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Microbiology Section

Comparative Evaluation of EUCAST RAST and VITEK 2 AST in Cancer Patients with Bloodstream Infections: A Prospective Observational Study

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ABSTRACT

Introduction: Early initiation of targeted antibiotic therapy is crucial for managing sepsis effectively, as it significantly reduces morbidity, mortality, treatment costs, and the risk of antibiotic resistance. The current microbiological reporting system requires 48-72 hours to provide antimicrobial susceptibility results from positive blood cultures. Rapid Antimicrobial Susceptibility Testing (RAST) by European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides antimicrobial susceptibility results at four, six, and eight hours after a blood culture bottle flags positive, significantly reducing Turnaround Time (TAT) and supporting timely, targeted therapy.

Aim: To evaluate the performance of RAST by EUCAST directly from positive blood cultures, compared to the automated VITEK 2 Antimicrobial Susceptibility Testing (AST) system, in a tertiary care cancer hospital.

Materials and Methods: A prospective observational study was conducted from June 2022 to May 2023 in the Department of Microbiology at the National Cancer Institute, Nagpur, Maharashtra, India. RAST procedure was applied to 148 positive blood culture samples showing monomicrobial Gram-negative bacilli or Gram-positive cocci on Gram staining. RAST results were compared with the reference method (Vitek 2 result). Categorical Agreement (CA), Very Major Errors (VME) (false susceptible), Major Errors (ME) (false resistant), and Minor Errors (mE) (intermediate discrepancies) were calculated according to standard definitions.

Results: Out of 1,109 blood culture samples, 148 were positive, and the EUCAST RAST method was evaluated for *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The overall CA at six hours was 92.13%, with VME 2 (1.11%), ME 6 (3.61%), and mE 20 (5.61%).

Conclusion: EUCAST RAST can significantly reduce the TAT for AST reporting while maintaining a CA (>90%) with the VITEK 2 system and can be effectively implemented in routine laboratory workflow with minimal clinically relevant errors. It enables timely and targeted antimicrobial selection at early stage, with good agreement for meropenem, imipenem, and gentamicin at six hours, except for a slightly higher mE rate observed with amikacin. Its implementation also supports antimicrobial stewardship by promoting timely optimisation of antibiotic therapy and reducing inappropriate antimicrobial use.

Keywords: Antimicrobial stewardship, Oncology, Rapid antimicrobial susceptibility testing, Sepsis, Treatment outcome

INTRODUCTION

Sepsis remains a major global health concern, accounting for over 5.3 million deaths annually, making it one of the leading causes of mortality worldwide, with Bloodstream Infections (BSIs) contributing substantially to hospital-related deaths, particularly among critically ill patients [1,2]. Prompt initiation of appropriate antibiotic therapy is crucial, as each hour of delay in antibiotic administration increases mortality by 7.6% [3]. Early initiation of targeted antibiotic therapy has been shown to improve patient outcomes in cases of bacteraemia and help mitigate the emergence of antibiotic resistance [4]. The gold standard method for diagnosing BSIs includes performing culture and AST from blood sample [5]. However, the conventional microbiological workflow requires a TAT of 48-72 hours, even with automation, leading to prolonged empirical use of broad-spectrum antibiotics. This delay can increase mortality, extend hospital stays, and escalate treatment costs [6].

Although newer molecular techniques and rapid phenotypic methods facilitate faster identification of pathogen, they have limitations, including an inability to detect a broad range of pathogens and provide antimicrobial susceptibility results. Furthermore, these methods require substantial hands-on processing time and financial investment, making blood culture-based testing the preferred diagnostic standard [7].

The RAST method by EUCAST is based on standard disk diffusion technique but incorporates modifications in inoculum preparation, incubation time, specific RAST breakpoints, reading instructions and provides antimicrobial susceptibility results at four, six, and eight hours after a blood culture bottle flags positive significantly reducing TAT [8]. However, the interpretation of RAST results is species-specific, necessitating prior bacterial identification [9]. This presents a challenge in resource-limited settings where automated identification systems, like Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF), are unavailable. Its performance remains unexplored in oncology settings, particularly in immunocompromised and neutropenic patients with sepsis as well as its practical applicability in guiding timely therapeutic decision making.

