European Committee on Antimicrobial Susceptibility Testing (EUCAST) rapid antimicrobial susceptibility testing (RAST) directly from positive blood cultures in a Brazilian regional public hospital

Teste rápido de sensibilidade aos antimicrobianos EUCAST RAST direto do frasco de hemocultura positiva em um hospital público regional

Valéria Martins Soares[1], Aléxia Soares Varella[2], Diana Manzi Viegas[3]

[1,3] Fundação Hospitalar do Estado de Minas Gerais/Hospital Júlia Kubitschek, Setor de Microbiologia/Laboratório. Belo Horizonte, MG, Brazil.

[2] Universidade Federal de São João del-Rei, Campus Centro-Oeste, Curso de Medicina. Divinópolis, MG, Brazil.

Abstract

Since 2022 EUCAST has developed a method for rapid antimicrobial susceptibility testing directly from positive blood culture bottles (RAST) for extended incubation time. The objective of this study was to evaluate the performance of the test in blood cultures positive for *Escherichia coli* and *Klebsiella pneumoniae*, after 16 to 20 hours of incubation, by comparison with the conventional antimicrobial test that was performed. The study demonstrated that RAST can be useful in the routine of a microbiology laboratory with little access to automation, reducing the time to obtain the result by 1 day.

Keywords: Anti-Bacterial Agents. *Escherichia coli*. *Klebsiella pneumoniae*. Microbial Sensitivity Tests

Resumo

A partir de 2022, o EUCAST validou o teste rápido de sensibilidade aos antimicrobianos diretamente do frasco de hemocultura positiva (RAST) para tempo de incubação estendido. O objetivo deste estudo foi avaliar o desempenho do teste em hemoculturas positivas para *Escherichia coli* e *Klebsiella pneumoniae,* após 16-20 horas de incubação, por meio de comparação com o antibiograma convencional realizado. O estudo demonstrou que o RAST pode ser útil na rotina de um laboratório de microbiologia com pouco acesso à automação, diminuindo em 1 dia o tempo para obtenção do resultado do antibiograma.

Palavras-chave: Antibacterianos. *Escherichia coli*. *Klebsiella pneumoniae*. Testes de Sensibilidade Microbiana

Correspondence
Valéria Martins Soares
E-mail: valeria-msoares@hotmail.com

Received on 03/26/2024 | Approved on 04/26/2024 | DOI: 10.21877/2448-3877.202400175.en

INTRODUCTION

Clinical microbiology laboratories play a critical role in diagnosing bloodstream infections (BSIs). The choice of antimicrobial therapy relies on the results of in vitro antimicrobial susceptibility testing (AST) conducted after bacterial growth in routine cultures.[1] Faster methods can reduce the duration of empirical therapy and facilitate the timely initiation of targeted therapy with antimicrobials shown to be sensitive in vitro. This approach helps prevent the emergence and spread of resistant strains, reduces hospital costs, and lowers mortality rates.[2,3]

Disk diffusion is a classic and widely used method for performing AST.[4] It is simple, cost-effective, and reproducible, allowing for the simultaneous testing of multiple antibiotics.[2] However, AST requires a defined inoculum from a pure culture, overnight incubation,[5]and at least two days to provide results after a positive blood culture result.[2]

In November 2018, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized rapid antimicrobial susceptibility testing (RAST), which can be performed directly from positive blood cultures with readings taken after 4, 6, and 8 hours of incubation.[6] Initially, EUCAST RAST was restricted to microbiology laboratories capable of identifying bacteria within that timeframe, as interpretation depends on the bacterial species. In 2022, EUCAST RASTwasfurther validated for 16 to 20 hours of incubation with specific reading and interpretation criteria to address situations in which shorter incubation times are not feasible due to logistics and limitations of microbiology laboratories.[6,7]

The aim of the present study was to evaluate the performance of EUCAST RAST in routine laboratory practice by analyzing positive blood culture samples of *Escherichia coli* and *Klebsiella pneumoniae* after 16 to 20 hours of incubation. Findings were compared to conventional antibiograms obtained from bacterial growth on solid media.

