

Contribution of rapid antimicrobial susceptibility testing from positive blood cultures to appropriate antibiotic treatment

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Abstract

Introduction: This study aimed to evaluate the efficiency of antibiotic disks that are used to identify microorganisms, and compare rapid antimicrobial susceptibility testing (RAST) with routinely used automated systems in terms of time and accuracy.

Methodology: Antibiotic disk zone diameter results were determined at the 4th, 6th, 8th, or 16th–20th hours in samples taken from blood culture bottles that were a positive for the presence of microorganisms, and conventional methods were used to provide the initial diagnosis at this stage.

Results: The laboratory received a total of 5,049 blood culture bottles within a period of 5.5 months. During this period, 2,170 blood culture bottles were positive for the presence of microorganisms. The maximum sample reading time for bacteria was 6–8 hours for Gram-negative bacteria, 16–20 hours for *Streptococcus pneumoniae*, and 8 hours for other Gram-positive bacteria. The time (hours) difference between RAST and the automated identification system was as follows: *Escherichia coli* (20.9 hours), *Klebsiella pneumoniae* (20.8 hours), *Acinetobacter baumannii* (29.7 hours), *Pseudomonas aeruginosa* (19.2 hours), *Staphylococcus aureus* (15.3 hours), *Enterococcus faecalis* (10.8 hours), *Enterococcus faecium* (14.5 hours), *S. pneumoniae* (37 hours). *S. pneumoniae* demonstrated the greatest time advantage among the isolates tested, yielding results up to 37 hours earlier with the RAST method.

Conclusions: Although all Gram-negative bacteria could be reported using the RAST method by the end of the 8th hour, a significant proportion of Gram-positive bacteria still required waiting until the 16th–20th hour.

Key words: RAST; EUCAST; sepsis.

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Introduction

Microorganisms are traditionally identified using various culture media (such as MacConkey agar, blood agar, and chocolate agar). Microbial cultures typically require 24–48 hours to grow on the media, after which bacterial colonies become macroscopically visible.

The growing bacterial colonies are examined both with the naked eye and with the help of a microscope, and the bacterial species is identified based on the characteristic morphological features of the microorganisms. The Gram staining technique is used to classify microorganisms as Gram-positive or Gram-negative, depending on the cell wall structure. Antimicrobial susceptibility tests (ASTs) are usually performed within 24–48 hours after the growth of microorganisms in the culture medium [1]. The European Committee for Antimicrobial Susceptibility Testing-Rapid Antimicrobial Susceptibility Testing (EUCAST-RAST) is performed in parallel with routine testing using blood culture bottles stained as Gram-negative bacilli, and Gram-positive bacilli or cocci [2]. The method is currently validated for bacteria that most commonly cause sepsis, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*,

Staphylococcus aureus, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Acinetobacter baumannii* [3]. The RAST disk diffusion method was developed to provide a standardized protocol for performing AST directly from positive blood culture bottles with specific zone diameters and reading times (4th, 6th, 8th, and 16th–20th hours) determined by EUCAST for each species [4]. Disk diffusion, the most widely used method for AST, is favored for its cost-effectiveness, methodological versatility, practical implementation, and rapid turnaround time. Additionally, it enables the detection of synergistic and antagonistic interactions, heterogeneous resistance patterns, and potential contamination [5]. EUCAST-RAST is a reliable method with the potential to improve the clinical management of sepsis patients by providing rapid phenotypic susceptibility data. Early detection enables the timely identification of resistant isolates, contributing significantly to successful antimicrobial stewardship programs. With this method, categorical agreement (CA), very major error (VMjE), major error (MjE), and minor error (MnE) values can also be evaluated at the 4th, 6th, and 8th hours [6]. This study

compared the EUCAST-RAST method and the VITEK 2 automated system in terms of time to result and categorical agreement, aiming to evaluate the feasibility and diagnostic accuracy of implementing the EUCAST-RAST method alongside conventional microbiological techniques across diverse laboratory settings.

Methodology

This study was approved by the Non-Interventional Scientific Research Ethics Committee of Trakya University Faculty of Medicine (approval number GOBAEK-2023/29).

Sample processing and inclusion criteria

The blood culture samples from the patients admitted to the hospital with a preliminary diagnosis of sepsis were examined for presence of microorganisms using the BACT/ALERT® 3D system (bioMérieux, Lyon, France). The samples that tested positive were stained using the Gram staining method and examined under a microscope. Samples with Gram-negative bacilli, Gram-negative cocci, or Gram-positive cocci morphology were included in the study. The samples in which microorganisms with different and mixed morphology were observed in the Gram-stained preparations were excluded from the study.

Primary inoculation and incubation

The selected samples were inoculated onto one 5% sheep blood agar plate (Vision, İstanbul, Türkiye) and one eosin methylene blue agar plate (Vision, İstanbul, Türkiye) immediately after Gram staining, using the streak plate method. Both media were then incubated for 24 hours at 35–37 °C.

Disk placement protocol

AST by the EUCAST-RAST method was performed with at least 120 µL sample from the bottles with positive signals. This sample was distributed on two Müller Hinton Agar media (Vision, İstanbul, Türkiye) in 90 mm Petri dishes. Antibiotic disks were placed on these media using two disk dispensers (Bioanalyse, Ankara, Türkiye) with appropriate antibiotic disk cartridges. These Petri dishes were then incubated in an incubator at 37 °C.

AST reading time points and identification procedure

The AST results were determined at the 4th, 6th, and 8th hours of incubation when the antimicrobial disk zone diameters became measurable, and the colonies grown at this time were identified based on the bacterial morphology observed following Gram staining.