The present study aimed to evaluate the performance of RAST based on the guidelines of the EUCAST directly from positive blood cultures in comparison with the automated AST report by VITEK 2 System in cancer patients with bacteraemia. The primary objective of the study was to determine the CA and error rates (VME, ME, and mE) between EUCAST RAST results and the reference AST results obtained by the VITEK 2 system and the secondary objective was to assess the potential impact of EUCAST RAST on early optimisation of empirical antibiotic therapy in cancer patients with bacteraemia.

MATERIALS AND METHODS

A prospective observational study was conducted from June 2022 to May 2023 in the Department of Microbiology at the National Cancer Institute, Nagpur, Maharashtra, India. This study was approved by the National Cancer Institute Ethics Committee, Nagpur, under Institutional Ethics Committee reference number NCI/EC/2018/032/07/2022 and NCIEC registration number ECR/1130/Inst/MH 2018/RR 21. All procedures were conducted in accordance with the relevant guidelines and regulations of the institutional ethical committee. This was a laboratory-based study with no direct involvement of human subjects.

A total of 1,109 blood culture samples were received, out of which 148 tested positive. It was a time-bound study, so all the samples received during the study period were taken into consideration.

Inclusion criteria: All positively flagged blood culture bottles from both adult and paediatric patients and samples demonstrating monomicrobial organisms, i.e., either Gram- positive cocci or Gram-negative bacilli on Gram staining, were included in the study.

Exclusion criteria: Blood culture bottles showing polymicrobial growth or the presence of yeast cells on Gram staining, as well as samples flagged positive outside routine working hours (i.e., positive at night and public holidays), were excluded from the study.

Study Procedure

For the EUCAST RAST, 125±25 µL of blood-broth inoculum was aspirated into a 1 mL syringe and spread as a lawn culture on Mueller-Hinton Agar (MHA) plates [10]. Antibiotic discs (Himedia, India) as recommended by EUCAST RAST were placed on the MHA plates and incubated subsequently at 35±1°C, namely: meropenem (10 µg), imipenem (10 µg), piperacillin-tazobactam (30-6 µg), amikacin (30 µg), gentamicin (10 µg), cefotaxime (5 µg), ceftazidime (10 µg), trimethoprim-sulphamethoxazole (1.25-23.75 µg) and ciprofloxacin (5 µg) [10]. Zone diameters were manually measured at four, six and eight hours of incubation and interpreted according to RAST breakpoints (version 4.0) by EUCAST [11] retrospectively after identification of the isolate by the VITEK 2 system. Bacterial isolates for which the zone diameter fell in the Area of Technical Uncertainty (ATU, i.e., the range between breakpoints of susceptibility and resistance) were neither categorised as susceptible nor resistant [10]. Quality control was performed using American Type Culture Collection (ATCC) reference strains: *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 for the RAST method.

Simultaneously, inoculum from blood culture bottles was streaked onto blood agar and MacConkey agar plates, which were incubated at 37°C for 18-20 hours. Bacterial identification and AST were performed using the VITEK 2 automated system. The MIC values obtained were interpreted as per Clinical and Laboratory Standards Institute (CLSI) M100 guidelines [12].

Patient records were examined for empiric antibiotics prescribed at the time of blood culture positivity and compared with RAST results at six hours. It was then evaluated to determine whether early RAST reporting could have led to escalation (switching to a broader-spectrum or more appropriate antibiotic when initial empiric therapy was ineffective) or de-escalation (narrowing from broad-spectrum to targeted therapy based on susceptibility results) [13]. The proportion of cases in which therapy modification could have occurred and the potential time saved compared with conventional VITEK 2 reporting were calculated.

