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Original Article

Microbiology Section

Impact of Accelerated Antimicrobial Susceptibility Testing on Clinical Outcomes in Critically ill Septicaemia Patients in a Tertiary Care Centre in Andhra Pradesh, India: A Prospective Observational Study

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ABSTRACT

Introduction: Bloodstream infection is a major cause of mortality and morbidity worldwide. Timely reporting of blood culture results is of utmost importance for better patient outcomes. The recently introduced Rapid Antimicrobial Susceptibility Testing (RAST) method is poised to profoundly influence clinical outcomes.

Aim: To compare the results of RAST with Standard Antimicrobial Susceptibility Testing (SAST) and evaluate the impact of RAST reporting on the clinical management of septicaemia patients.

Materials and Methods: This prospective, observational study was conducted in the Department of Microbiology, Government Medical College, Kurnool, Andhra Pradesh, India from May 2021 to September 2022. All positive blood culture bottles with only a single morphotype in gram staining were further processed using the RAST method, followed by conventional identification and SAST. Categorical agreement and disagreement between the RAST and SAST results were compared, along with the difference in the time at which results were available.

Results: Out of 1,146 blood cultures received, 228 were flagged as positive. A total of 514 isolate and antimicrobial agent combinations were evaluated, of which 496 (96.5%) showed categorical agreement. Only 18 (3.5%) showed categorical disagreement, with the majority being Major Errors (ME) (1.56%), followed by Very Major Errors (VME) (0.97%) and minor Errors (mE) (0.97%).

Conclusion: RAST results demonstrated strong concurrence with SAST results. RAST is affordable, fast, and flexible and can potentially lead to a considerably shortened time for AST results to reach the bedside of the patient. This enables rapid modifications and adjustments in antibiotic therapy, including both escalation and de-escalation.

Keywords: Blood culture, Rapid antimicrobial susceptibility testing, Septicaemia, Turnaround time

INTRODUCTION

Septicaemia caused by drug-resistant bacterial infections is a significant contributor to mortality and morbidity worldwide [1]. It is crucial to promptly receive and assess blood culture results to ensure the selection of the most appropriate treatment for a better outcome. This is why it was imperative to develop a swift Antimicrobial Susceptibility Testing (AST) method [2]. Traditional methods without automation are extremely time-consuming with a turnaround time of 5-7 days or more. With the advent of automated blood culture systems, blood culture and sensitivity results are available 2-3 days earlier than those of conventional blood culture methods [3,4]. However, a subculture has to be performed from positive blood culture bottles to obtain pure growth so that identification and AST can be carried out. RAST is a newer methodology performed directly from blood culture bottles to get early AST results. It helps clinicians switch over to appropriate antimicrobials quickly, playing a pivotal role in decreasing the mortality rate of such patients. This disc diffusion-based method is cheap, flexible, and adapted for any newer antibiotics without much difficulty. Recently, both EUCAST (The European Committee of Antimicrobial Susceptibility Testing) and the Clinical and Laboratory Standards Institute (CLSI) have standardised this method and provided interpretation breakpoints that are being adopted by multiple laboratories worldwide [5,6]. The turnaround time for AST can be decreased to as low as 4 to 8 hours after a positive blood culture using the RAST method, as opposed to the SAST method, which takes 24-48 hours [7,8].

There has been an unprecedented increase in drug-resistant isolates from clinical samples. Invasive infections by these drug-resistant pathogens in critically-ill patients and patients with underlying co-morbidities are extremely difficult to treat unless definitive therapy with appropriate antibiotics is administered promptly [9]. RAST plays a major role in adjusting therapy by the treating clinician in such cases. While many genotypic methods are available for the early identification of drug-resistant pathogens, they are expensive, require expertise and equipment, and cannot be adapted in peripheral settings. This is why RAST has gained momentum recently in routine clinical microbiology laboratory setups. Researchers assessed the influence of RAST testing on clinical outcomes and observed positive findings in bloodstream infections [10,11].

There is a gap in knowledge regarding the impact of RAST testing on the clinical management of patients, especially in the Indian context when compared to standard testing methods. Moreover, there is a lack of understanding about the clinical outcomes for patients when RAST testing is employed for antibiotic monitoring and adjustment. Hence, the present study aimed to identify the importance of this methodology and evaluates its impact and significance in the clinical management of septicaemia cases in a tertiary care hospital. The objectives are as follows:

1. To compare the results of RAST with SAST.
2. To determine the impact of RAST reporting on the clinical management of septicaemia patients in terms of:

a. Difference in the time at which results are available from RAST and SAST.

b. Changes to appropriate antimicrobial agents.

