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Original Article

Evaluation of EUCAST rapid antibiotic susceptibility testing for positive blood cultures in the Autobio BC60 blood culture system

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ABSTRACT

Early diagnosis and effective treatment of bloodstream infections significantly improve prognosis. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has developed rapid antimicrobial susceptibility testing(RAST) based on the disk diffusion method. In the present study, we compared the data from the RAST study from positive blood cultures in the Autobio BC (Autobio-diagnostics, Zhengzhou, Chinese) blood culture system with those obtained using the standard disk diffusion method.

A total of 234 spiked blood culture samples (*Klebsiella penumoniae* [*n*= 50], *Escherichia coli* [*n*= 50], *Acinetobacter baumannii* [*n*= 20], *Pseudomonas aeruginosa* [*n*= 13], *Staphylococcus aureus* [*n*= 51], and *Enterococcus* spp. [*n*= 50]) were collected. The RAST test was performed on positive bottles, and the results were measured at 4, 8, and 20 h. Standard disk diffusion (sDD) was performed, and zone diameters were measured from subculture samples obtained from blood culture bottles. The RAST zone diameters were compared with the sDD zone diameters.

The categorical agreement based on the readable zone diameters for the 4th, 8th, and 20th h was *K.pneumoniae* (99.7 %,99.7 %,99.7 %), *E.coli* (99.4 %,99.6 %,99.7 %), *A.baumannii* (98.7 %,98.7 %,98.7 %), *P.aeruginosa* (100 %,99.3 %,100 %), *S. aureus* (100 %,100 %,100 %), and *Enterococcus* spp. (100 %,100 %,100 %). The minor error (mE), major error(ME), and very major error(VME) results obtained in the whole study were determined as 0.9, 0, and 1.3 for 4 h; 1.4, 0, and 1.3 for 8 h; 0.6, 0, and 1.3 for 20 h.

The EUCAST RAST study can be performed from positive bottles in the Autobio BC blood culture system, resulting in reliable outcomes. To report earlier antibiotic susceptibility test results, laboratories using Autobio BC can combine RAST with quality control studies.

1. Introduction

Currently, sepsis is one of the infections with the highest mortality rate [1]. The most common cause of sepsis is bloodstream infection [2]. It is essential to initiate the correct antibiotic treatment at the earliest period [3]. Treatment should generally be initiated with broad-spectrum antibiotics due to the rapidly increasing rates of antibiotic resistance. De-escalation is applied according to the identification and antibiotic susceptibility test results [4]. Nevertheless, it takes approximately 3 days for the blood culture sample to produce a positive signal, identify the causative bacterium, and consequently perform the antibiotic susceptibility test. This process is too time-consuming to guide the treatment [5].

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has developed a rapid antibiotic susceptibility test (RAST) that provides phenotypic test results from positive blood culture bottles. It is based on the EUCAST standard disk diffusion method (sDD), and susceptibility test results are evaluated at 4 and 8 h. This significantly shortens the time to start the correct sDD method, facilitating easy application and widespread use method [6–8].

Although the EUCAST RAST method has been validated with bacteria from positive signal bottles of Biomerieux and Becton Dickinson

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blood culture systems, other blood culture systems are applicable. Other blood culture systems, including the new Autobio Blood Culture System, are routinely and widely used. In this study, we investigated whether the EUCAST RAST method applies to Autobio BC-positive bottles. The EUCAST recommendations were followed to prepare spiked blood culture bottles from routinely cultured bacteria. The results of RAST and sDD were compared and the performance of RAST results was evaluated. Results from this study can improve the practicality of the EUCAST RAST method in hospitals have Autobio BC in Turkey and provide the report of antimicrobial susceptibility test in a short time.