The results of the EUCAST RAST method were compared to those obtained from the VITEK 2 system, which served as the gold standard. CA, VME; false susceptible, ME; false resistant, and mE; intermediate discrepancies were calculated according to standard definitions. CA is considered when the interpretive category (susceptible, intermediate, or resistant) is the same for a particular drug-bug combination by both methodologies. Results that did not match were further categorised as VME, ME and mE [14].

The VME is defined as false susceptible, i.e., resistant by VITEK 2, which was falsely categorised as susceptible by RAST. ME is falsely resistant, i.e., susceptible by VITEK 2, and was falsely categorised as resistant by RAST. The total number of resistant isolates and total susceptible isolates by VITEK 2 was taken as the denominator to calculate VME and ME rates, respectively. mE were defined as intermediate results by one method and susceptible or resistant results by another method [15].

The total number of drug-bug combinations tested, excluding those in the ATU category, was used as the denominator to calculate CA and the ME rates. As per EUCAST recommendations, results in the ATU category were not included in the final analysis. The result should meet the acceptable performance criteria of FDA and CLSI M52 guidelines (CA ≥90%, VME ≤1.5%, ME ≤3%) [16].

STATISTICAL ANALYSIS

The overall agreement between RAST and VITEK 2 results was assessed as percentage agreement or CA and error rate analysis to evaluate concordance between the methods.

RESULTS

A total of 1,109 blood culture samples were received. Out of these samples, 148 (13.3%) flagged positive. The EUCAST RAST method was applicable to 50 isolates, which included *Escherichia coli* 26 (52%), *Klebsiella pneumoniae* 17 (34%) and *Pseudomonas aeruginosa* 7(14%) [Table/Fig-1].

Total blood culture samples	1109
Positive blood culture samples	148 (13.3%)
Pathogens for which RAST was applicable	50
	E. coli - 26 (52%)
	K. pneumoniae - 17 (34%)
	P. aeruginosa - 7 (14%)

[Table/Fig-1]: Overview of positive blood cultures.

Four isolates of *P. aeruginosa* showed no growth after six hours of incubation and were subsequently analysed at eight hours. A total of 356 drug-bug combinations were tested for 26 *E. coli*, 17 *K. pneumoniae*, and 3 *P. aeruginosa* isolates at the 6-hour time point, excluding those in the ATU.

Categorical Agreement (CA) and Errors

The CA was 94.5% for *E. coli*, 95.45% for *P. aeruginosa*, and 88.06% for *K. pneumoniae* [Table/Fig-2]. For CA calculation, drug-bug combinations classified as ATU were excluded, resulting in denominators of 200 for *E. coli*, 134 for *K. pneumoniae*, and 22 for *P. aeruginosa*.

Organisms and antibiotic tested (n*Ab = N)	Categorical agreement n (%)	Categorical disagreement, n (%)		
		Very Major Error (VME) (VME) %	Major Error (ME) %	Minor error (mE) %
Escherichia coli (26*8 =208)	94.5% (189/200)	1.26% (1/79)	3.30% (4/121)	3% (6/200)
Klebsiella pneumoniae (17*8 =136)	88.06% (118/134)	1.01% (1/99)	4.54% (1/22)	10.44% (14/134)
Pseudomonas aeruginosa (3*8 = 24)	95.45% (21/22)	0 (0/1)	4.7% (1/21)	0 (0/22)
Overall CA	92.13% (328/356)	1.11% (2/179)	3.61% (6/166)	5.61% (20/356)

[Table/Fig-2]: Comparison of result of RAST methodology and standard methodology (Vitek 2 compact system) for gram negative isolates.
n: Number of isolates (Bug); Ab: Number of antibiotics tested (drug); N: Total drug–bug combinations

The overall CA across all tested isolates was 92.13%. A good CA (≥90) was observed for imipenem, meropenem, gentamicin and amikacin in *E. coli* and for imipenem, meropenem, piperacillin-tazobactam, and gentamicin in *K. pneumoniae* [Table/Fig-3,4].