Classical identification of microorganisms was based on the Gram staining results and macroscopic growth characteristics observed on Petri dishes. Gram-negative bacilli with macroscopically specific colony morphology, typical odor, and oxidase (+) were considered as *P. aeruginosa*. Those with oxidase (-), lactose (+), and colonies showing a green metallic sheen were classified as *E. coli*. Those forming lactose (-) colonies on Petri dishes in addition to the microscopic characteristics of Gram-negative cocci or coccobacilli, were identified as *A. baumannii*. The catalase test was applied to bacteria showing Gram-positive cocci morphology on Gram stain and growing on 5% sheep blood agar. The catalase (+) samples were then subjected to a latex coagulase test (Liofilchem, Teramo, Italy), and the catalase (+) and coagulase (-) samples were identified as coagulase negative staphylococci (CNS) and excluded from the study. Catalase (+) and coagulase (+) samples were classified as *S. aureus*. The L-pyrrolidonyl-β-naphthylamide (PYR) test (ADR, İstanbul, Türkiye) was performed when Gram-positive cocci were found to be catalase (-). Gram-positive cocci with catalase (-) and PYR (+) were classified as *Enterococcus* spp. The samples that were resistant to ampicillin were evaluated as *E. faecium*, and those that were susceptible to ampicillin were evaluated as *E. faecalis*. When the alpha-hemolytic colonies were observed on 5% sheep blood agar, a disk diffusion test with an optochin disk was performed, and the catalase-negative, PYR-negative, optochin-sensitive Gram-positive cocci were identified as *S. pneumoniae* [7].

Reporting schedule and routine workflow

The AST results, along with the results of classical identification, were provided to the clinicians at the end of the 4th, 6th, 8th, and 16th–20th hours. Thus, results obtained from blood culture bottles with a positive signal were routinely presented to clinicians within the first 4, 6, or 8 hours after the signal, rather than 48 hours later, thus providing early AST results and classical identification information of the microorganism. In addition, routine procedures in the laboratory (including subculturing from conventional blood culture bottles, species-level identification after 18–24 hours of incubation, and AST) continued without interruption.

Antibiotic selection and RAST panels

The antibiotic disks were selected following the recommendation of the Hospital Infection Control Committee based on the antibiotic resistance profiles in

the hospital, and among the antibiotics standardized by EUCAST for RAST. Piperacillin-tazobactam (TZP), ceftazidime (CAZ), meropenem (MEM), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), amikacin (AK) and imipenem (IPM) were used to determine antimicrobial resistance of the Gram-negative bacteria at 4th, 6th, and 8th hours. The antibiotics selected according to EUCAST-RAST breakpoint tables v. 5.0 (Bioanalyse, Ankara, Türkiye) were: TZP, CAZ, MEM, CIP, SXT, AK, and IPM for *E. coli* and *K. pneumoniae*; TZP, CAZ, MEM, CIP, AK, and IPM for *P. aeruginosa*; MEM, CIP, AK, and IPM for *A. baumannii*; cefoxitin (FOX) for *S. aureus*, ampicillin (AM), vancomycin (VA), and linezolid (LNZ) for *E. faecalis* and *E. faecium*; and AM and erythromycin (E) for *S. pneumoniae* [8]. When the blood culture bottles from patients diagnosed with sepsis gave a positive signal, the antimicrobial susceptibility results were determined by disk diffusion test at the 4th, 6th, 8th, and 16th–20th hours from the time of the signal, and classical identification was performed.

Statistical analysis

This study did not include a control group. Descriptive statistics for continuous variables were reported as mean, standard deviation, minimum, and maximum values; while categorical variables were expressed as number and percentage. Comparisons of continuous variables across categorical groups were performed using either one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, depending on the fulfillment of the parametric test assumptions. After the variance analysis, Duncan's multiple comparison test was applied to determine the differences between the groups. Pearson's or Spearman's correlation coefficient was calculated, according to the fulfillment of parametric test assumptions, to evaluate the relationship between continuous variables. Relationships between categorical variables were analyzed using the Chi square test. Diagnostic test statistics were calculated along with survival analysis. $p < 0.05$ was accepted as the statistical significance level, and all calculations were performed using the SPSS (version 21) statistical package program [9].

Table 1. Evaluation periods of antibiotics and disks used.

