood cultures antibiotic sensitivity with a positive impact on patient management.

KEYWORDS : Antimicrobial Susceptibility, Blood Stream Infection, Intensive Care Unit, Multidrug Resistance.

1. INTRODUCTION:

Blood Stream Infection (BSI) with resistance bacteria is a major contributing factor of mortality and morbidity in Intensive Care Unit (ICU).[1] ICU is an ideal place for bacteria to develop antibiotic resistant primarily due to the immunocompromised state of the critically ill patients and use of broad-spectrum antibiotics as empirical antibiotic therapy. Use of broad-spectrum antibiotics for longer duration with inappropriate de-escalation causes dysbiosis of gut bacteria which not only impair the immune response and loss of gut wall integrity but also facilitate colonization of the gut with Multi Drug Resistant (MDR) bacteria.[2] Once these MDR bacteria colonize the gut, BSIs with these MDR bacteria are more likely anticipated.[3] Implementation of Antimicrobial Stewardship Programme (AMSP) in ICUs in combination with rapid microbiological test and early de-escalation of broad-spectrum antibiotics can reduce the emergence of MDR pathogens and, thereby, the mortality risk.[4]

Traditionally, microbiological processing of the blood cultures takes 48 hours from the positively flagged blood culture bottles in automated blood culture system to final sensitivity reporting. The initial 24 hours for the growth and identification of the bacteria and the next 24 hours for antibiotic sensitivity test. Numerous studies showed that the bacterial identification directly from positively flagged blood culture bottles by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can be obtained within few hours, but with poor accuracy for Gram positive bacteria.[5] Similarly, various other studies used Antimicrobial susceptibility testing (AST) directly from the positively flagged blood culture bottles in automated Vitek-2 and Phoenix platforms with poor accuracy.[6] Even molecular techniques like Reverse transcriptase polymerase chain reaction (RT-PCR) have been used for rapid detection of the pathogen with simultaneous antibiotics resistant genes detection. But these techniques cannot be used in routine laboratory due to many limitations (such as highly expensive and labor-intensive).[7]

Considering the value of rapid antimicrobial susceptibility testing reports in blood stream infections, a rapid antimicrobial susceptibility test (RAST) method has been developed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). This method, by using the disk diffusion technique, provides a rapid estimation of the antimicrobial susceptibility within 8 hours and since April 2022 also after 16-20 hours incubation, directly from the positive blood culture bottles.[8] However, outside the Europe, this rapid method has rarely been used.

The aim of our study is to reduce the microbiological reporting from 48 hours to 24 hours by using AST directly from positively flagged blood culture bottles applying the EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) guideline and compared with minimum inhibitory concentration (MIC) results obtained from automated Vitek-2 system. The concordant and discordant reports were expressed as "categorical agreement", "very major", "major" and "minor" errors.

2. MATERIALS & METHODS:

2.1 Study Design And Duration: A hospital-based study was done in the Department of Microbiology, Gauhati Medical College & Hospital (GMCH), Guwahati for duration of three months (May, 2022 to July, 2022).

2.2 Sample collection:

A total of 300 blood culture bottles, collected from 936 suspected patients having blood stream infection, were flagged positive during the study period by the automated (BacT/ALERT3D; bioMerieux, France) blood culture system. Once the bottle flagged positive, it was taken out from the system and subjected for Gram staining.

2.3 Inclusion and exclusion criteria:

Bottle showing morphologically uniform Gram positive cocci and Gram negative bacilli; and subculture showing single bacterial growth were included in the study. Positive blood bottles showing yeasts and more than one organism in Gram stain or after subculture (polymicrobial growth) were excluded from the study.

2.4 Standard Antibiotic Susceptibility Test (AST):

As a routine procedure all the positive blood culture broths were sub-cultured in 5% sheep Blood agar (BA) and MacConkey agar (MA) and incubated at 37˚C for 16-24 hours. After overnight incubation, the plates showing uniform single colonies were subjected for identification by the automated MALDI-TOF MS system followed by drug susceptibility testing by Vitek-2 automated platform (bioMerieux, France) using suitable Vitek panel (N628 panel for Gram positive isolates and N281 panel for Gram negative isolates). Data were collected retrospectively from the records maintained in the bacteriology section of Microbiology laboratory and entered in a pre-designed proforma.