Antibiotics	Categorical agreement, n (%)	Categorical disagreement, n (%)		
		Very Major Error (VME)	Major Error (ME)	Minor Error (mE)
MRP	92% (23/25)	0	0	8% (2/25)
IPM	96% (24/25)	0	0	4% (1/25)
PIT	88% (22/25)	0	17.64% (3/17)	0
CTX	100% (24/24)	0	0	0
AK	92% (23/25)	0	4.16% (1/24)	4% (1/25)
GEN	96.15% (25/26)	16.67% (1/6)	0	0
CIP	91.66% (22/24)	0	0	8.33% (2/24)
COT	100% (26/26)	0	0	0
Overall	94.5% (189/200)	1.26% (1/79)	3.30% (4/121)	3% (6/200)

[Table/Fig-3]: Comparison of RAST methodology results with standard methodology for *E. coli*.
MRP: Meropenem; CTX: Cefotaxime; PIT: Piperacillin-Tazobactam; IPM: Imipenem; CIP: Ciprofloxacin; AK: Amikacin; GEN: Gentamicin; and COT: Trimethoprim-sulphamethoxazole

Antibiotics	Categorical agreement, n (%)	Categorical disagreement, n (%)		
		Very Major Error (VME)	Major error (ME)	Minor error (mE)
MRP	94.11% (16/17)	7.69 % (1/13)	0	0
IPM	93.75% (15/16)	0	0	6.25% (1/16)
PIT	94.11% (16/17)	0	50 % (1/2)	0
CTX	100% (17/17)	0	0	0
AK	31.25% (5/16)	0	0	68.75% (11/16)
GEN	94.11% (16/17)	0	0	5.88% (1/17)
CIP	94.11% (16/17)	0	0	5.88% (1/17)
COT	100% (17/17)	0	0	0
Overall	88.06% (118/134)	1.01% (1/99)	4.54% (1/22)	10.44% (14/134)

[Table/Fig-4]: Comparison of RAST methodology results with standard methodology for *K. pneumoniae*.

A single VME was observed in the *K. pneumoniae*-meropenem combination and *E. coli*-gentamicin combination [Table/Fig-5]. ME errors were most notable in piperacillin-tazobactam (*E. coli* and *K. pneumoniae*). mE were predominantly observed with amikacin in *K. pneumoniae*.

Isolates	Very Major Error (VME) at 6h	Major Error (ME) at 6h	Minor Error (mE) at 6h
E.coli (26)	1 (GEN)	3 (PIT), 1 (AK)	2 (MRP), 1 (IPM), 2 (CIP), 1 (AK)
K. pneumoniae (17)	1 (MRP)	1 (PIT)	1 (IPM), 1 (CIP), 11 (AK), 1 (GEN)
P. aeruginosa (3)	0	1 (PIT)	0
Total	2	6	20

[Table/Fig-5]: Overall evaluation of various errors by RAST methodology for different pathogens.

Antibiotic Therapy Implications

Antibiotic therapy administered was analysed to assess the potential clinical impact of RAST in guiding early antibiotic escalation. Among 37 patients, 14 required escalation (switching to a broader-spectrum or more appropriate antibiotic when initial empiric therapy was ineffective), while 23 continued with empirical therapy [Table/Fig-6].

It was observed that RAST results at six hours were categorically concordant in 86% of cases requiring escalation, indicating that RAST could have facilitated early treatment adjustments. The majority of empirical antibiotic regimens (20/23 cases) were appropriately continued based on RAST findings at six hours.

Pathogen	No. of patients	Escalation		Continuation of the empirical antibiotic	
E. coli	22	6		16	
		4 CA	2 ME	14 CA	1 ME 1 mE
K. pneumoniae	15	8		7	
		8 CA	-	6 CA	1 ME
Total	37	14		23	
		12 CA	2 ME	20 CA	2ME 1 mE

[Table/Fig-6]: Impact of RAST on antibiotic therapy.
Abbreviations- CA: Categorical agreement; ME: Major error; mE: minor error

DISCUSSION

The data from this study indicate that early reading and reporting of antimicrobial susceptibility tests using RAST breakpoints by EUCAST can provide clinically relevant susceptibility results for BSI-related bacteria within six hours, significantly benefiting BSI patients. The overall CA and error rates adhered to the acceptable standards set by the FDA and CLSI M52 guidelines, i.e., CA ≥ 90%, ME ≤ 3% and VME ≤ 1.5% [16,17].