Microorganisms and antibiotics (Total number of disks used)	4 hours				6 hours				8 hours				16-20 hours			
	CA n/N, %	MjE n/N, %	VMjE n/N, %	MnE n/N, %	CA n/N, %	MjE n/N, %	VMjE n/N, %	MnE n/N, %	CA n/N, %	MjE n/N, %	VMjE n/N, %	MnE n/N, %	CA n/N, %	MjE n/N, %	VMjE n/N, %	MnE n/N, %
<i>E. coli</i> (595)	136/154, 88	1/154, 0,6	3/154, 2	14/154, 9	175/196, 89	6/196, 3	0/196, 0	15/196, 0	227/245, 93	2/245, 0,8	4/245, 2	12/245, 5	-	-	-	-
<i>K. pneumoniae</i> (1460)	431/490, 88	7/490, 1	7/490, 1	45/490, 9	505/529, 95	3/529, 0,5	0/529, 0	21/529, 4	392/441, 89	11/441, 2	10/441, 2	28/441, 6	-	-	-	-
<i>A. baumannii</i> (516)	108/114, 95	0/114, 0	2/114, 2	4/114, 4	242/258, 94	2/258, 0,8	4/258, 2	10/258, 4	137/144, 95	2/144, 1	0/144, 0	5/144, 3	-	-	-	-
<i>P. aeruginosa</i> (256)	0/0	0/0	0/0	0/0	88/102, 86	0/102, 0	2/102, 2	12/102, 12	133/154, 86	1/154, 0,6	2/154, 1	18/154, 12	-	-	-	-
TZP (423)	95/111, 86	0/111, 0	0/111, 0	16/111, 14	142/164, 87	1/164, 0,6	0/164, 0	21/164, 13	136/148, 92	3/148, 2	1/148, 0,7	8/148, 5	-	-	-	-
CAZ (337)	87/92, 95	0/92, 0	1/92, 1	4/92, 4	115/121, 95	4/121, 3	0/121, 0	2/121, 2	114/124, 92	0/124, 0	4/124, 3	6/124, 5	-	-	-	-
MEM (423)	105/111, 95	0/111, 0	0/111, 0	6/111, 5	158/164, 96	2/164, 1	0/164, 0	6/164, 4	132/148, 89	2/148, 1	0/148, 0	14/148, 9	-	-	-	-
CIP (422)	100/111, 90	0/111, 0	2/111, 2	9/111, 8	155/164, 95	0/164, 0	0/164, 0	9/164, 5	136/147, 93	0/147, 0	0/147, 0	11/147, 7	-	-	-	-
SXT (380)	102/111, 92	5/111, 5	3/111, 3	1/111, 0,9	140/147, 95	2/147, 1	0/147, 0	5/147, 3	109/122, 89	4/122, 3	7/122, 6	2/122, 2	-	-	-	-
AK (421)	91/111, 82	0/111, 0	4/111, 4	16/111, 14	153/162, 94	0/162, 0	6/162, 4	3/162, 2	141/148, 95	0/148, 0	6/148, 4	1/148, 0,6	-	-	-	-
IPM (421)	95/111, 86	3/111, 3	2/111, 2	11/111, 10	152/163, 93	0/163, 0	0/163, 0	11/163, 7	131/147, 89	0/147, 0	2/147, 1	14/147, 10	-	-	-	-
<i>S. aureus</i> (115)	6/6, 100	0/6, 0	0/6, 0	0/6, 0	15/15, 100	0/15, 0	0/15, 0	0/15, 0	81/83, 98	0/83, 0	2/83, 2	0/83, 0	11/11, 100	0/11, 0	0/11, 0	0/11, 0
FOX (115)	6/6, 100	0/6, 0	0/6, 0	0/6, 0	15/15, 100	0/15, 0	0/15, 0	0/15, 0	81/83, 98	0/83, 0	2/83, 2	0/83, 0	11/11, 100	0/11, 0	0/11, 0	0/11, 0
<i>E. faecalis</i> (132)	0/0	0/0	0/0	0/0	2/2, 100	0/2, 0	0/2, 0	0/2, 0	76/112, 68	9/112, 8	1/112, 0,8	26/112, 23	13/18, 72	0/18, 0	0/18, 0	5/18, 28
<i>E. faecium</i> (79)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	66/79, 84	1/79, 1	1/79, 1	11/79, 14	0/0	0/0	0/0	0/0
AM (75)	0/0	0/0	0/0	0/0	1/1, 100	0/1, 0	0/1, 0	0/1, 0	58/68, 85	9/68, 13	1/68, 1	0/68, 0	6/6, 100	0/6, 0	0/6, 0	0/6, 0
VA (61)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	20/55, 36	0/55, 0	0/55, 0	35/55, 60	0/6, 0	0/6, 0	0/6, 0	6/6, 100
LNZ (75)	0/0	0/0	0/0	0/0	1/1, 100	0/1, 0	0/1, 0	0/1, 0	64/68, 94	1/68, 1	1/68, 1	2/68, 3	6/6, 100	0/6, 0	0/6, 0	0/6, 0
<i>S. pneumoniae</i> (7)	-	-	-	-	-	-	-	-	-	-	-	-	7/7, 100	0/7, 0	0/7, 0	0/7, 0
AM (3)	-	-	-	-	-	-	-	-	-	-	-	-	3/3, 100	0/3, 0	0/3, 0	0/3, 0
E (4)	-	-	-	-	-	-	-	-	-	-	-	-	4/4, 100	0/4, 0	0/4, 0	0/4, 0

CA: categorical agreement; VMjE: very major error; MJE: major error; MnE: minor error; n/N: number of disks in the relevant category/total number of disks tested at the corresponding time point; TZP: piperacillin-tazobactam; CAZ: ceftazidime; MEM: meropenem; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; AK: amikacin; IPM: imipenem; AM: ampicillin; VA: vancomycin; LNZ: linezolid; E: erythromycin.

Results

The laboratory received a total of 5049 blood culture bottles within a period of 5.5 months, of which 2170 gave a positive signal. Among the blood culture samples with positive signals, 1200 (55%) were collected from male patients and 970 (45%) from female patients. Among all samples, 622 were analyzed using both the EUCAST-RAST method and VITEK 2 automated systems.

Table 1 presents the number of antibiotic disks evaluated at the 4th, 6th, and 8th hours based on the growth of Gram-negative bacteria, along with the total number of disks used for the identified microorganisms. The respective counts for the antibiotics were as follows: TZP, 423; CAZ, 337; MEM, 423; CIP, 422; SXT, 380; AK, 421; and IPM, 421. The total number of disks evaluated per microorganisms were 595 for *E. coli*, 1,460 for *K. pneumoniae*, 516 for *A. baumannii*, and 256 for *P. aeruginosa*.

The number of antibiotic disks evaluated based on microorganism growth at different reading times was as follows. A total of 154 disks for *E. coli*, 490 for *K. pneumoniae*, 114 for *A. baumannii*, and none for *P. aeruginosa* were assessed at the 4th hour. The number of evaluated disks increased to 196 for *E. coli*, 529 for *K. pneumoniae*, 258 for *A. baumannii*, and 102 for *P. aeruginosa* at the 6th hour. By the 8th hour, 245 disks for *E. coli*, 441 for *K. pneumoniae*, 144 for *A. baumannii*, and 154 for *P. aeruginosa* were evaluated.

The number of antibiotic disks evaluated at different reading times was as follows. A total of 111 disks were evaluated for TZP, MEM, CIP, SXT, AK, and IPM each; and 92 disks for CAZ at the 4th hour. By the 6th hour, the number of disks evaluated increased to 164 for TZP, MEM, and CIP; 163 for IPM; 162 for AK; 147 for SXT; and 121 for CAZ. The evaluated disk counts were 148 for TZP, MEM, and AK; 147 for CIP and IPM; 124 for CAZ; and 122 for SXT at the 8th hour.