2.5 Rapid Antibiotic Susceptibility Test (RAST):

The direct susceptibility test from positive blood culture bottle was done following the EUCAST RAST methods.[8] Antimicrobial susceptibility testing was done on 90-mm circular Muller-hinton agar plate directly from the positive blood culture bottles. Shortly, 125±25 µL of broth from positive blood culture bottle (undiluted) was inoculated in Muller-hinton agar plate by streaking the entire plate gently in three directions using sterile cotton swab. While streaking, the plate was rotated approximately 60 degrees to ensure uniform distribution of inoculum. Six antibiotic discs were applied in the Muller-hinton agar plate (Gentamicin 10 mcg, Cefoxitin 30 mcg, Ciprofloxacin 5 mcg, Teicoplanin 30 mcg, Linezolid 30 mcg and Levofloxacin 5 mcg for Gram positive cocci; and Ceftazidime 10 mcg, Cefepime 30 mcg, Piperacillin-tazobactum 30/6 mcg, Meropenem 10

mcg, Amikacin 30 mcg and Ciprofloxacin 5 mcg for Gram negative bacilli) and incubated at 35±1°C for 16 hours. Three sets of dRAST plates were prepared for each isolate and the average of the zone diameters measured at sixteenth hour of incubation were taken for the study.

In addition to clinical samples, two known strains of *Methicillin Resistant Staphylococcus Aureus* (MRSA) (ATCC 43300) and two Extended-spectrum beta-lactamases (ESBLs) producing strains of *Klebsiella pneumoniae* (ATCC 700603) were processed, simultaneously, both for RAST and antibiotics sensitivity testing in Vitek-2 plateform.

Working flow of direct Rapid Antibiotic Susceptibility Test (RAST) and standard antibiotic susceptibility test (AST) methods were shown in the Figure 1.

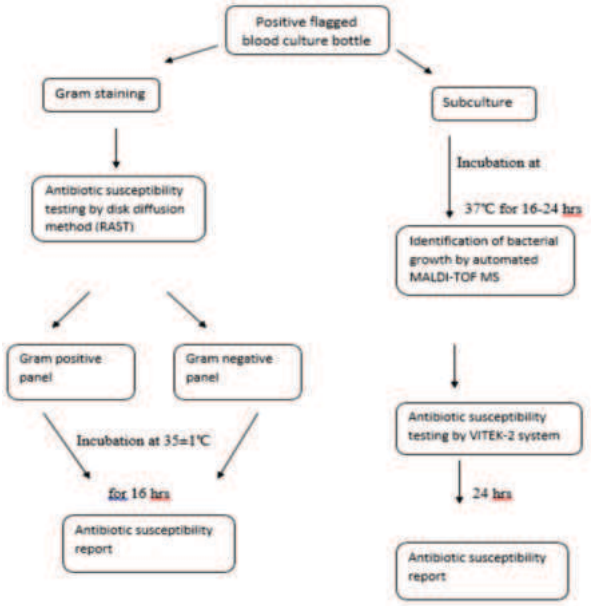

Figure 1: Working flow of direct Rapid Antibiotic Susceptibility Test (RAST) and standard antibiotic susceptibility test (AST) methods

The results of the two AST methods were compared using the term-

- Categorical agreement (CA): When the result was in concordance between the two methods.
- Very major error (VME) (False susceptibility): When sensitive in direct AST method but resistance in the automated AST method.
- Major error (ME) (False resistance): When resistance in direct AST method but sensitive in the automated AST method.
- Minor errors (mE): When intermediate in direct AST method but sensitive or resistance in the automated AST method.

2.6 Statistical Analysis:

The data collected in this study were summarized and entered in the Microsoft Office Excel 2007. Analysis of the data was carried out by using the GraphPad Prism software. The data, denoted as numbers and percentages, were taken as appropriate whenever applicable. Citations were done by using the Vancouver style.

3. RESULTS

During the study period a total of 936 blood culture bottles were received from the suspected cases of BSIs for aerobic culture of which 300 (32%) blood culture bottles flagged positive. Among the positive bottles, 167 (167/300; 55.6%) bottles showed single bacterial growth which were processed further for the study. The bottles which showed polymicrobial growth (34/300; 11.4%) and yeasts (99/300; 33%) were excluded from the study.

3.1 Gram staining: Subculture of these 167 positive blood culture bottles showed growth of Gram negative bacilli in 81 bottles (81/167; 48.5%) and Gram positive cocci in 86 bottles (86/167; 51.5%). Among the Gram negative bacteria, *Klebsiella pneumoniae* was found to be the most common isolate (31/81; 38.3%), followed by *Acinetobacter baumanni* (27/81; 33.3%), *Escherichia coli* (18/81; 22.2%), *Pseudomonas aeruginosa* (2/81; 2.5%), *Enterobacter hormaechiae* (2/81; 2.5%) and *Salmonella typhi* (1/81; 1.2%). Among the Gram positive bacteria, *Coagulase negative staphylococcus aureus* (CONS) was found to be the highest organisms isolated (73/86; 85%), followed by *Enterococcus faecium* (10/86; 11.6%) and *Methicillin resistant staphylococcus aureus* (3/86; 3.4%) (Table 1). There was 100% concordance between the results of Gram stain from the positive blood culture bottles and the Gram stain from the subculture colonies obtained after overnight incubation in solid media.

