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European Committee on Antimicrobial Susceptibility Testing-Recommended Rapid Antimicrobial Susceptibility Testing of *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* From Positive Blood Culture Bottles

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Background: Early diagnosis and treatment are important for a good prognosis of bloodstream infections. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends rapid antimicrobial susceptibility testing (RAST) based on the disk diffusion methodology for 4, 6, and 8 hours of incubation. We evaluated EUCAST-RAST of *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus* from positive blood culture bottles.

Methods: Twenty strains of *E. coli*, *K. pneumoniae*, and *S. aureus* were tested using EUCAST-RAST. Ten antimicrobial agents against *E. coli* and *K. pneumoniae* and four agents against *S. aureus* were tested. The diameter of the inhibition zone (mm) was compared with the minimal inhibitory concentration (μg/mL) obtained using the Sensititre AST system (TREK Diagnostic Systems, East Grinstead, UK).

Results: For *E. coli*, the percentage of total categorical agreement (CA) was 69.5% at 4 hours, and 87% at 8 hours. For *K. pneumoniae*, the total CA was 89% at 4 hours, and 95.5% at 6 hours. For *S. aureus*, the total CA was 100% after 4 hours. Discrepancies were observed mainly for *E. coli* with β-lactam antimicrobial agents, and the numbers of errors decreased over time.

Conclusions: EUCAST-RAST for *K. pneumoniae* and *S. aureus* met the United States Food and Drug Administration criteria at 6 and 4 hours, respectively, whereas that for *E. coli* did not meet the criteria for up to 8 hours. RAST can shorten the turn-around testing time by more than one day; therefore, if applied accurately according to laboratory conditions, antimicrobial agent results can be reported faster.

Key Words: Sepsis, European Committee on Antimicrobial Susceptibility Testing, Rapid antimicrobial susceptibility testing, Microbial sensitivity test, Categorical agreement, United States Food and Drug Administration, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*

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INTRODUCTION

Bacteremia can develop into sepsis, and bloodstream infections are a leading cause of high morbidity and mortality worldwide [1-4]. Therefore, early diagnosis and treatment are important for a good prognosis in sepsis.

Blood cultures are the reference method for determining the cause of bacteremia and provide critical information for initiating appropriate antimicrobial therapy [5-9]. Because of the increase in multidrug-resistant bacterial infections such as methicillin-resistant *Staphylococcus aureus*, rapid and accurate antimicrobial susceptibility testing (AST) for appropriate antimicrobial treatment is becoming increasingly important [10-15].

Typically, AST is performed using the broth microdilution (BMD) method or disk diffusion method [16-18]. The results are reported qualitatively using CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (susceptible, intermediate, resistant, or an area of technical uncertainty [ATU]) [19-22].

The BMD method takes 24 hours and the disk diffusion method takes 16-20 hours to complete. Therefore, it takes at least three days for the final report of the blood culture to be provided. The EUCAST provides rapid AST (RAST) guidelines based on the standard disk diffusion method to shorten the turn-around time (TAT) required for AST. This method is faster than the conventional method because culture-positive blood samples are directly inoculated into media and analyzed after 4, 6, and 8 hours of incubation [23]. The EUCAST-RAST method is currently available for testing *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Streptococcus pneumoniae*, with interpretation criteria for five to 13 antimicrobial agents according to the species [24]. However, RAST has to be evaluated before it can be readily implemented in clinical microbiology laboratories.

We aimed to evaluate EUCAST-RAST of *E. coli*, *K. pneumoniae*, and *S. aureus* from positive blood culture bottles. These three pathogens are the most prevalent in positive blood cultures in Korea [25]. To determine categorical agreement (CA), EUCAST-RAST results were compared with minimum inhibitory concentrations (MICs) obtained using the Sensititre AST System, which is widely used in clinical microbiology laboratories in Korea, according to the CLSI M100-S29 guidelines [26] as a reference method.

MATERIALS AND METHODS

Bacterial strains

This study was conducted between August 2019 and February 2020 at the Department of Laboratory Medicine, Severance Hospital (Seoul, Korea). Sixty strains of *E. coli*, *K. pneumoniae*, and *S. aureus* with various antibiotic susceptibilities were randomly selected from clinical blood culture samples. Quality control (QC) strains, including *E. coli* ATCC 25922 and *S. aureus* ATCC 29213, were tested for comparison. The study protocol was approved by the Institutional Review Board at Severance Hospital (approval number: 4-2019-0965).