The CA, MjE, VMjE, and MnE values derived from the comparison between the VITEK 2 automated system utilized in the laboratory and the EUCAST-RAST method were calculated for the respective bacteria and antimicrobials at the 4th, 6th, and 8th hours as follows. For *E. coli*, CA, MjE, VMjE, and MnE values were recorded as 4th hour (88-0.6-2-9), 6th hour (89-3-0-0), and 8th hour (93-0.8-2-5). In the case of *K. pneumoniae*, the values were 4th hour (88-1-1-9), 6th hour (95-0.5-0-4), and 8th hour (89-2-2-6). In the case of *A. baumannii*, the corresponding values were 4th hour (95-0-2-4), 6th hour (94-0.8-2-4), and 8th hour (95-1-0-3). For *P. aeruginosa*, the measurements were indeterminate (Ø) at the 4th hour, the remaining values

were: 6th hour (86-0-2-12) and 8th hour (86-0.6-1-12). In terms of antibiotics, the results were as follows: for TZP, 4th hour (86-0-0-14), 6th hour (87-0.6-0-13), and 8th hour (92-2-0.7-5); for CAZ, 4th hour (95-0-1-4), 6th hour (95-3-0-2), and 8th hour (92-0-3-5); for MEM, 4th hour (95-0-0-5), 6th hour (96-1-0-4), and 8th hour (89-1-0-9); for CIP, 4th hour (90-0-2-8), 6th hour (95-0-0-5), and 8th hour (93-0-0-7); for SXT, 4th hour (92-5-3-0.9), 6th hour (95-1-0-3), and 8th hour (89-3-6-2). In the case of AK, the values were 4th hour (82-0-4-14), 6th hour (94-0-4-2), and 8th hour (95-0-4-0.6). Finally, for IPM, the measurements were 4th hour (86-3-2-10), 6th hour (93-0-0-7), and 8th hour (89-0-1-10).

The total number of antimicrobial disks evaluated at the 4th, 6th, 8th, and 16th–20th hours for Gram-positive bacteria, along with the distribution of disks used for the identified microorganisms, were as follows: FOX 115, AM 78, VA 61, LNZ 75, and E 4; *S. aureus* 115, *E. faecalis* 132, *E. faecium* 79, and *S. pneumoniae* 7 (Table 1).

At the 4th hour, measurable zones were observed for 6 antibiotic disks used for *S. aureus*, while no zones were observed for *E. faecalis*, *E. faecium*, or *S. pneumoniae*. By the 6th hour, the number of measurable zones increased to 15 for *S. aureus* and 2 for *E. faecalis*, with none for *E. faecium* or *S. pneumoniae*. At the 8th hour, measurable zones were identified for 83 disks used for *S. aureus*, 112 for *E. faecalis*, 79 for *E. faecium*, and none for *S. pneumoniae*. Finally, at the 16th–20th hours, measurable inhibition zones were obtained for 11 disks evaluated for *S. aureus*, 18 for *E. faecalis*, none for *E. faecium*, and 7 for *S. pneumoniae*.

The number of antibiotic disks with measurable zones at different time points were as follows: at the 4th hour, 6 for FOX, and none for AM, VA, LNZ, or E; at the 6th hour, 15 for FOX, 1 each for AM and LNZ, and none for VA or E; at the 8th hour, 83 for FOX, 68 for AM and LNZ, 55 for VA, and none for E; and at the 16th–20th hours, 11 for FOX, 9 for AM, 6 each for VA and LNZ, and 4 for E.

The CA, MjE, VMjE, and MnE values derived from the comparison of the VITEK 2 automated system and the EUCAST-RAST method were determined for the relevant bacteria and antimicrobials at the 4th, 6th, 8th, and 16th–20th hours are listed below. The results for *S. aureus*, were as follows: 4th hour (100-0-0-0), 6th hour (100-0-0-0), 8th hour (98-0-0-2-0), and 16th–20th hour (100-0-0-0). The results for *E. faecalis* were indeterminate (Ø) at the 4th hour, followed by 6th hour (100-0-0-0), 8th hour (68-8-0.8-23), and 16th–20th hour (72-0-0-28). For *E. faecium*, the values were indeterminate (Ø) at the 4th, 6th, and 16th–20th hours; but

at the 8th hour, they were (84-1-1-14). The results for *S. pneumoniae* were indeterminate (Ø) at the 4th, 6th, and 8th hours, while at the 16th–20th hour, the values were (100-0-0-0). The results for FOX were 4th hour (100-0-0-0), 6th hour (100-0-0-0), 8th hour (98-0-2-0), and 16th–20th hour (100-0-0-0). The results for AM in *E. faecalis* and *E. faecium* were indeterminate (Ø) at the 4th hour, followed by 6th hour (100-0-0-0), 8th hour (85-13-1-0), and 16th–20th hour (100-0-0-0). In the case of *S. pneumoniae*, the values for AM were indeterminate (Ø) at the 4th, 6th, and 8th hours, and at the 16th–20th hour, the values were (100-0-0-0). In the case of VA, the values were indeterminate (Ø) at the 4th and 6th hours, followed by 8th hour (36-0-0-64) and 16th–20th hour (0-0-0-100). The results for LNZ were indeterminate (Ø) at the 4th hour, 6th hour (100-0-0-0), 8th hour (94-1-1-3), and 16th–20th hour (100-0-0-0). In the case of E, the values were indeterminate (Ø) at the 4th, 6th, and 8th hours, while at the 16th–20th hour, the value was (100-0-0-0).