2. Materials and methods

2.1. Bacterial isolates and quality control (QC) strains

The study was conducted in the clinical microbiology laboratory of a tertiary care oncology hospital. The bacteria used in the study were isolated from different clinical samples sent to our laboratory in 2024. Identification of bacteria at the species level was performed using VITEK MS Prime (Biomeriuex, France). Antibiotic susceptibilities of bacteria were studied using VITEK 2 (Biomerieux, France) and sDD method and interpreted according to the EUCAST Breakpoint tables v14. *Klebsiella pneumoniae* ($n = 50$), *Escherichia coli* ($n = 50$), *Acinetobacter baumannii* ($n = 20$), *Pseudomonas aeruginosa* ($n = 13$), *Staphylococcus aureus* ($n = 51$), *Enterococcus faecalis* ($n = 28$), and *Enterococcus faecium* ($n = 22$) were included (Table 1). In addition, standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used.

2.2. Spiked blood culture (BC) bottles

Bacteria and ATCC strains obtained from clinical specimens were passaged on 5 % sheep blood agar (RTA, Turkey). After overnight incubation, a 100 to 200 CFU bacterial suspension was inoculated into Autobio BC60 (Autobio-diagnostics, Zhengzhou, Chinese) aerobic blood culture bottles. Briefly, 5 mL of sterile horse blood (Ankara Uni Kazan Çitfliği; Ankara, Turkey) was added to the bottles before incubation. The bottles were loaded into the Autobio BC60 (Autobio-diagnostics, Zhengzhou, Chinese). BC bottles produced a positive signal between 3.7 and 17.2 h. The bottles were removed after 0 to 14 h [6,9].

2.3. RAST and standard disk diffusion study

RAST and sDD methods were performed according to the EUCAST recommendations. Mueller Hinton agar medium (RTA Laboratories, Kocaeli, Turkey) was used. Ampicillin (10 and 2 μg), amoxicillin-clavulanic acid (20-10 μg), piperacillin-tazobactam (30-6 μg), cefotaxime (5 μg), ceftazidime (10 μg), ceftazidime-avibactam (10-4 μg), ceftolozane tazobactam (30-10 μg), cefoxitin (30 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), norfloxacin (10 μg), amikacin (30 μg), gentamicin (10 and 30 μg), tobramycin (10 μg), clindamycin (2 μg), vancomycin (5 μg), and trimethoprim-sulfamethoxazole (1. 25-23.75 μg) (Oxoid, UK) antibiotic disks were used for both RAST and sDD methods. Muller Hinton agar media were read at the 4th h (6th h for *P. aeruginosa*) and 8th h for the RAST study. The results of sDD were evaluated after 18 to 22 h.

2.4. Quality control strains

Quality control strains were used to control all materials and RAST and standard disk diffusion procedures according to the EUCAST recommendations [9,10]. Quality control of the RAST method was ensured by inoculating four different QC strains into Autobio BC aerobic blood culture bottles. The RAST procedure was repeated 15 times for each QC strain throughout the experiments. Each RAST result was read by three different technicians.

Table 1
Bacterial isolates and their antimicrobial susceptibility results*.