Species identification

Blood samples were inoculated into BacT-ALERT FA Plus bottles and incubated on a BacT-ALERT VIRTUO blood culture system (BioMérieux, Durham, NC, USA). When a blood bottle was flagged as positive by the instrument, it was subjected to Gram staining, and a preliminary result was reported. The sample was then sub-cultured on sheep blood agar and MacConkey agar (Asan Pharmaceutical, Hwaseong, Korea). After overnight incubation at 35°C, all bacterial species were identified using Microflex with a Biotyper IVD MBT v.2.3 matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system (Bruker Daltonics, Bremen, Germany), according to the manufacturer's instructions.

EUCAST-RAST method

Strains for QC and clinically isolated strains were subjected to RAST according to the EUCAST guidelines, and the results were compared with conventional Sensititre BMD results.

Selection of antimicrobial agents and disks

Ten antimicrobial agents (piperacillin–tazobactam, cefotaxime, ceftazidime, imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, and trimethoprim–sulfamethoxazole) for *E. coli* and *K. pneumoniae* and four antimicrobial agents (cefoxitin, norfloxacin, gentamicin, and clindamycin) for *S. aureus* were used for AST. Because of differences in the concentrations of some antimicrobial agents between the EUCAST and CLSI guidelines (Table 1), BD BBL Sensi-Disc (Becton, Dickinson and Company) and Thermo Scientific Oxoid disc (Oxoid, Basingstoke, UK) were selectively used according to the EUCAST guidelines. For *E. coli* and *K. pneumoniae* (gram-negative bacilli), disk diffusion tests were performed using Oxoid disks containing piperacillin–tazobactam, cefotaxime, and ceftazidime

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Table 1. Comparison of antimicrobial agent concentration between the EUCAST and CLSI guidelines used in this study

Species (N antimicrobial agents tested)	Antimicrobial agent	Disk content (μg)	
		EUCAST	CLSI
Escherichia coli and *Klebsiella pneumoniae* (N=10)	Piperacillin–tazobactam	30-6	100-10
	Cefotaxime	5	30
	Ceftazidime	10	30
	Imipenem	10	10
	Meropenem	10	10
	Ciprofloxacin	5	5
	Amikacin	30	30
	Gentamicin	10	10
	Tobramycin	10	10
	Trimethoprim–sulfamethoxazole	1.25-23.75	1.25-23.75
Staphylococcus aureus (N=4)	Cefoxitin*	30	30
	Norfloxacin†	10	10
	Gentamicin	10	10
	Clindamycin	2	2

*Isolates susceptible to cefoxitin are reported as susceptible to all β-lactam agents with breakpoints in the EUCAST clinical breakpoint tables (standard methodology); †The norfloxacin disk diffusion test was used to screen for fluoroquinolone resistance.
Abbreviation: EUCAST, European Committee on Antimicrobial Susceptibility Testing.

and BD disks containing imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, and trimethoprim–sulfamethoxazole. For *S. aureus* (a gram-positive coccus), Oxoid disks containing norfloxacin and BD disks containing cefoxitin, gentamicin, and clindamycin were used for disk diffusion testing.

Spiked blood culture bottles

All *E. coli*, *K. pneumoniae*, and *S. aureus* strains were cultured on blood agar at 35°C for 24 hours. Using the direct colony suspension method, the number of bacterial particles in each suspension was set to McFarland scale 0.5 (1.5×10^8 colony-forming units [CFU]/mL). The bacterial suspension was diluted 1:1,000,000 by transferring 1 μL of the solution into 1 mL of saline, in duplicate. Blood culture bottles were inoculated with 1 mL of the final solution (100-200 CFU/mL suspension), and 5 mL of defibrinated sterile sheep blood (South Pacific Sera, Timaru, New Zealand) was added. Usually, the RAST method takes 0-18 hours to complete after the blood culture bottles are signaled as positive. In this study, the average time for detecting a positive blood culture using the instrument was 9.2 hours (8.4 hours for *E. coli*, 8.6 hours for *K. pneumoniae*, and 10.9 hours for *S. aureus*), and the culture bottle was removed 5.1 hours (4.5 hours for *E. coli*, 5.9 hours for *K. pneumoniae*, and 4.8 hours for *S. aureus*) after detecting the positive signal. RAST was performed immediately after the bottle was removed from the instrument.