Table 2 presents the percentage distribution (%) of antibiotic disks used for Gram-negative bacteria at the 4th, 6th, and 8th hour reading times. The percentages for each organism and antibiotic are as follows: *E. coli* (26-33-41), *K. pneumoniae* (34-36-30), *A. baumannii* (22-50-28), *P. aeruginosa* (0-40-60), TZP (26-39-35), CAZ (27-36-37), MEM (26-39-35), CIP (26-39-35), SXT (29-39-32), AK (26-38-35), and IPM (26-39-35). Statistically significant reading times according to *p* values were identified as the 8th hour for *E. coli*, the 6th hour for *K. pneumoniae*, the 6th hour for *A. baumannii*,

the 8th hour for *P. aeruginosa*, and the 6th–8th hours for TZP, CAZ, MEM, CIP, AK, and IPM, as well as the 6th hour for SXT.

Table 2 presents the percentage distribution (%) of antibiotic disks used for Gram-positive bacteria at the 4th, 6th, 8th, and 16th–20th hours. The corresponding percentages were as follows: *S. aureus* (5-13-72-10), *E. faecalis* (0-2-85-14), *E. faecium* (0-0-100-0), *S. pneumoniae* (0-0-0-100), AM (0-1-87-12), FOX (5-13-72-10), E (0-0-0-100), VA (0-0-90-10), and LNZ (0-1-91-8). Statistically significant reading times based on *p* values were identified as the 8th hour for *S. aureus*, *E. faecalis*, *E. faecium*, AM, FOX, VA, and LNZ; and the 16th–20th hour for *S. pneumoniae* and E.

In this study, the total number of isolates not validated in EUCAST-RAST and typed by VITEK 2 was 134. The numerical distribution of the 6 most common species among these isolates was: *Micrococcus* spp. 24, *Serratia marcescens* 16, *Proteus mirabilis* 14, *Enterobacter cloacae* 13, *Brucella* spp. 13, and *Klebsiella oxytoca* 11.

A total of 1,414 isolates were not validated by the EUCAST-RAST method and were also not typed by the VITEK 2 system. The distribution of these isolates was as follows: CNS, 798 isolates; mixed growth on Petri dishes, 238 isolates; mixed morphology observed directly, 127 isolates; yeast species, 97 isolates; Gram-positive diphtheroid bacilli, 94 isolates; no bacteria observed, 57 isolates; and *Bacillus* spp., 3 isolates.

The susceptibility percentages of Gram-negative

Table 2. Reading times (hours) of antibiotic disks used for Gram-negative and Gram-positive bacteria.

Microorganisms and antibiotics (Total number of disks used)	Reading times for Gram-negative bacteria				<i>p</i> value						Most significant reading hours
	4 hours n/N, %	6 hours n/N, %	8 hours n/N, %	16-20 hours n/N, %	4-6 hours hours	4-8 hours hours	4-[16-20] hours hours	6-8 hours hours	6-[16-20] hours hours	8-[16-20] hours hours	
<i>E. coli</i> (595)	154/595, 26	196/595, 33	245/595, 41	-	0.007	0.001	-	0.003	-	-	8
<i>K. pneumoniae</i> (1460)	490/1460, 34	529/1460, 36	441/1460, 30	-	0.130	0.052	-	0.001	-	-	6
<i>A. baumannii</i> (516)	114/516, 22	258/516, 50	144/516, 28	-	0.001	0.031	-	0.001	-	-	6
<i>P. aeruginosa</i> (256)	0/256, 0	102/256, 40	154/256, 60	-	0	0	-	0	-	-	8
TZP(423)	111/423, 26	164/423, 39	148/423, 35	-	0.001	0.006	-	0.254	-	-	6,8
CAZ(337)	92/337, 27	121/337, 36	124/337, 37	-	0.016	0.008	-	0.810	-	-	6,8
MEM(423)	111/423, 26	164/423, 39	148/423, 35	-	0.001	0.006	-	0.254	-	-	6,8
CIP(422)	111/422, 26	164/422, 39	147/422, 35	-	0.001	0.007	-	0.225	-	-	6,8
SXT(380)	111/380, 29	147/380, 39	122/380, 32	-	0.01	0.39	-	0.06	-	-	6
AK(421)	111/421, 26	162/421, 38	148/421, 35	-	0	0.01	-	0.32	-	-	6,8
IPM(421)	111/421, 26	163/421, 39	147/421, 35	-	0	0.01	-	0.25	-	-	6,8
<i>S. aureus</i> (115)	6/115, 5	15/115, 13	83/115, 72	11/115, 10	0.038	0.001	0.206	0.001	0.404	0.001	8
<i>E. faecalis</i> (132)	0/132, 0	2/132, 2	112/132, 85	18/132, 14	0.154	0.001	0.001	0.001	0.001	0.001	8
<i>E. faecium</i> (79)	0/79, 0	0/79, 0	79/79, 100	0/79, 0	0.999	0.001	0.999	0.001	0.999	0.001	8
<i>S. pneumoniae</i> (7)	0/7, 0	0/7, 0	0/7, 0	7/7, 100	0.999	0.999	0.001	0.999	0.001	0.999	16-20
AM (78)	0/78, 0	1/78, 1	68/78, 87	9/78, 12	0.999	0.001	0.001	0.001	0.018	0.001	8
FOX (115)	6/115, 5	15/115, 13	83/115, 72	11/115, 10	0.038	0.001	0.001	0.001	0.001	0.001	8
E (4)	0/4, 0	0/4, 0	0/4, 0	4/4, 100	0.999	0.999	0.029	0.999	0.029	0.029	16-20
VA (61)	0/61, 0	0/61, 0	55/61, 90	6/61, 10	0.999	0.001	0.027	0.001	0.027	0.001	8
LNZ (75)	0/75, 0	1/75, 1	68/75, 91	6/75, 8	0.999	0.001	0.028	0.001	0.116	0.001	8

n/N: number of disks in the relevant category/total number of disks tested at the corresponding time point; TZP: piperacillin-tazobactam; CAZ: ceftazidime; MEM: meropenem; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; AK: amikacin; IPM: imipenem; FOX: cefoxitin; AM: ampicillin; VA: vancomycin; LNZ: linezolid; E: erythromycin.