Bacteria (n)	Antibiotics	S (%)	I (%)	R (%)
***K. pneumoniae* (50)**	Amikacin	100	0	0
	Amoxicillin-clavulanic acid	60	0	40
	Cefotaxime	76	0	24
	Ceftazidime	76	0	24
	Ceftazidime-avibactam	96	0	4
	Ciprofloxacin	72	4	24
	Gentamicin	92	0	8
	Imipenem	96	0	4
	Levofloxacin	80	0	20
	Meropenem	96	4	0
	Piperacillin-tazobactam	84	0	16
	Tobramycin	84	0	16
	Trimethoprim-sulfamethoxazole	60	0	40
***E. coli* (50)**	Amikacin	100	0	0
	Amoxicillin-clavulanic acid	74	0	26
	Ampicillin	50	0	50
	Cefotaxime	84	2	14
	Ceftazidime	88	6	6
	Ceftazidime-avibactam	100	0	0
	Ciprofloxacin	78	2	20
	Gentamicin	92	0	8
	Imipenem	100	0	0
	Levofloxacin	78	2	20
	Meropenem	100	0	0
	Piperacillin-tazobactam	92	0	8
	Tobramycin	96	0	4
	Trimethoprim-sulfamethoxazole	70	0	30
A. baumannii (20)	Amikacin	90	0	10
	Ciprofloxacin	0	50	50
	Gentamicin	90	0	10
	Imipenem	80	0	20
	Levofloxacin	60	0	40
	Meropenem	40	20	40
	Tobramycin	80	0	20
	Trimethoprim-sulfamethoxazole	60	0	40
***P. aeruginosa* (13)**	Amikacin	100	0	0
	Cefepime	0	80	20
	Ceftazidime	0	96	4
	Ceftazidime-avibactam	92	0	8
	Ceftolozane-tazobactam	100	0	0
	Ciprofloxacin	0	92	8
	Imipenem	0	92	8
	Levofloxacin	0	92	8
	Meropenem	88	4	8
	Piperacillin-tazobactam	0	88	12
	Tobramycin	100	0	0
***S. aureus* (51)**	Amikacin	9		2
	Cefoxitin	75		25
	Clindamycin	89		11
	Gentamicin	100		0
	Norfloxacin	87		13
	Tobramycin	100		0
***Enterococcus* spp. (50)**	Ampicillin	58		42
	Gentamicin	60		40
	Imipenem	0	54	46
	Vancomycin	92		8

*Antibiotic susceptibilities of bacteria were studied using VITEK 2 (Biomerieux, France) and sDD method and interpreted according to the EUCAST Breakpoint tables v14.

2.5. Analysis

The area of technical uncertainty (ATU) (uninterpretable) results were not considered in the presence of categorical errors. The results were compared with those obtained from the sDD test (reference method). Categorical errors were defined as very major error (VME; RAST = S and reference = R), major error (ME; RAST = R and reference = S) or minor error (mE; RAST = S or R and reference = I) [6].

2.6. Ethics committee approval

A local ethical review of this study has been provided by Health Sciences Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Ethical Review Committee (Research Number:2025-03/308, 06.03.2025).

3. Results

The mean positive signal time of 234 (*K. pneumoniae* [$n = 50$], *E. coli* [$n = 50$], *A. baumannii* [$n = 20$], *P. aeruginosa* [$n = 13$], *S. aureus* [$n = 51$], and *Enterococcus* spp. [$n = 50$]) isolates was 5 h. All isolates were included in the RAST study at 0 to 14 h. The RAST results of isolates at the 4th (6th h for *P. aeruginosa*), 8th h, and 16–20th h are shown in Table 2.

3.1. RAST results

The zone readability of isolates at 4th h (6th h for *P. aeruginosa* and *Enterococcus* spp.) was determined to be *Enterococcus* spp. (91.2 %), *P. aeruginosa* (99.6 %), and all zone diameters were readable for other bacteria. For unreadable zone diameters, insufficient growth implied insufficient growth or unclear zone edges. All zone diameters were readable at 8 and 16 to 20 h of RAST evaluation. All zone diameters were readable at 8 and 16 to 20 h. A challenge encountered during the evaluation of RAST results was the ATU. Although ATUs were detected in all bacteria, their rates decreased significantly at 8 and 16 to 20 h (Table 2). Among gram-negative bacteria, *A. baumannii* and *P. aeruginosa* had ATU rates of 33.8 % and 60 %, respectively, at 4 h. The ATU rate for these two bacteria decreased at 8 and 16 to 20 h. Although it decreased compared to the readings at 8 and 16 to 20 h for these two bacteria, the rate of decrease was very low, especially in *P. aeruginosa*. Among gram-positive bacteria, *Enterococcus* spp. had an ATU rate of 45.2 % at 4 h, which decreased to 36.5 % at 20 h. The ATU rate was frequently determined in *K. pneumoniae* (10.5 %) and *E. coli* (27.0 %) in piperacillin-tazobactam and *P. aeruginosa* (12.5 %). *P. aeruginosa* was the most common bacterium with ATU and the most common agents were cefepime (15.6 %), piperacillin-tazobactam (12.5 %), meropenem (12.5 %), ceftazidime-avibactam (12.5 %), ceftalozone-tazobactam (9.3 %) and ceftazidime (9.3 %).

