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Original Research Article

Implementation and diagnostic evaluation of EUCAST rapid antimicrobial susceptibility testing directly from positive blood cultures for early targeted therapy in bloodstream infections

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Abstract:

Background: Bloodstream infections (BSIs) are medical emergencies associated with high morbidity and mortality, where delays in initiating appropriate antimicrobial therapy adversely affect outcomes. Although automated blood culture systems reduce time to pathogen detection, conventional antimicrobial susceptibility testing (AST) requires additional incubation, leading to delayed targeted therapy. The EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) method was developed to provide early phenotypic susceptibility results directly from positive blood culture bottles.

Aim: To evaluate the diagnostic accuracy of the EUCAST RAST method, compare its performance with conventional disc diffusion AST using FDA Class II criteria, and determine the prevalent pathogens and resistance patterns in bloodstream infections.

Methods: A prospective laboratory-based observational study was conducted over six months on monomicrobial positive blood cultures. RAST was performed directly from flagged blood culture bottles, with inhibition zones read at 8 hours and interpreted using EUCAST RAST breakpoints. Conventional Kirby–Bauer disc diffusion testing, interpreted as per CLSI guidelines, served as the reference standard. Categorical agreement and error rates were analyzed.

Results: EUCAST RAST showed high categorical agreement (>90%) across all tested organisms. Perfect agreement was observed for *Enterococcus faecium*, *Acinetobacter baumannii*, and *Salmonella enterica*. Minor discrepancies were mainly associated with fluoroquinolones among Enterobacterales.

Conclusion: EUCAST RAST is a rapid, reliable AST method that significantly reduces turnaround time and supports early targeted therapy and antimicrobial stewardship in BSIs.

Keywords: Bloodstream infections; EUCAST RAST; Rapid antimicrobial susceptibility testing; Antimicrobial resistance; Diagnostic stewardship

Introduction

Bloodstream infections (BSIs) remain a major cause of morbidity and mortality worldwide, particularly among critically ill patients, where delays in diagnosis and targeted antimicrobial therapy significantly worsen outcomes. Evidence suggests that mortality increases by approximately 7–8% for every hour of delay in initiating appropriate therapy in sepsis. Although automated blood culture systems have reduced the time required for detection of microbial growth, conventional antimicrobial susceptibility testing (AST) still requires an additional 18–48 hours due to mandatory subculture and incubation steps. This delay often necessitates prolonged empirical broad-spectrum antibiotic use, which contributes to antimicrobial resistance (AMR) and adversely affects antimicrobial stewardship efforts. To address this diagnostic gap, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) introduced the Rapid Antimicrobial Susceptibility Testing (RAST) method, which enables phenotypic susceptibility testing directly from positive blood culture bottles with shortened incubation times and modified interpretive criteria [1, 2].

The EUCAST RAST approach has the potential to transform routine microbiology workflows by providing reliable susceptibility results within 6–8 hours, thereby supporting early escalation or de-escalation of antimicrobial therapy and aligning with national and global AMR action plans [2, 3]. The aim of the present study was to implement and evaluate the diagnostic accuracy of the EUCAST RAST method directly from positive blood cultures, compare its performance with conventional disc diffusion testing using FDA Class II criteria, and identify the common pathogens and resistance profiles associated with BSIs.

Methodology

This study was conducted as a laboratory-based prospective observational study in the Department of Microbiology at CMR Institute of Medical Sciences over a six-month period from May 2025 to October 2025, after obtaining approval from the Institutional Ethics Committee (IEC/CMRIMS/2025/043). Blood culture samples received from patients with clinical suspicion of sepsis admitted to intensive care units, pediatric intensive care units, and general wards were included. Only the first positive blood culture per septic episode was considered. Blood cultures showing monomicrobial growth of Gram-positive cocci or Gram-negative bacilli (GNB) on Gram staining were included, while polymicrobial cultures, fungal isolates, anaerobes, fastidious organisms, contaminated samples, and repeat cultures from the same patient within 48 hours were excluded. Blood samples (5–10 mL from adults and 2–3 mL from pediatric patients) were aseptically collected and inoculated into automated blood culture bottles, which were incubated in the BacT/ALERT system until flagged positive.