Table 3. Resistance rates in Gram-negative bacteria (%).

	TZP			CAZ			MEM			CIP			SXT			AK			IPM		
	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S
	%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK		
<i>E. coli</i>	29-36	19-0	51-64	53-47	2-6	45-47	1-2	1-0	98-98	44-45	8-13	48-42	49-42	1-2	49-55	0-4	7-0	93-96	0-1	0-0	100
<i>K. pneumoniae</i>	78-82	9-0	13-18	81-83	2-1	17-16	63-62	4-1	33-37	69-73	6-5	25-22	71-67	1-0	27-33	39-43	8-1	53-56	43-50	12-2	44
<i>A. baumannii</i>	-	-	-	-	-	-	93-84	0-7	7-9	93-93	2-6	5-1	83-80	0-2	17-17	74-85	5-0	21-15	93-94	1-0	6
<i>P. aeruginosa</i>	11-30	87-70	2-0	9-9	89-91	2-0	30-22	17-9	52-69	11-9	89-91	0-0	-	-	-	4-0	0-0	96-100	22-27	78-73	0

TZP: piperacillin-tazobactam; CAZ: ceftazidime; MEM: meropenem; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; AK: amikacin; IPM: imipenem; R: resistant; ATU: area of technical uncertainty; I: increased exposure; S: susceptible; RAST: rapid antimicrobial susceptibility testing; VITEK: VITEK 2 automated system.

bacteria according to the EUCAST-RAST method used in the laboratory were presented as resistant-area of technical uncertainty-susceptible (R-ATU-S), respectively; TZP (29-19-51), CAZ (53-2-45), MEM (1-1-98), CIP (44-8-48), SXT (49-4-49), AK (0-7-93) and IPM (0-0-100) in *E. coli*; TZP (78-9-13), CAZ (81-2-17), MEM (63-4-33), CIP (69-6-25), SXT (71-1-27), AK (39-8-27) and IPM (43-12-44) in *K. pneumoniae*; MEM (93-0-7), CIP (93-2-5), SXT (83-0-17), AK (74-5-21) and IPM (93-1-6) in *A. baumannii*; and TZP (11-87-2), CAZ (9-89-2), MEM (30-17-52), CIP (11-89-0), AK (4-0-96) and IPM (22-78-0) in *P. aeruginosa* (Table 3).

According to the VITEK 2 automated system used in the laboratory, the susceptibility percentages in Gram-negative bacteria presented as resistant-increased exposure-susceptible (R-I-S) were as follows; TZP (36-0-64), CAZ (47-6-47), MEM (2-0-98), CIP (45-13-42), SXT (42-2-55), AK (4-0-96) and IPM (1-0-99) in *E. coli*; TZP (82-0-18), CAZ (83-1-16), MEM (62-1-37), CIP (73-5-22), SXT (67-0-33), AK (43-1-56) and IPM (50-2-47) in *K. pneumoniae*; MEM (84-7-9), CIP (93-6-1), SXT (80-2-17), AK (85-0-15) and IPM (94-0-6) in *A. baumannii*; TZP (30-70-0), CAZ (9-91-0), MEM (22-9-69), CIP (9-91-0), AK (0-0-100) and IPM (27-73-0) in *P. aeruginosa*.

The susceptibility percentages of Gram-positive bacteria according to the EUCAST-RAST method used in the laboratory, expressed as R-ATU-S, were as follows: FOX (37-0-63) in *S. aureus*; AM (35-0-65), VA (0-86-14) and LNZ (2-2-96) in *E. faecalis*; AM (96-0-4), VA (20-44-36) and LNZ (4-0-96) in *E. faecium*; AM (100-0-0) and E (25-0-75) in *S. pneumoniae* (Table 4).

The susceptibility percentages of Gram-positive bacteria according to the VITEK 2 automated system used in the laboratory, expressed as R-ATU-S, were as follows: FOX (39-0-61) in *S. aureus*; AM (18-0-82), VA (0-8-92) and LNZ (2-2-96) in *E. faecalis*; AM (100-0-0), VA (19-0-81) and LNZ (0-0-100) in *E. faecium*; AM (100-0-0) and E (25-0-75) in *S. pneumoniae*.

A total of 115 *S. aureus* were identified in this study. Of these isolates, 45 (39.1%) were classified as methicillin resistant *S. aureus* (MRSA) and 70 (60.9%) as methicillin susceptible *S. aureus* (MSSA).

The time difference between the EUCAST-RAST method and the VITEK 2 automated identification system was as follows: *E. coli* (20.9 hours), *K. pneumoniae* (20.8 hours), *A. baumannii* (29.7 hours), *P. aeruginosa* (19.2 hours), *S. aureus* (15.3 hours), *E. faecalis* (10.8 hours), *E. faecium* (14.5 hours), *S. pneumoniae* (37 hours).

Discussion

Sepsis is a severe infectious disease with high mortality rates in the absence of appropriate antimicrobial treatment and timely intervention [10,11]. It is currently not possible to comprehensively predict complex resistance phenotypes in Gram-negative microorganisms using simple molecular tests due to heterogeneity of the genetic determinants associated with acquired resistance. Therefore, phenotypic analysis is still mandatory to obtain conclusive evidence on antibiotic susceptibility [6]. Routinely used ASTs require approximately 2 working days after the blood culture becomes positive and before any results are available. This is a significant disadvantage because results are needed quickly to guide the antimicrobial

Table 4. Resistance rates in Gram-positive bacteria (%).