MATERIALS AND METHODS

This study was a prospective, observational study conducted in the Department of Microbiology, Government General Hospital and Kurnool Medical College, Kurnool, Andhra Pradesh, India from May 2021 to September 2022. The study was approved by the Institutional Ethics Committee, Kurnool Medical College, Kurnool (IEC No. 36/2021 dated 26.04.2021).

Inclusion criteria: All positive blood cultures of septicaemia patients admitted to Intensive Care Units (ICU) were included in the study.

Exclusion criteria: Positive blood cultures with two or more morphotypes, positive blood cultures with fungal growth, positive blood cultures with gram positive bacilli and gram negative cocci, and repeated blood cultures from the same patient were excluded from the study.

Sample size estimation: Convenience sampling was employed to gather a dataset consisting of 87 blood samples. This method was chosen for its ease of access and quick implementation, allowing for a relatively efficient collection process.

Study Procedure

All the positive blood culture bottles flagged by the automated blood culture system (BacT/ALERT3D; bioMerieux, France) were subjected to Gram staining. Gram staining that showed only a single organism (single morphotype) was processed further by RAST.

RAST using the Kirby Bauer disc diffusion method [12-14]: A 125±25 µL of undiluted blood culture broth was taken from the positive blood culture bottle to each 90-mm circular Muller-Hinton agar plate, and it was spread over the agar surface by swabbing in three directions. After the plate surface dried, antibiotic discs were placed and incubated at 35±1°C for 8 hours. Reading and interpretation were done after 4 hours, 6 hours, and 8 hours±five minutes of incubation following EUCAST RAST guidelines. Inhibition zones were read only when the growth was confluent, and zone edges were clearly visible. The RAST QC procedure was performed to calibrate and validate the implementation of the procedure by using QC strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213). Internal QC was performed daily to validate the procedure and AST materials.

Standard Conventional Identification and AST [6,15]: Simultaneously, broth from the positive blood culture bottle was sub-cultured onto MacConkey Agar and Blood Agar. Pure growth from solid plates was subjected to Standard Conventional identification and AST as per EUCAST and CLSI guidelines. Gram negative bacilli were tested for a panel of six antibiotics such as amikacin 30 µg (AK), gentamicin 10 µg (GEN), ceftriaxone 30 µg (CTR)/ceftazidime 30 µg (CAZ), ciprofloxacin 5 µg (CIP), meropenem 10 µg (MRP), and piperacillin-tazobactam 100/10 µg (PIT). All these antibiotics were included for analysis if the pathogen was a member of the Enterobacteriaceae family, *Pseudomonas* species, or *Acinetobacter* species. CTR was excluded from the analysis for *Pseudomonas* species.

All RAST results were informed to treating clinicians either through a phone call or by posting in the specific ICU WhatsApp group.

Results of the RAST were compared with those of SAST. The performance of RAST compared with SAST was expressed in terms of categorical agreement and categorical disagreement according to ISO 20776-2:2007 guidelines [16,17].

Categorical agreement between RAST and SAST was classified as shown in [Table/Fig-1].

The time difference between the availability of RAST and SAST was recorded. The impact of the RAST result on clinical decision-making was evaluated by reviewing patients' case sheets for the modification of antimicrobial agents, i.e., initiation of appropriate antimicrobial agents.

Type of error	RAST	SAST
Very Major Error (VME)	S	R
Major Error (ME)	R	S
Minor Error (mE)	S/R	I
	I	S/R

[Table/Fig-1]: Terminologies used for comparison of RAST and SAST.
RAST:Rapid antimicrobial susceptibility testing;SAST:Standard antimicrobial susceptibility testing; S:Susceptible;R:resistant;I:Intermediate

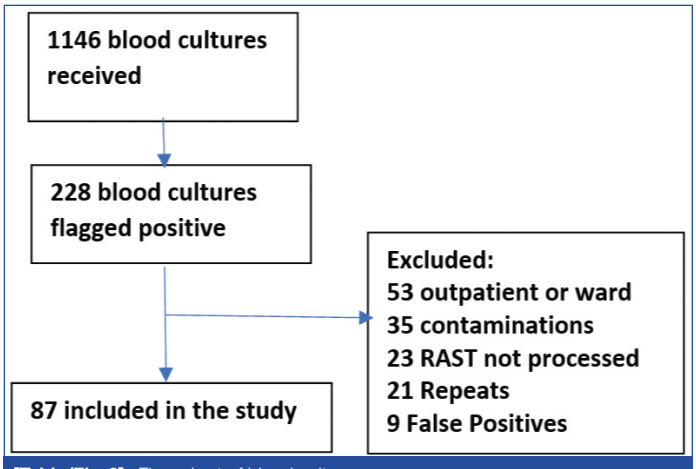
STATISTICAL ANALYSIS

All the data collected was entered into a Microsoft Excel sheet. The analysis of the data was performed using Statistical Package for Social Sciences Software (SPSS) version 22.0.