Upon positivity, Gram staining was performed immediately, and RAST was initiated within one hour directly from the positive blood culture bottle. A volume of 125 ± 25 µL of undiluted blood culture broth was inoculated onto 90-mm Mueller–Hinton agar plates and evenly spread using sterile swabs. A maximum of four to six antibiotic discs were applied per plate to prevent overlapping inhibition zones. The RAST plates were incubated aerobically and inhibition zones were read at 8 hours only when confluent growth and clearly demarcated zone edges were observed. Zone diameters were measured from the front of the plate using reflected light, and interpretation was performed using EUCAST RAST-specific breakpoint tables. In parallel, conventional subcultures were made on blood agar and MacConkey agar plates, incubated for 18–20 hours, and organisms were identified by standard biochemical tests. Conventional antimicrobial susceptibility testing was carried out using the Kirby–Bauer disc diffusion method, and results were interpreted according to CLSI M100 guidelines, which served as the reference standard for comparison [2 – 4].

The performance of EUCAST RAST was evaluated by comparing its results with those of conventional disc diffusion testing. Antimicrobial susceptibility results were categorized as susceptible or resistant, and discrepancies were assessed using FDA Class II performance metrics. Categorical agreement (CA), very major error (VME), major error (ME), and minor error (mE) rates were calculated for each organism–antibiotic combination. ESBL production among Escherichia coli, Klebsiella pneumoniae, and Salmonella enterica was screened using cefotaxime and ceftazidime cut-off values, while carbapenemase production was screened using meropenem cut-off values at 8 hours as per EUCAST recommendations. All data were entered into Microsoft Excel and analyzed descriptively to assess diagnostic accuracy and agreement parameters. This methodological approach was designed to evaluate the feasibility, accuracy, and clinical applicability of EUCAST RAST in routine microbiology workflows for timely therapeutic decision-making and antimicrobial stewardship [2, 5].

Results:

A total of 546 blood culture bottles were received during the study period, of which 53 (9.9%) flagged positive and yielded monomicrobial growth. Among these, 34 (64.2%) isolates were Gram-positive cocci (GPC) and 19 (35.8%) were GNB. Staphylococcus aureus was the most common pathogen isolated, followed by Escherichia coli and Klebsiella pneumoniae. RAST was successfully performed on all isolates, and results were compared with conventional Kirby–Bauer disc diffusion testing. Overall, EUCAST RAST demonstrated high diagnostic accuracy, with categorical agreement (CA) exceeding 90% for all organisms. Perfect agreement (100% CA) with no errors was observed for Enterococcus faecium, Acinetobacter baumannii, and Salmonella enterica. Staphylococcus aureus showed excellent concordance (CA) (98.8%) with one ME (1.2%) and no VMEs. Among Enterobacterales, *Escherichia coli* demonstrated a 96.9% CA%, two (5.8%) VMEs observed for fluoroquinolones. *Klebsiella pneumoniae* showed slightly lower agreement (95.45%), with both very major and MEs primarily associated with fluoroquinolones. *Pseudomonas aeruginosa* exhibited the lowest CA (90.0%) due to single ME, though no false-susceptible results were observed.