The ATU rate for cefoxitin in *S. aureus* was 26.1 % at 4 h, and all isolates could be evaluated for clinical limit at 8 h. The ATU rate for clindamycin for *S. aureus* could be categorized at 20 h. The ATU rates for *Enterococcus* spp. were 61.1 % for gentamicin (61.1 %) and 23.4 % for vancomycin (23.4 %) and it especially reduced to 20.3 % at 20 h for gentamicin.

In RAST evaluations, mE was detected in ciprofloxacin for *K. pneumoniae*, ceftazidime, and ciprofloxacin for *E. coli*, and ciprofloxacin for *P. aeruginosa*. ME was not detected in our study. vME was detected only in tobramycin for *A. baumannii*. The categorical agreement according to the readable zone diameters for the 4th, 8th, and 20th h was *K. pneumoniae* (99.7 %, 99.7 %, 99.7 %), *E. coli* (99.4 %, 99. 6 %, 99.7 %), *A. baumannii* (98.7 %, 98.7 %, 98.7 %), *P. aeruginosa* (100 %, 99.3 %, 100 %), *S. aureus* (100 %, 100 %, 100 %), and *Enterococcus* spp. (100 %, 100 %, 100 %).

3.2. QC Results

The RAST results obtained from blood cultures prepared with QC strains and the quality control results for the sDD were compatible with the published EUCAST results. The readings acquired by three different personnel were averaged during the first 5 days of the study, and the agreement between the readings was 99.6 %, 99.7 %, and 100 % for 4, 8, and 20 h, respectively.

Table 2
The actual number of tests performed, the proportion of tests that could be read and interpreted after 4, 8, and 20 h, and categorical errors with RAST at each reading time for isolates.

Bacteria (n)	Incubation time	4 h*	8 h	16–20 h
***K. pneumonia* (50)**	Completed tests	650	650	650
	Readable zones, n (% of completed tests)	650 (100)	650	650
	Not interpreted to S or R (ATU) (%)	38 (5.8)	24 (3.7)	18 (2.8)
	Interpreted to S** (%)	518 (79.7)	536 (82.5)	532 (81.8)
	Interpreted to R (%)	94 (14.5)	90 (13.8)	100 (15.4)
	mE (%)	2 (0.3)	2 (0.3)	2 (0.3)
	ME (%)	0	0	0
	VME (%)	0	0	0
	Total errors (%)	2 (0.3)	2 (0.3)	2 (0.3)
***E.coli* (50)**	Completed tests	685	700	700
	Readable zones, n (% of completed tests)	685 (100)	700 (100)	700 (100)
	Not interpreted to S or R (ATU) (%)	37 (5.4)	16 (2.3)	24 (3.4)
	Interpreted to S** (%)	566 (82.6)	598 (85.4)	590 (84.3)
	Interpreted to R (%)	82 (12)	86 (12.3)	86 (12.3)
	mE (%)	4 (0.6)	3 (0.4)	2 (0.3)
	ME (%)	0	0	0
	VME (%)	0	0	0
	Total Errors (%)	4 (0.6)	3 (0.4)	2 (0.3)
***A. baumannii* (20)**	Completed tests	160	160	160
	Readable zones, n (% of completed tests)	160 (100)	160 (100)	160 (100)
	Not interpreted to S or R (ATU) (%)	54 (33.8)	18 (11.3)	16 (10)
	Interpreted to S** (%)	80 (50)	100 (62.5)	100 (62.5)
	Interpreted to R (%)	26 (16.3)	42 (26.3)	44 (27.5)
	mE (%)	0	0	0
	ME (%)	0	0	0
	VME (%)	2 (1.3)	2 (1.3)	2 (1.3)
	Total errors (%)	2 (1.3)	2 (1.3)	2 (1.3)
P. aeruginosa* **(13)**	Completed tests	550	550	550
	Readable zones, n (% of completed tests)	548 (99.6)	550 (100)	550 (100)
	Not interpreted to S or R (ATU) (%)	300 (54.7)	278 (50.5)	330 (60)
	Interpreted to S** (%)	218 (39.8)	238 (43.3)	186 (33.8)
	Interpreted to R (%)	30 (5.5)	34 (6.2)	34 (6.2)
	mE (%)	0	4 (0.7)	0
	ME (%)	0	0	0
	VME (%)	0	0	0
	Total errors (%)	0	4 (0.7)	0
***S. aureus* (51)**	Completed tests	330	330	330
	Readable zones, n (% of completed tests)	330 (100)	330 (100)	330 (100)
	Not interpreted to S or R (ATU) (%)	23 (7)	5 (1.5)	3 (0.9)
	Interpreted to S** (%)	283 (85.8)	298 (90.3)	300 (90.9)
	Interpreted to R (%)	24 (7.3)	27 (8.2)	30 (9.1)
	mE (%)	0	0	0
	ME (%)	0	0	0
	VME (%)	0	0	0
	Total errors (%)	0	0	0
Enterococcus* spp. **(50)**	Completed tests	250	250	250
	Readable zones, n (% of completed tests)	228 (91.2)	250 (100)	250 (100)
	Not interpreted to S or R (ATU) (%)	103 (45.2)	92 (36.8)	91 (36.5)
	Interpreted to S** (%)	67 (29.4)	96 (38.4)	96 (38.6)
	Interpreted to R (%)	58 (25.4)	62 (24.8)	62 (24.9)