Direct inoculation on agar plates from blood culture bottles

The test volume for direct RAST is suggested by the EUCAST [23]. After transferring the content of the positive blood culture bottle into an empty tube, 125±25 μL of undiluted blood culture broth was transferred to a 90-mm circular Mueller-Hinton agar plate (Asan Pharmaceutical, Hwaseong, Korea), or 350 μL was transferred to a 150-mm plate. The plates were inoculated using a Retro C80 Inoculator (AB BIODISK, Solna, Sweden), and disks for each antimicrobial agent were placed on the agar surface using BD BBL Sensi-Disc dispensers (Becton, Dickinson and Company).

Incubation and reading of plates

Plates were incubated for 4, 6, and 8 hours at 35°C under ambient air and re-incubated within 10 mins after the stated reading time. In total, 1,440 AST results (600 for *E. coli*, 600 for *K. pneumoniae*, 240 for *S. aureus*) were obtained and compared with 480 reference AST results (200 for *E. coli*, 200 for *K. pneumoniae*, and 80 for *S. aureus*). Inhibition zones with a visible zone edge and confluent growth were inspected manually at the front side of the plate, with the lid removed, using a caliper (Sylvac SA, Yverdon-les-Bains, Switzerland).

Sensititre AST system as a reference method

BMD was performed for all isolates using the Sensititre AST system (TREK Diagnostic Systems, East Grinstead, UK) based on the CLSI M100-S29 guidelines [26]. The Sensititre AIM Automated Inoculation Delivery System, Sensititre VIZION Digital MIC Viewing System, Sensititre DKMGN, and GPALL1F panel were used, and the MIC results were analyzed using the Sensititre SWIN software.

EUCAST standard disk diffusion method

The EUCAST standard disk diffusion method was used as a reference to compare the susceptibilities of *S. aureus* to cefoxitin and norfloxacin. Each *S. aureus* strain was cultured on a blood agar plate at 35°C for 24 hours, and the direct colony suspension method was used to suspend the microorganism in saline at a density of 1.5×10^8 CFU/mL (0.5 McFarland). The prepared suspension was used immediately. A sterile cotton swab was

dipped in the suspension and streaked over the plates using a Retro C80 Inoculator. The plates were incubated at 35°C for 18-24 hours [21].

QC

To determine the accuracy of the RAST method, QC was conducted according to EUCAST recommendations for 30 days. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were tested using EUCAST-RAST and the standard disk diffusion method, respectively. The results were interpreted according to EUCAST-RAST QC version 5.0 and EUCAST standard QC version 12.0 [24, 27].

Data analysis

Discrepancies between EUCAST-RAST and Sensititre BMD results were, based on the CLSI guidelines, classified as very major errors (VMEs; susceptible in RAST and resistant in the reference method), major errors (MEs; resistant in RAST and susceptible in the reference method), or minor errors (mEs; susceptible or resistant in RAST and intermediate in the reference method). According to the United States Food and Drug Administration (FDA) recommendations, the acceptability criteria are >89.9% for CA (same susceptible, intermediate, resistant classification), >89.9% for essential agreement (EA; MICs within one two-fold dilution of the values obtained using the reference method), ≤1.5% for VMEs (false susceptibility based on the number of resistant organisms), and ≤3% for MEs (false resistance based on the number of susceptible isolates) [28].

After interpretation according to the AST breakpoint table, the EUCAST-RAST results were compared with the Sensititre BMD results based on the CLSI criteria. However, some antimicrobial agents against *S. aureus* strains cannot be analyzed using this method. Neither method could be accurately compared for cefoxitin because the MIC cutoff in the Sensititre GPALL1F panel is ≤6, which is higher than the cutoff of ≤4 in the CLSI guidelines. In addition, norfloxacin is not included in the Sensititre GPALL1F panel. Therefore, the EUCAST standard disk diffusion method was used as the reference method for cefoxitin and norfloxacin.

RESULTS

Comparison of EUCAST-RAST with Sensititre as a reference BMD method

The number of *E. coli* strains classified as susceptible according to the EUCAST-RAST criteria was 84 at 4 hours, 109 at 6 hours, and 119 at 8 hours, increasing with incubation time, whereas the number of strains within the ATU gradually decreased with time: 40 at 4 hours, 22 at 6 hours, and 12 at 8 hours (Supplemental Data Table S1).