Table 1: Distribution of organisms isolated from positive blood culture:

ORGANISMS		NUMBER n=167, %)	PERCENTAGE (%)
Methicillin Resistant Staphylococcus aureus (MRSA)	Total Gram-Positive cocci (86; 51.5%)	3	3.4
Enterococcus faecium		10	11.6
Coagulase-negative Staphylococcus aureus (CONS)		73	85
Klebsiella pneumonia	Total Gram-Negative bacilli (81; 48.5%)	31	38.3
Escherichia coli		18	22.2
Pseudomonas aeruginosa		2	2.5
Acinetobacter baumanni		27	33.3
Enterobacter hormaechiae		2	5
Salmonella typhi		1	1.2
TOTAL	167	167	100

3.2 Comparison of interpretative results with RAST and standard AST:

The comparison of RAST results with standard automated Vitek-2 results was done. For the Gram negative bacilli, the CA for individual antimicrobials was found to be ranging from 96.3% for amikacin to 100% for meropenem, piperacillin/tazobactam, ceftazidime, cefepime and ciprofloxacin. VME and ME were detected for the antimicrobial, amikacin with 1.2% and 2.5% respectively. Minor error was not detected in this study. For the Gram positive cocci, all the 86 isolates showed 100% categorical agreement for all the antimicrobials applied in this study. Comparison of interpretative results with RAST and standard AST for the isolates were shown in the Table 2.

Table 2: Agreement between direct RAST and automated VITEK-2 AST:

Gram negative bacilli (n=81)								
Antimicrobials	RAST		VITEK-2		CA	VME	ME	mE
	S	R	S	R				
Amikacin	23	58	24	57	78 (96.3%)	1 (1.2%)	2 (2.5%)	-
Ciprofloxacin	18	63	18	63	81 (100%)	-	-	-
Meropenem	28	53	28	53	81 (100%)	-	-	-
Piperacillin-tazobactum	24	57	24	57	81 (100%)	-	-	-
Ceftazidime	9	72	9	72	81 (100%)	-	-	-
Cefepime	11	70	11	70	81 (100%)	-	-	-
Gram positive cocci (n=86)								
Gentamicin	80	6	80	6	86 (100%)	-	-	-
Ciprofloxacin	52	34	52	34	86 (100%)	-	-	-
Cefoxitin	65	21	65	21	86 (100%)	-	-	-
Teicoplanin	71	15	71	15	86 (100%)	-	-	-
Linezolid	84	2	84	2	86 (100%)	-	-	-
Levofloxacin	44	42	44	42	86 (100%)	-	-	-
CA: Categorical agreement, VME: Very major error, ME: Major error, mE: Minor error								

4. DISCUSSION

In the present study we evaluated the EUCAST RAST method in routine Microbiology Laboratory for both Gram positive cocci and Gram negative bacilli associated with blood stream infection. We compared the results obtained from EUCAST RAST method with routinely used commercial method (automated Vitek2 system) for six commonly used antibiotics in blood stream infection.

Our study showed a very good categorical agreement for both the Gram positive cocci (100%) and the Gram negative bacilli (96.3%). Various previous published reports also showed good categorical agreement when compared the direct AST with various methods, both in-house and commercials, at least for the fast growing bacteria.[9,10] However, many of these studies, showed less categorical agreement against Gram positive bacteria than Gram negative bacteria.[11,12] In our study, discrepancy was observed for the antimicrobial, amikacin, against the Gram negative bacilli. The rates of VME, ME and mE for Gram negative bacilli were found to be 0.2%, 0.4% and 0% respectively. Interestingly, error rate of amikacin was also found in previous studies using diverse AST methods. Chandrasekaran et al. found 23.1% VME in amikacin when used disk diffusion method directly from positive blood culture bottle to estimate the susceptibility.[13] Similarly Kevin et al. observed significant error in all aminoglycosides susceptibility not only in disk diffusion method but also in automated methods.[14] The disagreement and inconsistent results of amikacin sensitivity may be attributable to complex mechanisms of aminoglycosides resistance which are working simultaneously having variable action and aminoglycosides alone may not be an appropriate treatment at least for Gram negative bacilli.

Overall these observations indicate a good agreement between RAST from positive blood culture bottle and standard AST method. Since the first proposal of EUCAST RAST in 2017, this method has been successfully using by European countries and reduce the AST report approximately to 20 hours. However, in India standard AST has been using according to the Clinical & Laboratory Standards Institute (CLSI) guideline which take minimum of 72 hours to report AST.[15] The alternative use of EUCAST RAST may significantly help the clinicians for early de-escalation of broad spectrum antibiotics in critically ill patients. However, evaluating clinical benefit of RAST will be more significant for it to be used as routine method in laboratory.