	AM			FOX			VA			LNZ			E		
	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S	R	ATU-I	S
	%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK			%RAST - %VITEK		
<i>S. aureus</i>	-	-	-	37-39	0-0	63-61	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	35-18	0-0	65-82	-	-	-	0-0	86-8	14-92	2-2	2-2	96-96	-	-	-
<i>E. faecium</i>	96-100	0-0	4-0	-	-	-	20-19	44-0	36-81	4-0	0-0	96-100	-	-	-
<i>S. pneumoniae</i>	100-100	0-0	0-0	-	-	-	-	-	-	-	-	-	25-25	0-0	75-75

AM: ampicillin; FOX: cefoxitin; VA: vancomycin; LNZ: linezolid; E: erythromycin; R: resistant; ATU: area of technical uncertainty; I: increased exposure; S: susceptible; RAST: rapid antimicrobial susceptibility testing; VITEK: VITEK 2 automated system.

treatment plan of patients with bloodstream infections. Although the concept of AST directly from blood culture bottles was first investigated in 1984, much work has been done on this topic in recent years [2–5,12]. Finally, EUCAST validated the RAST method and defined it as a technique that allows antimicrobial susceptibility results to be obtained within a few hours for specific microorganisms and antibiotics [4]. In this study, the validated EUCAST-RAST method was applied in the laboratory.

Sepsis is one of the most common causes of intensive care unit admission [13]. The risk of severe sepsis and the likelihood of intensive care unit hospitalization increase with age. In one study, when the distribution of samples according to the type of service was analyzed, it was found that the highest rate of samples came from intensive care services (63.81%) [14]. In this study, 72.8% of clinical samples were taken from patients aged 60 years and older. When the age groups were analyzed at 10-year intervals, it was observed that these samples were most frequently taken from patients aged 71–80 years (31.5%). The fact that sepsis is more common in the elderly is an expected result due to the slowing down of metabolism with increasing age, increase in chronic diseases, and high number of comorbid factors.

According to a study by Cortes *et al.* that investigated the prevalence of microorganisms in patients with bacteremia in intensive care units, the most frequently isolated microorganisms were CNS [15]. In the study by Vendemiato *et al.* CNS was most frequently isolated from blood cultures of sepsis patients [16]. In this study, the most frequently isolated microorganism type was CNS. However, these microorganisms were excluded from this study because they were not defined in the EUCAST-RAST method. In this study, *S. aureus* was the most frequently isolated Gram-positive microorganism identified in the EUCAST-RAST method, and similar results were obtained in both studies [15,16].

The most frequently isolated Gram-negative bacteria in this study were *K. pneumoniae* (211 isolates), *A. baumannii* (86), *E. coli* (85), and *P. aeruginosa* (46). Similarly, in the study conducted by Cortes *et al.* in intensive care unit patients with bacteremia, the most frequently isolated Gram-negative microorganisms were *K. pneumoniae*, *E. coli*, *A. baumannii*, and *P. aeruginosa* [15]. In the same study, the three most frequently isolated Gram-negative bacteria not covered by the EUCAST-RAST method were *E. cloacae*, *S. marcescens*, and *K. oxytoca* [15]. Similarly, in this study, these bacteria were not

identified in the EUCAST-RAST method and were among the most frequently isolated Gram-negative bacteria. The isolation of similar strains in the present study and in other intensive care unit-based studies suggests that the patient population was comparable and that bacterial identification procedures were properly conducted.

In the present study, the results obtained with the EUCAST-RAST method were compared with the routinely used VITEK 2 automated device in terms of categorical agreement and turnaround time. Categorical agreement was greater than 86% for Gram-negative bacteria and 84% for Gram-positive bacteria. Shan *et al.* [17] reported that the categorical agreement between the EUCAST-RAST technique and the liquid microdilution method for *E. coli* and *K. pneumoniae* ranged between 90–99%. Cherkaoui *et al.* [18] found in their study that the categorical agreement between the EUCAST-RAST technique and the automated WASPLab (Copan, Brescia, Italy) system ranged between 80–100%. These differences between the studies may be due to reasons such as ambient temperature, humidity level, medium content, and calibration of the devices used. In addition, the manual application of the EUCAST-RAST method may cause errors or typing mistakes when reading disk zones. These problems and these differences can be reduced by integrating the laboratory automation system into the EUCAST-RAST system. In this study, *P. aeruginosa* was the Gram-negative bacterium with the lowest categorical agreement, while *E. faecalis* was the bacterium with the lowest categorical agreement among all microorganisms.

However, the errors in the present study were mostly due to MnEs. These MnEs were due to isolates other than *P. aeruginosa*-isolates that gave intermediate values in VITEK 2 but produced susceptible or resistant results in the EUCAST-RAST method. Due to the wide margin in *P. aeruginosa*, most antimicrobial results were reported as intermediate in VITEK 2 reports. However, a higher rate of ATU was detected in the EUCAST-RAST method compared to the VITEK 2 system. When the categorical agreement of antimicrobial agents was analyzed, the lowest categorical agreement in Gram-negative bacteria was obtained at the 4th hour for amikacin, and the lowest categorical agreement in Gram-positive bacteria was obtained at the 8th hour for vancomycin. The analysis showed that these errors were mostly due to MnEs. These MnEs were not followed up in the following hours as repeated readings are not recommended in the EUCAST-RAST method.