For *K. pneumoniae*, the numbers of susceptible strains were higher: 111 at 4 hours, 122 at 6 hours, and 125 at 8 hours, whereas the numbers of strains within ATU were lower: 16 at 4 hours, 18 at 6 hours, and 16 at 8 hours. Overall, the distribution of the categories approached that of the reference method over time. For *S. aureus*, the AST results at 4, 6, and 8 hours were in the same category as those obtained using the reference method.

CA and discrepancy of EUCAST-RAST

To evaluate the RAST method, the AST results at 4, 6, and 8 hours were compared with those obtained using standard methods.

For the 200 *E. coli* samples, the total number of samples showing CA was 139 (69.5%) at 4 hours, but it gradually increased to 164 (82%) at 6 hours and 174 (87%) at 8 hours. The proportions of samples showing CA for piperacillin–tazobactam, ceftazidime, and amikacin were <40% at 4 hours. The proportion of samples showing CA for amikacin gradually increased to 90% over time, whereas those for piperacillin–tazobactam and ceftazidime remained low (60%) after 8 hours (Table 2).

The total number of mEs was high: 47 (23.5%) at 4 hours, 29 (14.5%) at 6 hours, and 19 (9.5%) at 8 hours. mEs were mainly observed for piperacillin–tazobactam, ceftazidime, and amikacin (50%-70%) at 4 hours, but the proportions decreased <35% after 8 hours.

The total number of MEs was 12 (6%) at 4 hours and five (2.5%) at both 6 and 8 hours. MEs were observed for piperacillin–tazobactam, cefotaxime, ceftazidime, and tobramycin (5%-30%) at 4 hours, and their proportions decreased <15% after 6 hours. Two VMEs were observed, including one for cefotaxime (5%) and one for trimethoprim–sulfamethoxazole (5%), at all time points.

The percentage of CA varied depending on the antimicrobial agent, from 0% to 100% at 4 hours, 30%-100% at 6 hours, and 60%-100% at 8 hours, but it improved over time. None of the percentages CA for *E. coli* met the FDA criteria at 4, 6, or 8 hours, but MEs satisfied the criteria at 6 hours and VMEs at 4 hours.

For *K. pneumoniae*, the total number of samples showing CA was very high: 178 (89%) at 4 hours, 191 (95.5%) at 6 hours, and 190 (95%) at 8 hours. In the case of piperacillin–tazobactam, the proportion of samples showing CA was 40% at 4 hours, but it significantly increased to 85% and 90% at 6 and 8 hours, respectively. Additionally, in the case of tobramycin, the proportion of samples showing CA was 65% at 4 hours and 85% at both 6 and 8 hours.

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Table 2. CA and discrepancy of RAST