Table 1: Distribution of Bloodstream Isolates (n = 53)

Organism	Number (%)
Staphylococcus aureus	29 (54.7)
Enterococcus faecium	5 (9.4)
Escherichia coli	6 (11.3)

Klebsiella pneumoniae	4 (7.5)
Acinetobacter baumannii	4 (7.5)
Salmonella enterica serotype Typhi	4 (7.5)
Pseudomonas aeruginosa	1 (1.9)

Table 2: Overall Performance of EUCAST RAST (FDA Class II Metrics)

Organism	CA (%)	VME (%)	ME (%)	mE (%)
Staphylococcus aureus	98.8	0	1.2	0
Enterococcus faecium	100	0	0	0
Escherichia coli	96.9	5.8	0	0
Klebsiella pneumoniae	95.45	3.2	7.7	0
Acinetobacter baumannii	100	0	0	0
Salmonella enterica	100	0	0	0
Pseudomonas aeruginosa	90	0	16.7	0

Table 3: RAST vs conventional AST summary among the GPC

Organism	AT	CA (%)	EO
Staphylococcus aureus	6	98.8	1 ME
Enterococcus faecium	5	100	Nil

AT: Antibiotics tested; EO: Errors Observed

Table 4: RAST vs conventional AST summary among the GNB

Organism	AT	CA (%)	EO
Escherichia coli	11	96.9	2 VME*
Klebsiella pneumoniae	11	95.45	1 VME, 1 ME
Acinetobacter baumannii	8	100	Nil
Salmonella enterica	9	100	Nil
Pseudomonas aeruginosa	10	90	1 ME

AT: Antibiotics tested; EO: Errors Observed; *Fluoroquinolones

Discussion

The present evaluation demonstrates that EUCAST RAST provides a significant reduction in diagnostic delay without compromising accuracy. This method allowed susceptibility profiling directly from positive blood culture bottles at 8 hours incubation, bypassing the 18–20 h subculture period which required for conventional Kirby–Bauer disc diffusion. The standard work flow require 72 – 96 hours from initial sample collection to final reporting. The present study evaluated the categorical agreement and error distribution of RAST across major bacterial pathogens in routine clinical care in comparison with Kirby Baeur disc diffusion method.

Predominantly, the pathogens in this study were isolated from adult ICUs, PICU and wards. *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Salmonella enterica* serotype typhi and *Pseudomonas aeruginosa* are the common isolates. The RAST findings were communicated to the concern treating specialist at the end of 8-hour incubation. Importantly, these pathogen groups represent high-impact causes of bacteraemia in critically ill patients and require early escalation or de-escalation to optimize outcomes of the patient.

Staphylococcus aureus (n = 29) was the most common isolate, all were Methicilllin resistant *Staphylococcus aureus* (MRSA). Similar findings were reported by Jasuja et al. [6] and Gangar et al. [7]. *Staphylococcus aureus* demonstrated excellent reliability with 98.8% CA and 1.2% ME for Clindamycin and Gentamicin which is as per the FDA class II requirements. MEs were not detected, indicating the rapidity in reporting the susceptibility results in staphylococcal infections. *Enterococcus faecium* (n=5) achieved 100% concordance and zero error across all categories. These findings support the RAST utility in enterococcal susceptibility testing.

Among the Gram negative rods, *Acinetobacter baumannii* (n=4) and *Salmonella enterica* (n=4) achieved 100% correlation and zero error. The excellent performance with multidrug-resistant strains particularly relevant finding given the clinical significance of carbapenem-resistant Acinetobacter species. *Escherichia coli* (n=6) exhibited 96.9% CA, 5.8% VME with Ciprofloxacin and Levofloxacin and all were Extended spectrum beta lactamase

(ESBL) producers. The high VME in this study correlated with Bin Najeeb et al. [8], showed similar VME (4.7%) and 2.5% ME for Piperacillin-Tazobactam and Ciprofloxacin. Bin Najeem et al. [8] showed 3.3% VME for Ciprofloxacin and Bianco G et al. [9] reported 3%.

Klebsiella pneumoniae (n=4) demonstrated 95.45 % CA with modest discordance of VME 3.2%, ME 7.7% with 2 isolates being ESBL and other 2 being ESBL and Metallo beta lactamases (MBL's). Whereas 11.1% and 7% ME were reported by Jasuja et al. [6] and Noor et al., [10] respectively and just 1.9% VME was reported by Najeem et al . [11] As per Takahasi et al. [12] Fluoroquinolones have complex time dependant/concentration dependant antimicrobials whose zone sizes may change with the time of reading. Other reasons like not fully upgraded efflux pump [13], gradual expression of qnr proteins which will be full active at 16-18 hours incubation [14] were also reported. Hwang et al. [15] suggested that RAST performed directly from blood culture which may influence the inoculum compared to the standard 0.5 McFarland suspension which used for overnight incubation. This small inoculum difference in RAST can move isolate across a breakpoint producing discrepant categorical results.