MATERIALS AND METHODS

The study was conducted in the microbiology laboratory at Hospital Júlia Kubitschek (HJK). HJK is a public general hospital with regional coverage and is part of the specialty complex managed byFundação Hospitalar do Estado de Minas Gerais (FHEMIG).

The aim of this descriptive, comparative study was to evaluate the performance of EUCAST RAST compared with disk diffusion (the standard method) used in routine laboratory practice. Positive blood culture samples of*E. coli* and *K. pneumoniae* were included. The protocol for the study was approved by the FHEMIG Human Research Ethics Committee under certificate of ethics review (CAAE) no. 74524523.6.0000.5119,and data collection began following approval.

Blood culture bottles (BD BACTEC™ Plus Aerobic, BD BACTEC™ Plus Anaerobic, and BD BACTEC™ Peds Plus; Beckton-Dickinson, USA) were incubated at 35°C in the BD BACTEC™ FX Blood Culture System (Beckton-Dickinson, USA). Bottles flagged as positive were removed and subjected to Gram staining. The results of positive cultures and Gram staining were reported to clinicians by the microbiology laboratory following the hospital's protocol.

EUCAST RAST was performed following the protocol,[6] with a modification in the volume used.[2] A total of 250 µL of liquid medium from positive blood cultures was streaked onto a 150 mm Mueller-Hinton agar plate (Plastlabor, Rio de Janeiro, Brazil) to ensure uniform inoculum distribution. Antimicrobial disks of amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), co-trimoxazole (23.75/1.25 µg), ciprofloxacin (5 µg), piperacillin-tazobactam (30/6 µg), and cefotaxime (5 µg)were applied. Plates were incubated at 35±1°C.

Since bacterial identification is required prior to RAST reading due to species-specific interpretation, the liquid medium from positive blood cultures was also inoculated onto CHROMagar™ Orientation, a nonselective chromogenic culture medium (Plastlabor, Rio de Janeiro, Brazil).

After 16 to 20 hours of incubation, if the chromogenic medium indicated the growth of *E. coli* or *K. pneumoniae*, RAST plates were read visually by microbiologists who measured the inhibition zones. Chromogenic medium plates were also used as the primary medium for performing traditional biochemical tests for bacterial identification and for conducting the antibiogram using the standard method, following the annual guidelines by the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST).[8]

The level of agreement was assessed using the kappa (κ) coefficient with the aid of the online tool available from https://epitools.ausvet.com.au/comparetwotests. Errors were classified as very major (standard method resistant and RAST susceptible), major (standard method susceptible and RAST resistant), or minor (reported as susceptible, increased exposure by one method but susceptible or resistant by another). Some inhibition zone diameters fell within the

area of technical uncertainty (ATU) and were therefore excluded from analyses.

Tests were validated based on criteria established by the U.S. Food and Drug Administration (FDA), which require ≥90% agreement, very major errors rates of ≤1.5%, major errors rates of ≤3.0%, and minor errors rates of <10%.[2]

Quality control of antimicrobial disks and culture media was performed according to the annual BrCAST guidelines, using a 0.5 McFarland suspension with 16 to 20 hours of incubation at 35±1°C.[8]

To calibrate and validate the RAST protocol, quality control was performed on consecutive days by inoculating 1 mL of a bacterial suspension containing 100 to 200 colony-forming units (CFUs) (0.5 McFarland suspension diluted 1:1,000,000) of *E. coli* ATCC 25922 into a blood culture bottle with 5 mL of sterile blood. Inoculated vials were incubated in the instrument and processed using the RAST method once flagged as positive.[6,9]

RESULTS

Between August 2023 and January 2024, 47 positive blood culture samples containing Gram-negative bacilli were identified. Fourteen samples were excluded from analyses due to the isolation of other *Enterobacterales* (6/14), nonfermenting Gram-negative bacilli (6/14), or mixed cultures (2/14). A total of 33 samples were included in the study, of which 22 (66.7%) were identified as *E. coli* and 11 (33.3%) as *K. pneumoniae*.