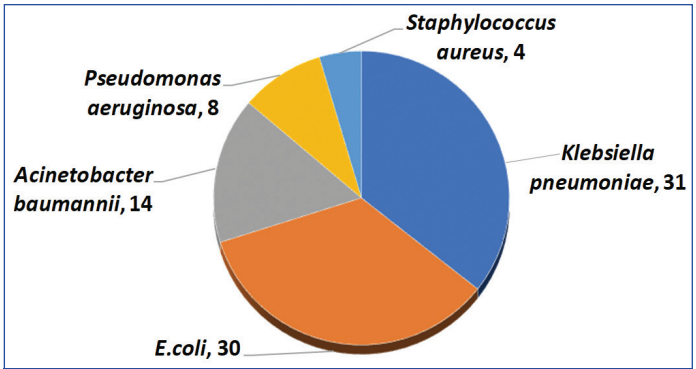
RESULTS

A total of 1,146 blood cultures were received, out of which 228 flagged positive (19.9% positivity rate). Out of the 228 positive blood cultures, 53 positives were excluded because they were collected from Outpatient or Wards, 35 positives grew contaminants, 23 bottles were not processed by the RAST method, 21 were repeats, and nine were false positives, leaving a total of 87 eligible blood cultures for the analysis [Table/Fig-2]. In the present study, there were 61 Enterobacteriaceae group of organisms (31 *Klebsiella pneumoniae* and 30 *E. coli*), 22 non fermenters (14 *Acinetobacter* spp. and 8 *Pseudomonas* spp.), and four *Staphylococcus aureus* included [Table/Fig-3]. A total of 514 isolate and antimicrobial agent combinations were evaluated, of which 496 (96.5%) showed categorical agreement, which was extremely satisfactory. Only 18 (3.5%) showed categorical disagreement, with the majority being ME 8 (1.56%), followed by VME 5 (0.97%) and mE 5 (0.97%).

A total of 366 isolate and antimicrobial agent combinations were obtained from the susceptibility results for the Enterobacteriaceae



[Table/Fig-2]: Flow chart of blood cultures.



[Table/Fig-3]: Distribution of organisms isolated from positive blood cultures.

group of organisms. Out of these, 358 (97.81%) combinations showed categorical agreement, whereas 8 (2.18%) combinations showed categorical disagreement, with 2 (0.55%) being VMEs, 4 (1.1%) MEs, and 2 (0.55%) mEs [Table/Fig-4]. The categorical agreement for individual antimicrobials against *Enterobacteriaceae* showed amikacin 61 (100%), gentamicin 59 (96.72%), ceftriaxone 60 (98.36%), ciprofloxacin 59 (96.72%), meropenem 60 (98.36%), and piperacillin tazobactam 59 (96.72%).

Antimicrobial agent	Categorical agreement	Categorical disagreement				Kappa value of agreement	p-value
		VME	ME	mE	Total		
Amikacin	61	0	0	0	0	1.000	0.0001
Gentamicin	59	1	1	0	2	0.934	0.0001
Ciprofloxacin	59	0	1	1	2	0.934	0.0001
Ceftriaxone	60	0	0	1	1	0.967	0.0001
Meropenem	60	1	0	0	1	0.967	0.0001
Piperacillin-tazobactum	59	0	2	0	2	0.934	0.0001

[Table/Fig-4]: Performance of Rapid Antimicrobial Susceptibility Testing (RAST) compared to Standard Antimicrobial Susceptibility Testing (SAST) for Enterobacteriaceae (n=61).

A total of 132 isolate and antimicrobial agent combinations were obtained for non fermenters (*Acinetobacter* and *Pseudomonas*). Among these, 123 (93.18%) combinations showed categorical agreement, whereas 9 (6.81%) combinations showed categorical disagreement, with 3 (2.27%) being VMEs, 3 (2.27%) MEs, and 3 (2.27%) mEs [Table/Fig-5]. The categorical agreement for individual antimicrobials against non fermenters showed amikacin 20 (90.91%), gentamicin 20 (90.91%), ceftazidime 22 (100%), ciprofloxacin 19 (86.36%), meropenem 21 (95.45%), and piperacillin tazobactam 21 (95.45%). Among gram positive organisms, four *Staphylococcus aureus* were tested for cefoxitin, norfloxacin, gentamicin, and clindamycin. Out of 16 isolate and antimicrobial agent combinations, only one (6.25%) ME for cefoxitin was observed with a categorical agreement of 93.8%.