Common pan drug resistant Gram-negative bacteria worldwide are *Acinetobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp. [19]. Consistent with this, the majority of the isolates resistant to all antimicrobials used in this study were *K. pneumoniae* and *A. baumannii* isolates. It was found that the reading time was generally shorter in isolates resistant to all antimicrobials. In addition, accurate and rapid results were obtained with the EUCAST-RAST method in these isolates since no inhibition zone was formed most of the time. The EUCAST-RAST method and VITEK 2 device gave highly compatible results in the case of the MEM results of carbapenem-resistant *E. coli* and *K. pneumoniae* strains. The dominant carbapenem resistance gene identified in studies conducted in Türkiye is *bla*_{OXA-48} [20,21]. It was reported that the EUCAST-RAST method gave variable results in strains containing *bla*_{OXA-48} [2]. However, this study showed that the EUCAST-RAST method is reliable in screening for decreased susceptibility to carbapenems. This is even more important considering that carbapenem is a broad-spectrum antibiotic and is frequently used in sepsis patients. Although categorical agreement was high in *A. baumannii* and *P. aeruginosa* strains, some of the results were within the ATU or reported as resistant in the EUCAST-RAST method. In addition, although the reporting time was longer for Gram-positive bacteria than for Gram-negative bacteria, categorical agreement was quite high. In the case of *Enterococcus* spp. strains, vancomycin results frequently fell within the ATU when assessed by the EUCAST-RAST method. Although this may be viewed as a limitation for such a critical antimicrobial agent, it is nonetheless reassuring from a clinical perspective, as it avoids potentially significant errors in treatment decisions. This low categorical agreement in *Enterococcus* spp. may, in part, be attributed to this high rate of ATU results. ATU represents the zone in which the susceptibility and resistance breakpoints overlap, rendering it impossible to definitively categorize isolates as susceptible or resistant [3]. While the presence of an ATU zone in EUCAST-RAST tables does not provide a conclusive result, it effectively reduces the risk of VMjEs and MEs. With increasing antimicrobial resistance, empirical therapy will fail more frequently, and it is important to shorten the time until effective treatment can be administered, especially in patients with severe illnesses. In these situations, safer options are preferred for empirical treatment, accelerating the development of resistance to last-line agents such as carbapenems, polymyxins, and, more recently, β -lactam/ β -lactamase inhibitor agents.

The EUCAST-RAST method provides results from positive blood culture bottles within a standard working day. Thus, laboratories can quickly report reliable S and R results [4]. The EUCAST-RAST method was found to reduce the time needed to obtain by 19–29 hours for Gram-negative bacteria and 10–37 hours for Gram-positive bacteria. Since the doubling time in Gram-negative bacteria is shorter than in Gram-positive bacteria, the EUCAST-RAST method can provide earlier results for Gram-negative bacteria. Therefore, it has become possible to save more time in Gram-negative bacteria. In the case of Gram-positive bacteria, the EUCAST-RAST method was mostly able to give results in the 8th and 16th–20th hours. In addition, routine antimicrobial susceptibility results of *S. pneumoniae*, which is a Gram-positive bacterium, can be quite prolonged due to its small colony size, slower growth, and sometimes the need for extra passages. Therefore, although *S. pneumoniae* results could only be reported at the 16th–20th hours using the EUCAST-RAST method, it was still the organism for which the greatest time advantage was achieved, compared to conventional methods. Among the evaluated pathogens, *S. pneumoniae* exhibited the most significant time gain with the EUCAST-RAST method, allowing for susceptibility results up to 37 hours earlier compared to the conventional approach. This finding is particularly valuable in clinical practice, as *S. pneumoniae* infections can lead to severe outcomes, and early initiation of appropriate antimicrobial therapy is crucial. The substantial reduction in turnaround time may contribute to faster clinical decision-making and potentially improved patient outcomes. A recent meta-analysis of 31 studies and a systematic review of 5,920 sepsis patients found that rapid diagnostic testing of positive blood cultures was associated with a significant reduction in mortality risk only when combined with a systematic antimicrobial stewardship program [22]. Therefore, since the laboratory is a 24-hour facility, an active antimicrobial stewardship program can play an important role in reducing the time required to change the antimicrobials used in the clinic.

Limitations

This study has some limitations. Firstly, devices such as syndromic tests and mass spectrometry were not used. These devices can immediately identify bacteria from blood culture devices that give a signal and which have been used in some laboratories in recent years, but which are not available in all laboratories due to their high costs. Instead, conventional bacterial identification was performed using conventional identification

methods familiar to the staff and their experience. The identification process could have been faster and with higher accuracy if rapid identification devices had been used instead of conventional methods. However, this study investigated whether the EUCAST-RAST method is a viable option for laboratories without rapid identification facilities. In addition, since some commonly used antibiotics in the hospital are used in accordance with the recommendations of the Hospital Infection Control Committee, all antibiotics in the EUCAST-RAST technique were not included in the study. This can be considered as the second limitation of the study. However, this can also be considered a positive aspect since each hospital's selection of the antibiotics to be used according to its antibiotic surveillance results prevents unnecessary antibiotic tests. In future studies, it may be useful to subject the isolates to further investigations in terms of resistance genes and resistance mechanisms.

Conclusions

EUCAST-RAST can be considered as a rapid and highly accurate method for the determination of antimicrobial resistance in positive blood cultures. All Gram-negative bacteria became reportable by the EUCAST-RAST method at the end of the 8th hour. However, in the case of Gram-positive bacteria, it was still necessary to wait until the 16th–20th hour for a significant observation. Although researchers plan to replace routine AST in blood culture by the EUCAST-RAST method, it is currently not possible due to the waiting time needed for Gram-positive bacteria and the presence of microorganisms that have not yet been validated. However, its ability to detect important resistances—such as carbapenem resistance, especially in Gram-negative bacteria—with high accuracy may be useful in selected patients. The results of this study show that the RAST method performed according to EUCAST recommendations is similar to the standard technique, although many issues may affect AST results when performed directly on blood culture. The method is not difficult to incorporate into standard clinical microbiology laboratories, but the workflow needs to be adapted. It is inexpensive to run, faster than other available methods and is based on known and accepted material. Implementation of the EUCAST-RAST method in laboratories while taking into account their facilities and workload, will contribute to the patient's life expectancy by enabling accurate diagnosis of sepsis and ensuring that the appropriate antibiotic is used by the clinician based on evidence, and not empirically. However, it should be kept in mind that the EUCAST-

RAST method will only become meaningful if it is combined with an active antimicrobial stewardship program.

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Authors' contributions

FA, study concept and design, data collection, statistical analysis, data analysis and interpretation, manuscript draft; HG, study concept and design, statistical analysis, data analysis and interpretation, manuscript draft, critical revision of the text for important intellectual content, supervision; ID, study concept and design, manuscript draft, critical revision of the text for important intellectual content.

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Conflict of interest

No conflict of interest is declared.

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