Amikacin and piperacillin-tazobactam demonstrated the lowest κ agreement and the highest rates of very major errors (6.1% and 6.2%, respectively) compared to the standard method. Another very major error was observed for gentamicin (3%). Regarding meropenem, only one minor error was identified, involving a strain classified as sensitive with increased exposure by the standard method but resistant by RAST. No errors were detected for the other antimicrobials tested.

Table 1

Comparison between RAST with reading at 16 to 20 hours and the standard method.

Antimicrobial (n)	Method	Sensitivity profile			ATU	Kappa	Number (%) of		
		S	I	R			VME	ME	mE
Amikacin (n=33)	RAST	28	0	5	0	0.798	2 (6.1%)	0	0
	SM	26	0	7	0				
Imipenem (n=33)	RAST	29	0	4	0	1.000	0	0	0
	SM	29	0	4	0				
Meropenem (n=33)	RAST	29	0	4	0	0.86	0	0	1 (3%)
	SM	29	1	3	0				
Co-trimoxazole (n=33)	RAST	17	0	16	0	1.000	0	0	0
	SM	17	0	16	0				
Ciprofloxacin (n=28)	RAST	18	0	10	5	1.000	0	0	0
	SM	18	0	10	0				
Cefotaxime (n = 32)	RAST	18	0	14	1	1.000	0	0	0
	SM	18	0	14	0				
Piperacillin-tazobactam (n = 32)	RAST	27	0	5	1	0.796	2 (6.2%)	0	0
	SM	25	0	7	0				
Gentamicin (n=33)	RAST	27	0	6	0	0.904	0	0	0
	SM	26	0	7	0				

ATU = area of technical uncertainty; I = susceptible with increased exposure; ME = major error; mE = minor error; R = resistant; S = susceptible; VME = very major error.

DISCUSSION

Conventional disk diffusion antibiograms require two days of work after a positive blood culture result. New methods are needed to reduce the time needed to obtain reliable microbiological results for Gram-negative BSIs. Rapid escalation of therapy is critical for treating infections caused by resistant bacteria. Conversely, timely de-escalation can enable the use of narrow-spectrum antimicrobials, thereby reducing the risk of developing resistance by minimizing selective pressure on the microbiota.[1]

If performed directly from positive blood culture bottles, EUCAST RAST has the potential to significantly reduce the time required to obtain AST results. Standardization of an extended reading time to 16 to 20 hours of incubation enabled the use of a chromogenic medium for the presumptive identification of *E. coli* and *K. pneumoniae*, allowing RAST readings based on inhibition zone values specific to each bacterium.

With regard to imipenem, findings were consistent with previous research[7] and showed absolute agreement in 38 samples after 16 to 20 hours of incubation. For meropenem, only one minor error was identified: RAST classified the strain as resistant while the standard method classified it as sensitive, increasing exposure. This occurred in a *K. pneumoniae* strain producing *Klebsiella pneumoniae* carbapenemase (KPC). The detection of resistance to imipenem and meropenem by RAST also allowed KPC detection using the NG-Test CARBA 5 chromatographic assay (NG-Biotech, France) from growth on Mueller-Hinton agar or chromogenic medium. Among the other three concordant strains, two were identified as KPC producers, and one was a KPC + New Delhi metallo-beta-lactamase (NDM)co-producer. These results suggest that RAST may be a valuable tool for guiding antimicrobial escalation.[5]

Some research has reported major errors in 16 out of 31 samples for piperacillin-tazobactam, but under shorter incubation times.[10] Bianco *et al*. suggest that extending the reading time to 16 to 20 hours improves test performance by increasing agreement, reducing very major errors, and lowering the percentage of results within the ATU. Herein, two very major errors (6.2%) were found for piperacillin-tazobactam, and the test for this antimicrobial did not meet the validation criteria used.