Zhang F et al. [16] suggested that early RAST readings at 8 hours predominantly show susceptible readings for majority of the bacteria, failing to reveal resistant subpopulations that become evident after extended incubation, hence RAST readings appear susceptible while overnight plates show resistant in conventional disc diffusion.

Hooper et al. [17] suggested that bacteria with target site mutations such as gyrA/par C mutations are partially inhibited early but recover faster and resume growth by 16 – 18 hours. As per the existing literature, early incubation in RAST results in Fluoroquinolone sensitive, the prolonged incubation in the conventional disc diffusion method in our study turns it to be resistant.

In this study, *Pseudomonas aeruginosa* showed lowest CA (90.0%), with a higher ME (16.7%) which could be due to limited isolation rate. Herroleon et al. [18] also reported a low CA (84–88%). Najeeb et al. [8] showed 5.6% VME. Nevertheless, the absence of VME in *Pseudomonas* is clinically significant, demonstrating consistent identification of resistant phenotypes with no false susceptible interpretations.

Multicentre studies conducted across 55 European laboratories [6, 9, 18] validated the performance of EUCAST RAST for key pathogens including *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and reported categorical agreement rate >95% at 8 hours incubation. The Indian studies [7, 8, 10] also evaluated EUCAST RAST directly from flagged blood culture bottles and early subculture growth, demonstrating high CA at 6–8 hrs and substantial reduction in turnaround time. Vishwakarma et al. [19] further contributed a practical solution for resource-limited settings by showing direct inoculum processing in an automated identification system that can provide early species identification compatible with RAST workflow in the absence of MALDI-TOF mass spectrometry, thereby enabling earlier, RAST-based reporting of AST from positive blood cultures in Indian hospitals.

Limitations: This single-centre, six-month study may limit generalizability to institutions with different blood culture positivity rates. Small numbers of certain isolates (e.g., *A. baumannii*, *Salmonella*, *P. aeruginosa*) may magnify the impact of individual discordant results on categorical agreement and error rates. The predefined exclusion of polymicrobial cultures, fungi, anaerobes, and fastidious organisms restricts extrapolation to these groups. Observed fluoroquinolone underdetection among Enterobacterales warrants cautious reporting pending confirmatory testing. Nevertheless, overall interpretive accuracy remained within acceptable standards, with no very major errors for key Gram-negative pathogens and excellent Gram-positive performance, supporting clinical utility in resource-limited settings.

However the current study findings also align with the broader goals of antimicrobial stewardship frameworks promoted by Indian Association of Medical Microbiologists, ICMR, and national AMR surveillance networks. By demonstrating dependable accuracy across multiple pathogen groups, the method may help streamline AST workflows, reduce turnaround times, and enhance diagnostic precision critical components in improving patient outcomes in Indian healthcare settings.

Conclusion: Investment in AMR action is always a prudent step toward ensuring a safer and healthier future. The implementation of EUCAST RAST in the Microbiology laboratory enhances diagnostic stewardship and ultimately facilitates the establishment and strengthening of an antimicrobial stewardship team to conduct regular audits of antibiotic usage and resistance patterns. In alignment with the World Antimicrobial Awareness Week 2025 theme, "Act Now: Protect Our Present, Secure Our Future," AMR containment remains a shared global responsibility. This study demonstrates that EUCAST RAST can be routinely implemented in microbiology laboratories to optimize turnaround time, promote early targeted therapy, reduce unnecessary empirical antimicrobial pressure, and ultimately reinforce and sustain the effectiveness of antimicrobial stewardship programs.

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