EUCAST RAST was applied to only 50 isolates (out of 148 positive cultures) because the method is validated primarily for certain Gram-negative bacteria (*E. coli, K. pneumoniae*, and *P. aeruginosa*) that are clinically relevant and meet the growth and inoculum requirements for early reading at 6-8 hours. Isolates of other species, mixed cultures, or those with insufficient growth at the early time point were excluded from RAST analysis. Six Gram-positive Coagulase Negative *Staphylococcus* (CoNS) species isolates were identified, but due to the lack of EUCAST RAST breakpoints, they were not included in the final analysis.

Four isolates of *P. aeruginosa* showed no growth after six hours of incubation and were subsequently analysed at eight hours. This finding highlights a potential limitation of early reading for certain strains of *P. aeruginosa*, which may exhibit slower growth kinetics under standard RAST conditions. The delayed growth could be influenced by intrinsic strain characteristics, low initial bacterial inoculum, or the physiological state of the bacteria in BSIs. A total of 356 drug-bug combinations were tested for 26 *E. coli*, 17 *K. pneumoniae*, and 3 *P. aeruginosa* isolates at the 6-hour time point, excluding those in the ATU.

The CA for *K. pneumoniae* (88.06%) was lower than for *E. coli* (94.5%) and *P. aeruginosa* (95.45%), likely due to uneven early growth leading to less distinct inhibition zones, variable expression of resistance mechanisms such as Extended-Spectrum Beta-Lactamases (ESBLs) or carbapenemases by *Klebsiella* species, and inoculum-related effects. These factors can lead to less distinct inhibition zones at six hours, resulting in minor errors.

Several studies have evaluated the performance of the EUCAST RAST method across different settings and pathogens between 2020 and 2025. These studies provide a benchmark for interpreting our findings [Table/Fig-7] [8,18-21].

The present study demonstrated an overall CA of 92.13% across all tested isolates, with specific rates of 94.5% for *E. coli*, 88.06% for *K. pneumoniae*, and 95.45% for *P. aeruginosa*. These findings are consistent with the high CA rates reported in the aforementioned studies [8,18-21] reinforcing the reliability of the EUCAST RAST method across diverse clinical settings. The six hour reporting time was chosen as it was most feasible for our laboratory workflow and covered the maximum number of positive blood cultures.

Unlike most published studies on EUCAST RAST, which have utilised MALDI-TOF for bacterial identification [19,20], the current study was conducted in a resource-limited setting using in-house biochemical tests, later correlated with Vitek ID results the following day. Identification of isolates before AST in the absence

Study	Pathogens studied	Categorical Agreement (CA)	Very Major Errors (VME)	Major Errors (ME)	Minor Errors (mE)
Åkerlund A et al., 2020 [8]	Various (e.g., *E. coli*, *K. pneumoniae*)	≥90% at 4-6 hours	≤0.3-1.1%	≤1.9-2.8%	Not separately reported (included in total error)
Tays¸i MR et al., 2022 [18]	Various Gram-negative bacteria	>90% overall at 6 h	Very few (Not specified)	Few (Not specified)	Not specified
Shan Y et al., 2022 [19]	BSI-causing bacteria	≥90% at 8 hours	1.5%	~1.1%	~0.2–0.7%
Cherkaoui A et al., 2022 [20]	Various pathogens	>95% at key time points	Low to few VMEs	Low	Low
Strubbe G et al., 2023 [21]	Various pathogens in low multidrug-resistant (MDR) prevalence settings	96.2% (6 h); 96.6% (8 h)	Not specified	Not specified	Not specified
Choudhari S et al., (2026)	*E. coli, K. pneumoniae, P. aeruginosa*	Overall CA 92.13% at 6 hours, *E. coli* 94.5%, *K. pneumoniae* 88.06%, *P. aeruginosa* 95.45%	1.11%	3.61%	5.61%