The main limitation of our study was less number of study samples and using limited number of antimicrobials. However this panel of antimicrobials may be modified according to the local antibiotics policy available in the hospital. Also, we compared the RAST results with CLSI M100 breakpoint tables. Additionally we did not analyse the clinical outcome with RAST report.

5. CONCLUSION

Overall, this approach with high degree of concordance is promising and contributed to reduce the turnaround time of blood cultures antibiotic sensitivity with a positive impact on patient management especially in the intensive care units. As the disk diffusion method reduces the cost, it can be used in all the level of health care facilities. Even this method will also help in properly implementation of the Antimicrobial Stewardship Programme.

Funding source:
The present study is self-financed and partially supported by the Department of Microbiology, Gauhati Medical College and Hospital Guwahati, Assam.

Ethical Board Number:
The ethical clearance was obtained from the Institutional Ethics Committee of Gauhati Medical College and Hospital, Guwahati, Assam bearing the Ethics Board No. MC/190/2007/Pt.II/March 2023/7.

Acknowledgements:
The authorswould like to thank all my faculties, colleague trainees and staff of Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, for their support.

6. REFERENCES

1. Bassetti M, Righi E, Carnelutti A. Bloodstream infections in the Intensive Care Unit. Virulence. 2016 Feb 9;7(3):267-79
2. Ramirez J, Guarner F, Bustos Fernandez L, Maruy A, Sdepanian VL, Cohen H. Antibiotics as Major Disruptors of Gut Microbiota. Frontiers in Cellular and Infection Microbiology. 2020 Nov 24;10.
3. Wuethrich I, W. Pelzer B, Khodamoradi Y, Vehreschild MJGT. The role of the human gut microbiota in colonization and infection with multidrug-resistant bacteria. Gut Microbes. 2021 Jan 1;13(1):1911279.
4. Ture Z, Güner R, Alp E. Antimicrobial stewardship in the intensive care unit. Journal of Intensive Medicine. 2022 Nov;
5. Altun O, Carlsson S, Ullberg M, Özenci V, Botero-Kleiven S. Rapid identification of bacteria from positive blood culture bottles by MALDI-TOF MS following short-term incubation on solid media. Journal of Medical Microbiology. 2015 Nov 1;64(11):1346-52.
6. Blondel-Hill E, Hetchler C, Andrews D, Lapointe L. Evaluation of VITEK 2 for analysis of enterobacteriaceae using the Advanced Expert System (AES) versus interpretive susceptibility guidelines used at Dynacare Kasper Medical Laboratories, Edmonton, Alberta. Clin Microbiol Infect. 2003;9(11):1091-103.
7. Chakravorty S, Aladegbami B, Burday M, Levi M, Marras SAE, Shah D, et al. Rapid universal identification of bacterial pathogens from clinical cultures by using a novel sloppy molecular beacon melting temperature signature technique. J Clin Microbiol. 2010;48(1):258-67.
8. Methodology - EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles. Version 3.0 April 2022
9. Kansak N, Adaleti R, Nakipoglu Y, Aksaray S. Evaluation of the performance of rapid antibiotic susceptibility test results using the disk diffusion directly from the positive blood culture bottles. Indian Journal of Medical Microbiology. 2021 jun;
10. Kumar M, Shergill S.P.S., Tandel K, Sahai K, Gupta R.M. Direct antimicrobial susceptibility testing from positive blood culture bottles in laboratories lacking automated antimicrobial susceptibility testing systems. Medical Journal Armed Forces India 75 (2019) 450-457
11. Quesada MD, Gimenez M, Molinos S, Fernandez G, Sanchez MD, Rivelo R, et al. Performance of VITEK-2 compact and overnight MicroScan panels for direct identification and susceptibility testing of Gram-negative bacilli from positive FAN BacT/ALERT blood culture bottles. Clin Microbiol Infect. 2010;16(2):137-40.
12. de Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascual A. Use of positive blood cultures for direct identification and susceptibility testing with the Vitek 2 system. J Clin Microbiol. 2004;42(8):3734-8.
13. Chandrasekaran S, Abbott A, Campeau S, Zimmer BL, Weinstein M, Thrupp L, et al. Direct-from-blood-culture disk diffusion to determine antimicrobial susceptibility of gram-negative bacteria: Preliminary report from the clinical and laboratory standards institute methods development and standardization working group. Journal of Clinical Microbiology. 2018;56(3). 10.1128/JCM.01678-17.
14. Akers KS, Chaney C, Barsoumian A, Beckius M, Zera W, Yu X, et al. Aminoglycoside resistance and susceptibility testing errors in Acinetobacter baumannii-calcoaceticus complex. Journal of Clinical Microbiology. 2010 Apr 1 [cited 2020 Jul 1];48(4):1132-8.
15. Clinical and Laboratory Standards Institute (CLSI). M100 Performance standards for Antimicrobial Susceptibility Testing. 32nd Edition, Feb 2022