With respect to amikacin, two very major errors (6.1%) were identified. However, other research reported no very major or major errors for amikacin when the reading time was extended to 16 to 20 hours.[7]For gentamicin, despite a very good agreement (κ = 0.904), one very major error (3%) was observed. No errors were detected for the other antimicrobials assessed; however, RAST results could not be interpreted for ciprofloxacin in five strains as they fell within the ATU.

One factor that may lead to discordant results is the lack of a controlled inoculum in positive blood culture bottles, unlike traditional AST, which standardizes the bacterial inoculum at 0.5 on the McFarland scale.[2]Additional factors, such as the quality of the culture medium and technical issues during method execution, may also contribute. Nevertheless, the results of this study indicate that RAST is comparable to the standard method for testing susceptibility to imipenem, meropenem, co-trimoxazole, ciprofloxacin, and cefotaxime.

One limitation of this study was the small number of resistant strains, particularly for carbapenems (4/33). However, additional samples will be included as the test undergoes validation for routine use. Furthermore, future studies could be conducted to assess the clinical impact of implementing RAST.

CONCLUSION

This study demonstrated that RAST is a valuable tool for microbiology laboratories with limited access to automation, thereby reducing the time required to obtain AST on*E. coli* and *K. pneumoniae* by one day in a large hospital setting.However, further studies with larger sample sizes are neededto validate results for antimicrobials that showed very major errors.

FINANCIAL SUPPORT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

This research was conducted with the support of the staff of the HJK microbiology laboratory and FHEMIG.

REFERENCES

1. Anton-Vazquez V, Suarez C, Planche T. Impact of rapid susceptibility testing on antimicrobial therapy and clinical outcomes in Gram-negative bloodstream infections. J AntimicrobChemother. 2022 Feb;77(3):771-781. doi: 10.1093/jac/dkab449.
2. Martins A, Wink P, Pereira D, Souza A, Aquino V, Barth A. Rapid antimicrobial susceptibility of Enterobacteriaceae by disk diffusion directly from blood culture bottles using the EUCAST RAST breakpoints. J Glob Antimicrob Resist. 2020 Sep:22:637-642. doi: 10.1016/j.jgar.2020.05.015.

3. 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 Apr;60(4):e0255921. doi: 10.1128/jcm.02559-21.
4. Song D, Lei Y. Mini-review: Recent advances in imaging-based rapid antibiotic susceptibility testing. Sens Actuators Rep. 2021;3:100053. doi: 10.1016/j.snr.2021.100053.
5. 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 Apr;76(5):1332-1338. doi: 10.1093/jac/dkab026.
6. European Committee on Antimicrobial Susceptibility Testing. Methodology - EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles. Version 4.0 [Internet]. Basel: The European Committee on Antimicrobial Susceptibility Testing; 2023Apr 25 [cited 2024 02 01. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/RAST/2023/EUCAST_RAST_methodology_v4.0_final.pdf.
7. Bianco G, Lombardo D, Ricciardelli G, Boattini M, Comini S, Cavallo R, et al. Multicentre Evaluation of the EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) Extending Analysis to 16-20 Hours Reading Time. Antibiotics (Basel). 2022 Oct;11(10):1404. doi: 10.3390/antibiotics11101404.
8. Brazilian Committee on Antimicrobial Susceptibility Testing. Tabelas de pontos de corte para interpretação de CIMs e diâmetros de halos [Internet].Rio de Janeiro: Brazilian Committee on Antimicrobial Susceptibility Testing; 2024 Apr 13 [cited 2024 02 01 Available from: https://brcast.org.br/wp-content/uploads/2022/09/Tabela-pontos-de-corte-BrCAST-13-04-2024.pdf.
9. European Committee on Antimicrobial Susceptibility Testing.Quality control criteria for the implementation of the RAST method.Version 5.0 [Internet]. Basel: The European Committee on Antimicrobial Susceptibility Testing; 2022 Apr 12 [cited 2024 02 01. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/RAST/2022/EUCAST_RAST_QC_v_5.0_final.pdf.
10. 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 Oct;78(5):385-391. doi: 10.1080/17843286.2023.2197314.