[Table/Fig-7]: Comparison of Categorical Agreement (CA) and error rates of rapid AST methods across different studies [8,18-21].

of automation remains a challenge in such settings. However, previous studies have demonstrated that bacterial identification can be performed at an early stage using a direct blood-broth mixture or pellets obtained after centrifugation, with acceptable accuracy [18,22]. In the current study's setting, Gram-negative pathogens were the predominant cause of sepsis, aligning with findings from other Indian studies [23-25]. Due to the limited number of Gram-positive isolates recovered and the absence of EUCAST breakpoints for coagulase-negative *Staphylococcus* species, Gram-positive bacteria were excluded from analysis in the present study.

The rates of major and minor errors were slightly higher for *K. pneumoniae*, with the highest minor error rates for amikacin. Notably, all amikacin-resistant isolates by the RAST method were categorised as intermediate by the VITEK 2 system, likely due to the thick mucoid growth with poorly demarcated zone edges on disk diffusion when using direct blood broth as inoculum [8,19]. This finding suggests potential difficulties in determining amikacin susceptibility and necessitates cautious interpretation. Similarly, Chandrasekaran S et al., reported high minor error rates (26.7%) for amikacin at six hours [14].

Another ME was observed for piperacillin-tazobactam, likely due to incomplete diffusion of the antimicrobial agent through agar, as beta-lactams require porin-mediated translocation to act on the bacterial cell wall. The presence of blood components in the inoculum may have interfered with this process [14].

A single VME was noted for the *K. pneumoniae*-meropenem combination during the early phase of the study, potentially due to technical inconsistencies or drug diffusion dynamics in agar. A similar finding was reported by Ekwall-Larson A et al., who attributed this discrepancy to isolates with minimum inhibitory concentration close to the susceptibility breakpoint, where a minor error in testing condition or interpretation may result in misclassification [26].

Antibiotic therapy implications were analysed retrospectively for 37 patients with BSI caused by *Escherichia coli* and *K. pneumoniae.* Among these, 14 patients required antibiotic escalation after VITEK 2 results, while 23 patients continued on empirical therapy. Of the 14 escalated cases, 12 showed CA between RAST and Vitek results, suggesting that 86% of escalations could have been advised on the same day using RAST. These findings highlight that RAST is more effective for guiding escalation rather than de-escalation, particularly in cases of multidrug-resistant organisms. Cardot Martin E et al., found that RAST led to multiple therapy escalations and de-escalations compared with conventional AST, and Berinson B et al., found that RAST allowed for rapid antibiotic escalation [27,28]. By significantly reducing the TAT for positive blood cultures, RAST facilitates early antibiotic adjustments, potentially improving patient outcomes.

Limitation(s)

RAST is a simple, cost-effective test that does not require expensive instrumentation and can be integrated into conventional laboratory setups. However, certain limitations exist, particularly identifying bacterial isolates before AST interpretation in the absence of automation, inapplicability to polymicrobial infections and slow-growing organisms, limited availability of breakpoints for certain drug-bug combinations and less distinct zone diameters prone to errors (e.g., absence of 4-hour breakpoints for *Pseudomonas aeruginosa*). This study was constrained by a low number of isolates. In addition, the impact of RAST on antibiotic escalation and de-escalation was assessed through a post hoc review of antimicrobial therapy rather than a real-time assessment of clinical decision-making. Despite these limitations, the findings of current study confirm that RAST, when performed according to EUCAST guidelines, is a reliable and implementable method in routine laboratory workflows with minimal clinically relevant errors.