Antimicrobial agent	N (%) at 4 hr				N (%) at 6 hr				N (%) at 8 hr			
	CA	mEs	MEs	VMEs	CA	mEs	MEs	VMEs	CA	mEs	MEs	VMEs
E. coli												
TZP	0 (0)	14 (70)	6 (30)	0 (0)	6 (30)	13 (65)	1 (5)	0 (0)	12 (60)	7 (35)	1 (5)	0 (0)
CTX	17 (85)	1 (5)	1 (5)	1 (5)	19 (95)	0 (0)	0 (0)	1 (5)	19 (95)	0 (0)	0 (0)	1 (5)
CAZ	8 (40)	10 (50)	2 (10)	0 (0)	12 (60)	7 (35)	1 (5)	0 (0)	12 (60)	7 (35)	1 (5)	0 (0)
IPM	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
MEM	18 (90)	2 (10)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
CIP	17 (85)	3 (15)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	0 (0)	19 (95)	1 (5)	0 (0)	0 (0)
AN	7 (35)	13 (65)	0 (0)	0 (0)	15 (75)	5 (25)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	0 (0)
GM	19 (95)	1 (5)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
NN	14 (70)	3 (15)	3 (15)	0 (0)	15 (75)	2 (10)	3 (15)	0 (0)	15 (75)	2 (10)	3 (15)	0 (0)
SXT	19 (95)	0 (0)	0 (0)	1 (5)	19 (95)	0 (0)	0 (0)	1 (5)	19 (95)	0 (0)	0 (0)	1 (5)
Total (N=200)	139 (69.5)	47 (23.5)	12 (6)	2 (1)	164 (82)	29 (14.5)	5 (2.5)	2 (1)	174 (87)	19 (9.5)	5 (2.5)	2 (1)
K. pneumoniae												
TZP	8 (40)	11 (55)	1 (5)	0 (0)	17 (85)	3 (15)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	0 (0)
CTX	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
CAZ	19 (95)	1 (5)	0 (0)	0 (0)	19 (95)	1 (5)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	0 (0)
IPM	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
MEM	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
CIP	18 (90)	2 (10)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	0 (0)	17 (85)	2 (10)	0 (0)	1 (5)
AN	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
GM	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
NN	13 (65)	7 (35)	0 (0)	0 (0)	17 (85)	3 (15)	0 (0)	0 (0)	17 (85)	3 (15)	0 (0)	0 (0)
SXT	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
Total (N=200)	178 (89)	21 (10.5)	1 (0.5)	0 (0)	191 (95.5)	9 (4.5)	0 (0)	0 (0)	190 (95)	9 (4.5)	0 (0)	1 (0.5)
S. aureus												
FOX*	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
NOR*	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
GM	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
CC	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)
Total (N=80)	80 (100)	0 (0)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)	0 (0)	80 (100)	0 (0)	0 (0)	0 (0)

*EUCAST-RAST disk diffusion test results were compared with those of the EUCAST standard disk diffusion method.
Abbreviations: N, number; CA, categorical agreement; EUCAST, European Committee on Antimicrobial Susceptibility Testing; mEs, minor errors; MEs, major errors; VMEs, very major errors; TZP, piperacillin–tazobactam; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; AN, amikacin; GM, gentamicin; NN, tobramycin; SXT, trimethoprim–sulfamethoxazole; FOX, cefoxitin; NOR, norfloxacin; CC, clindamycin; RAST, rapid antimicrobial susceptibility testing.

The total number of mEs was 21 (10.5%) at 4 hours and was nine (4.5%) at 6 and 8 hours; mEs were observed mainly for piperacillin–tazobactam (55%) and tobramycin (35%), and their proportion decreased to ≤15% after 6 and 8 hours. The was only one ME and one VME (5%) for piperacillin–tazobactam at 4 hours and for ciprofloxacin at 8 hours, respectively. The FDA criteria for CAs, MEs, and VMEs were met at 4, 6, and 8 hours, except for CA at 4 hours.

For *S. aureus*, the total number of samples showing CA was high (N=80, 100%) at 4, 6, and 8 hours. There were no MEs or VMEs at 4, 6, and 8 hours. All categories were consistent between the two methods, and CA, MEs, and VMEs satisfied the

Table 3. VMEs and MEs for *E. coli* and *K. pneumoniae*

Antimicrobial agent	Zone diameter (mm), S/I/R, and discrepancy of RAST									Reference (MIC, S/I/R)	
	4 hr			6 hr			8 hr				
E. coli											
TZP	12	R	ME	14	R	ME	14	R	ME	8	S
TZP	13	R	ME	16	ATU	mE	18	S	CA	2	S
TZP	11	R	ME	15	ATU	mE	16	ATU	mE	2	S
TZP	12	R	ME	15	ATU	mE	16	ATU	mE	8	S
TZP	11	R	ME	15	ATU	mE	16	ATU	mE	8	S
TZP	13	R	ME	18	S	CA	19	S	CA	2	S
CTX	18	S	VME	21	S	VME	22	S	VME	>8	R
CTX	12	R	ME	19	S	CA	21	S	CA	≤0.5	S
CAZ	11	R	ME	18	S	CA	20	S	CA	≤0.5	S
CAZ	11	R	ME	13	R	ME	14	R	ME	4	S
NN	11	R	ME	11	R	ME	11	R	ME	4	S
NN	11	R	ME	12	R	ME	12	R	ME	4	S
NN	11	R	ME	12	R	ME	12	R	ME	4	S
SXT	21	S	VME	22	S	VME	24	S	VME	>8	R
K. pneumoniae											
TZP	12	R	ME	15	ATU	mE	15	ATU	mE	16	S
CIP	15	ATU	mE	16	ATU	mE	19	S	VME	1	R
Total number of discrepancies											
ME	13			5			5				
VME	2			2			3				

Abbreviations: CA, categorical agreement; VME, very major error; ME, major error; mE, minor error; S, susceptible; I, intermediate; ATU, area of technical uncertainty; R, resistant; TZP, piperacillin–tazobactam; CTX, cefotaxime; CAZ, ceftazidime; NN, tobramycin; SXT, trimethoprim–sulfamethoxazole; CIP, ciprofloxacin; MIC, minimal inhibitory concentration; RAST, rapid antimicrobial susceptibility testing.