(*continued on next page*)

Table 2 (*continued*)

Bacteria (n)	Incubation time	4 h*	8 h	16–20 h
	mE (%)	0	0	0
	ME (%)	0	0	0
	VME (%)	0	0	0
	Total errors (%)	0	0	0

*6 h for *P. aeruginosa and Enterococcus* spp., **Including species–agent combinations for which WT isolates are categorized as I. S, susceptibility; R, resistance; mE: minor error; ME, major error; VME, very major error.

4. Discussion

A rapid increase in antibiotic resistance has led to an increase in the use of carbapenems, polymyxins, and new β-lactam/β-lactamase inhibitors in the empirical treatment of serious infectious diseases [11]. Incorrect and long-term use of these antibiotics could cause increased antibiotic resistance. In this case, shortening the duration of bacterial diagnosis and antibiotic susceptibility testing is crucial for the safety of patients and the progress from empirical treatment to effective treatment [12]. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI TOF MS) and syndromic panels for rapid identification of microorganisms have considerably shortened the diagnostic process [13]. The RAST test, developed by EUCAST, can be conducted directly using positive blood culture bottles to shorten the transition from empirical treatment to effective treatment [6]. The EUCAST RAST method and RAST clinical cut-off values were assessed with different media, antibiotic disks, and blood culture systems and the results were presented [6].

Antibiotic resistance rates in Turkey are considerably high and have been increasing exponentially over the years [14]. BD and Biomerieux blood culture systems were used in the EUCAST study [6]. We conducted this study because Autobio blood culture systems have been widely used in our country. The study was based on the EUCAST study [6,7,15]. We isolated bacteria from patients in our hospital in 2024. Because bacteria isolated from blood cultures are usually multidrug-resistant, we included those isolated from different clinical samples. *P. aeruginosa* and *A. baumannii* were in small numbers because these isolates are less common in our institution compared with other bacteria. Another limitation of our study is that *Streptococcus pneumoniae* was not included.

The routine application of the EUCAST RAST study has shortcomings. One of these is the insufficient growth for the detection of certain zones in the short evaluation period, and ATU is frequently observed especially during the early reading periods [6]. The first readings of *P. aeruginosa* were made at the 6th h. Table 2 shows the ratios of readable zone diameters in all completed bacterial isolate matches. Among the bacteria included in the study, the most common problem of readable zone diameter was observed for *Enterococcus* spp. at 4 h. The rates we determined were in parallel with those obtained in EUCAST studies [6,7].