CONCLUSION(S)

EUCAST RAST can significantly reduce the TAT for AST reporting while maintaining a high level of CA with the VITEK 2 system and can be implemented in routine laboratory workflow with fewer clinically relevant errors. The test is rapid, cost-effective, validated and standardised by EUCAST and can be effectively implemented in conventional and resource-limited laboratory settings where access to advanced molecular diagnostics is limited. Earlier availability of susceptibility results supports timely escalation of appropriate therapy, particularly in immunocompromised cancer patients with bacteraemia. Importantly, integration of RAST into clinical workflows can strengthen antimicrobial stewardship efforts by promoting earlier optimisation of therapy and reducing unnecessary broad-spectrum antibiotic use. These findings underscore its value as a practical tool for improving early antimicrobial decision-making. Further studies with larger sample sizes and prospective clinical outcome measurements are required to validate these findings and optimise RAST-based workflows in routine clinical practice.

Authors' contribution: SC: Conceptualisation, supervision, reviewing and editing of the final manuscript; PB: Conducting the study, data entry, data analysis and drafting the original manuscript; JW: Procurement, supervision, reviewing and editing; RG and EF: Data entry and review. All authors contributed equally to the study and approved the final manuscript.

Availability of data and materials: The data generated and analysed during this study will be made available upon reasonable request from the corresponding author.

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REFERENCES

[1] Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al.; International Forum of Acute Care Trialists. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. Am J Respir Crit Care Med. 2016;193(3):259-72. Doi: 10.1164/rccm.201504-0781OC.

[2] Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med. 2002;28(2):108-21. Doi: 10.1007/s00134-001-1143-z.

[3] Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34(6):1589-96. Doi: 10.1097/01.CCM.0000217961.75225.E9.

[4] Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J Clin Microbiol. 1999;37(5):1415-18. Doi: 10.1128/JCM.37.5.1415-1418.1999.

[5] Klouche M, Schröder U. Rapid methods for diagnosis of bloodstream infections. Clin Chem Lab Med. 2008;46(7):888-908.

[6] Gupta A, Siddiqui F, Saxena B, Purwar S, Saigal S, Sharma JP, et al. A prospective study evaluating the effect of a diagnostic stewardship care-bundle for automated blood culture diagnostics. J Glob Antimicrob Resist. 2023;34:119-26. Doi: 10.1016/j.jgar.2023.07.001.

[7] Kavipriya D, Prakash SS, Dhandapani S, Rajshekar D, Sastry AS. Evaluation of the performance of direct susceptibility test by VITEK-2 from positively flagged blood culture broth for gram-negative bacilli. J Lab Physicians. 2021;13(4):374-79. Doi: 10.1055/s-0041-1732489.

[8] Åkerlund A, Jonasson E, Matuschek E, Serrander L, Sundqvist M, Kahlmeter G; RAST Study Group. EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: Validation in 55 European laboratories. J Antimicrob Chemother. 2020;75(11):3230-38. Doi: 10.1093/jac/dkaa333. PMID: 32789506; PMCID: PMC7566356.

[9] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Implementation of the EUCAST method for rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles using designated breakpoints. Version 2.0, April 2022.

[10] European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST methodology: Rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles. Version 2.0, January 2022.

[11] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Zone diameter breakpoints for rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles. Version 4.0. Basel: EUCAST; 2022.

[12] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne, PA: CLSI; 2022.

[13] Tabah A, Cotta MO, Garnacho-Montero J, Schouten J, Roberts JA, Lipman J, et al. A systematic review of the definitions, determinants, and clinical outcomes of antimicrobial de-escalation in the intensive care unit. Clin Infect Dis. 2016;62(8):1009-17.

[14] 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. J Clin Microbiol. 2018;56(3):e01678-17. Doi: 10.1128/JCM.01678-17.

[15] Clark RB, Lewinski MA, Loeffelholz MJ, Tibbetts RJ. Cumitech 31A: Verification and validation of procedures in clinical microbiology laboratory. In: Sharp SE, editor. Washington, DC: ASM Press; 2009.