Antimicrobial agent	Categorical agreement	Categorical disagreement				Kappa value of agreement	p-value
		VME	ME	mE	Total		
Amikacin	20	1	0	1	2	0.818	0.0001
Gentamicin	20	0	1	1	2	0.818	0.0001
Ciprofloxacin	19	1	1	1	3	0.727	0.0001
Ceftazidime	22	0	0	0	0	1.000	0.0001
Meropenem	21	0	1	0	1	0.909	0.0001
Piperacillin-tazobactum	21	1	0	0	1	0.909	0.0001

[Table/Fig-5]: Performance of Rapid Antimicrobial Susceptibility Testing (RAST) compared to Standard Antimicrobial Susceptibility Testing (SAST) for *Acinetobacter* and *Pseudomonas* (n=22).

Modification of treatment based on RAST results was evaluated, in which 13/87 (14.9%) cases had an escalation of antibiotics. De-escalation was not done in any of the cases, but discontinuation of antimicrobials with coverage other than that of the isolated pathogen was done in 49 cases (56.3%), i.e., discontinuation of gram positive specific antimicrobials like vancomycin if gram negative bacilli were reported in RAST [Table/Fig-6]. The mean time difference in obtaining results between RAST and SAST was 36.8±4.96 hours. RAST results were available 28 to 42 hours earlier than the SAST results.

Change of antimicrobial treatment	Total	%
Escalation of Antimicrobials	13/87	14.9
De-escalation	0/87	0
Discontinuation of Antimicrobials with coverage other than that of Pathogen isolated	49/87	56.3

[Table/Fig-6]: Modification of treatment based on RAST results (n=87).

DISCUSSION

Early diagnosis, susceptibility testing, and appropriate initiation of treatment are essential for the survival of septicaemia patients, with the blood culture report playing a vital role. Conventional SAST methodology is time-consuming, with a turnaround time of more than 48 hours [18]. Every hour of delay in initiating appropriate therapy decreases the survival rate of septicaemia patients [19]. Rapid susceptibility testing directly from blood culture can significantly help in this regard.

In India, there is a lack of literature on RAST testing methods, with very few studies [20,21] comparing RAST and SAST testing methods. Due to potential variations in interlaboratory testing among different facilities, it is advisable to test a larger number of isolates from various regions of India to generate cumulative RAST results for comparison with SAST results. This will enhance understanding of the methodologies and their routine application. Therefore, the present study aimed to correlate RAST testing with conventional SAST methodology.

In the present study, RAST and SAST exhibited very good categorical agreement. Out of 514 isolate and antimicrobial agent combinations tested, 496 (96.5%) showed categorical agreement, with only 18 (3.5%) showing categorical disagreement. The majority of disagreements were minor errors (ME) in 8 cases (1.56%), followed by very major errors (VME) in 5 cases (0.97%) and major errors (mE) in 5 cases (0.97%). Present study findings were consistent with a study by Rajasekhar D et al., where a total of 965 pathogens and 7106 organism-antibiotic combinations were evaluated, resulting in a 96% categorical agreement. Categorical disagreements were found in only 4% of organism-antibiotic combinations, primarily minor errors (2.1%), followed by very major errors (1%) and major errors (0.9%) [20]. In contrast, Chandrasekaran S et al., reported a lower categorical agreement of 87.9% between RAST and SAST with errors found in their study including VME (0.5%), ME (3.5%), and mE (10%) [21].

Providing adequate and appropriate antimicrobial therapy is crucial in reducing mortality and improving outcomes for sepsis patients. Present study also aimed to identify changes in clinicians' prescription practices after the RAST report was released. A good correlation was observed for the escalation of antibiotics with RAST reporting, while clinicians generally did not practice de-escalation therapy, possibly due to concerns about patient deterioration if de-escalation was done early given the severity of the cases. Nevertheless, based on gram stain and RAST reporting, antibiotics with coverage other than the pathogen were discontinued in the majority of cases in present study.

Limitation(s)

This study was conducted at a single centre and included a limited number of isolates. Since the bacteriology laboratory operates only during specified working hours, some delays in obtaining/reporting blood culture positives during off-hours can be expected, which might have led to subsequent delays in RAST testing and informing clinicians.

CONCLUSION(S)

Present study findings underscore the potential of RAST in facilitating the early initiation of targeted therapies, as demonstrated by its strong correlation with SAST results. This method has the potential to significantly reduce mortality and morbidity rates among septicaemia patients. RAST is affordable, fast, and flexible, potentially leading to a considerably shortened time for AST results to reach the patient's bedside. This enables rapid modifications

and adjustments in antibiotic therapy, including both escalation and de-escalation, thereby facilitating the effective implementation of antimicrobial stewardship protocols.

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