The ATU results were the highest at the 4th h. At 8th and 20th h, the ATU values decreased significantly. Thus, ATU prevents clinical errors originating from the laboratory by reducing the VME and ME rates. In our study, *K. penumoniae* ($n = 38$), *E. coli* ($n = 37$), *A. baumannii* ($n = 44$), *P. aeruginosa* ($n = 64$), *S. aureus* ($n = 23$), and *Enterococcus* spp. ($n = 77$) were detected at 4 h (6 h for *P. aeruginosa* and *Enterococcus* spp.).

The Food and Drug Administration (FDA) states that categorical agreement ≥90 %, VME ≤1.5 %, ME ≤3.0 % and mE ≤10 % for early reading of commercial antibiotic susceptibility test results [16]. Cumitech reports a CA ≥90 %, VME and ME ratio separately ≤3 %, and the total ratio for ME and mE ratios ≤7 % [17]. These rates have been demonstrated in large numbers of EUCAST RAST studies [6,7,18,19]. When comparing the RAST method performed using positive signal bottles in the Autobio BC blood culture system with the sDD method, both categorical agreement and mE, ME, and VME rates were within the acceptable range.

In our study, vME was detected only in tobramycin for *A. baumannii*. Zone diameters for tobramycin in Acinetobacter8 spp. were found to be "14″ ($S \geq 14$, ATU 12-13, $R < 12$) [20]. This is a borderline value. As is known, zone diameters of aminoglycosides are greatly affected by medium performance. In addition, one of the biggest bias of our study is that minimal inhibitory concentration (MIC) values were not studied by broth microdilution.

The RAST study was defined for eight identified bacteria. Tayşi et al. demonstrated that the RAST study can be performed even in a microbiology laboratory with limited facilities and no automated systems. The study stated that RAST is an effective method for treating isolates not identified at the species level [21].

The turnover time of multidrug-resistant carbapenemase-producing bacteria frequently encountered in blood cultures was significantly shorter than that obtained using a combination of MALDI TOF and RAST. The sensitivity of carbapenemase presence with RAST was 100 % and the specificity was 88.7 % [22]. The *OXA-48*-producing isolates can be identified at the 4th h with temocillin and ertapenem disks in addition to meropenem during RAST screening [23]. RAST enables the identification of carbapenemase type in a carbapenemase-producing bacterium and the safe use of ceftazidime-avibactam. It is possible to obtain accurate results in a maximum of 8 h for the safe use of ceftazidime avibactam for KPC- and *OXA-48*-producing isolates [24]. We found that even if the 4th-h result of ceftazidime-avibactam was ATU, the categorical compliance with sDD at the 8th h was 100 %.

Following the studies demonstrating the clinical contribution of the EUCAST-developed RAST study, the Clinical and Laboratory Standards Institute (CLSI) developed RAST. The CLSI RAST stated that antibiotic susceptibility results in a maximum of 10 h, especially for Enterobacterales isolates, which is a significant tool in management of patients with bloodstream infections [25].

5. Conclusion

The RAST study is used worldwide to provide safe and effective treatment to patients, with different versions used routinely with several modified studies. We demonstrated that the EUCAST RAST study can be performed using vials with positive signals from Autobio BC; the results are compatible with sDD. We believe that RAST studies can be easily conducted using the Autobio BC in the laboratories after verification studies in their systems in line with EUCAST RAST quality control studies.

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CRediT authorship contribution statement

Serap Süzük Yıldız: Writing – original draft, Validation, Project administration, Methodology. **Esra Tavukcu:** Writing – original draft, Validation, Methodology. **Mert Emre Ölmez:** Methodology, Formal analysis. **Sevgi Şahin:** Writing – original draft, Methodology, Formal analysis. **Habibe Kurtaran Tek:** Methodology, Formal analysis. **Can Hüseyin Hekimoğlu:** Software, Data curation. **Ayşe Semra Güreser:** Validation. **İpek Mumcuoğlu:** Supervision, Conceptualization. **Tuba Dal:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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