[16] Clinical and Laboratory Standards Institute. Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems; Approved Standard. CLSI document M52:2010(R2020). Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

[17] U.S. Food and Drug Administration. Antimicrobial susceptibility test (AST) systems-Class II special controls guidance for industry and FDA staff. Silver Spring (MD): U.S. Food and Drug Administration; 2019.

[18] Tayşi MR, Şentürk GÇ, Çalişkan E, Öcal D, Miroglu G, Şencan İ. Implementation of the EUCAST rapid antimicrobial susceptibility test (RAST) directly from positive blood culture bottles without the advanced identification systems. J Antimicrob Chemother. 2022;77(4):1020-26. Doi: 10.1093/jac/dkac003.

[19] Shan Y, Hu B, Guo W, Wang B, Zhou C, Huang S, et al. Evaluation of the EUCAST rapid antimicrobial susceptibility test for Enterobacterales-containing blood cultures in China. J Clin Microbiol. 2022;60(4):e0255921. Doi: 10.1128/jcm.02559-21.

[20] Cherkaoui A, Schorderet D, Azam N, Crudeli L, Fernandez J, Renzi G, et al. Fully automated EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) from positive blood cultures: Diagnostic accuracy and implementation. J Clin Microbiol. 202260:e00898-22.https://doi.org/10.1128/jcm.00898-22.

[21] Strubbe G, Messiaen AS, Vandendriessche S, Verhasselt B, Boelens J. EUCAST rapid antimicrobial susceptibility testing (RAST) compared to conventional susceptibility testing: Implementation and potential added value in a tertiary hospital in Belgium. Acta Clin Belg. 2023;78(5):385-91. Doi: 10.1080/17843286.2023.2197314.

[22] Höring S, Massarani AS, Löffler B, Rödel J. Rapid antibiotic susceptibility testing in blood culture diagnostics performed by direct inoculation using the VITEK®-2 and BD Phoenix™ platforms. Eur J Clin Microbiol Infect Dis. 2019;38(3):471-78. Doi: 10.1007/s10096-018-03445-3.

[23] Chatterjee S, Bhattacharya M, Todi SK. Epidemiology of adult-population sepsis in India: A single center 5 year experience. Indian J Crit Care Med. 2017;21(9):573-77. Doi: 10.4103/ijccm.IJCCM_240_17.

[24] Agrawal R, Ranjan KP. Bacteriological profile of sepsis and their antibiotic susceptibility pattern in adult patients in a tertiary care hospital of Madhya Pradesh, India. Natl J Med Res. 2019;9(2):65-69.

[25] Jain P, Galiya A, Luke Philip S, Mateti UV, P S S, Gudi SK, et al. Bacteriological profile and antimicrobial resistance pattern among patients with sepsis: A retrospective cohort study. Int J Clin Pract. 2021;75(10):e14701. Doi: 10.1111/ijcp.14701.

[26] Ekwall-Larson A, Fröding I, Mert B, Åkerlund A, Özenci V. Analytical performance and potential clinical utility of EUCAST rapid antimicrobial susceptibility testing in blood cultures after four hours of incubation. Microbiol Spectr. 2023;11(2):e0500122. Doi: 10.1128/spectrum.05001-22. Epub 2023 Feb 21. PMID: 36809027; PMCID: PMC10100889.

[27] Cardot Martin E, Colombier MA, Limousin L, Daude O, Izarn O, Cahen P, et al. Impact of EUCAST rapid antimicrobial susceptibility testing (RAST) on management of Gram-negative bloodstream infection. Infect Dis Now. 2022;52(8):421-25. Doi: 10.1016/j.idnow.2022.09.002.

[28] Berinson B, Olearo F, Both A, Brossmann N, Christner M, Aepfelbacher M, et al. EUCAST rapid antimicrobial susceptibility testing (RAST): Analytical performance and impact on patient management. J Antimicrob Chemother. 2021;76(5):1332-38. Doi: 10.1093/jac/dkab026.

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