FDA criteria at 4, 6, and 8 hours.

In general, the numbers of mEs, MEs, and VMEs decreased over time (Supplemental Data Figs. S1, S2, and S3).

Analysis of VMEs and MEs for *E. coli* and *K. pneumoniae*

Discrepancies were observed mainly for *E. coli*. The total number of MEs for *E. coli* was 12 (6%) at 4 hours and five (2.5%) at 6 and 8 hours (Table 3). Six MEs were observed for piperacillin–tazobactam at 4 hours, but these were changed to mEs or CA at 6 and 8 hours. Two VMEs were observed for cefotaxime and trimethoprim–sulfamethoxazole. For *K. pneumoniae*, one ME was observed for piperacillin–tazobactam at 4 hours, but this changed to an mE at 6 hours. Only one (5%) VME was observed for ciprofloxacin at 8 hours. For *S. aureus*, no mEs, MEs, or VMEs were observed at 4, 6, or 8 hours. These results showed that β-lactam susceptibility test results of *E. coli* are highly discrepant; therefore, additional tests are required to accurately compare the results.

DISCUSSION

In this study, 60 strains were tested for their susceptibility to antimicrobial agents using the EUCAST-RAST method, including 20 strains each of *E. coli*, *K. pneumoniae*, and *S. aureus*. Ten antimicrobial agents against *E. coli* and *K. pneumoniae* and four antimicrobial agents against *S. aureus* were tested. RAST inhibition zone diameters were compared with MICs obtained using the Sensititre AST system. The results were analyzed in terms of CA, mEs, MEs, and VMEs.

Comparing the results at 4, 6, and 8 hours for each species, large differences were observed depending on the antimicrobial agents. For *E. coli*, numerous mEs were observed for piperacillin–tazobactam, ceftazidime, and amikacin. In particular, β-lactam agents produced highly discrepant results in *E. coli*. As shown in Table 3, for some *E. coli* isolates, the EUCAST-RAST results were classified as resistant at 4 hours, which changed to sus-

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ceptible or ATU after 6 or 8 hours. This phenomenon is believed to occur because the blood in the sample confuses the initial inhibition zone measurement. In other cases, such as the phenomenon of susceptible-to-susceptible or resistant-to-resistant, this can be considered a limitation of the EUCAST-RAST method. It is peculiar that this phenomenon occurred more frequently in *E. coli*. These results indicate that the clinical performance of EUCAST-RAST varies depending on the bacterial species, and additional tests are required to accurately compare the results. For *K. pneumoniae*, mEs were mainly observed for piperacillin–tazobactam and tobramycin. For *S. aureus*, all categories were consistent. These differences depended on the type of antimicrobial agent used and reading time.

Although EUCAST-RAST provides rapid results, there are some limitations in evaluating the applicability of this method. First, compared with the CLSI or EUCAST standard disk diffusion methods, EUCAST-RAST cannot be performed for various strains and antimicrobial agents. The RAST method has only been validated for eight species to date: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii, S. aureus, E. faecium*, and *S. pneumoniae* [24]. In this study, only *E. coli*, *K. pneumoniae*, and *S. aureus* were examined. Further evaluations using *P. aeruginosa, A. baumannii, E. faecalis, E. faecium*, and *S. pneumoniae* are needed. Practical application of the RAST method requires interpretation criteria for more antibiotics than currently available. Second, EUCAST-RAST cannot be readily adopted in clinical microbiology laboratories in Korea because some antibiotic disks in the EUCAST-RAST method are not available in Korea (Table 1). Moreover, the concentrations of piperacillin–tazobactam, cefotaxime, and ceftazidime required for testing *E. coli* and *K. pneumoniae* differ. Third, result interpretation is difficult when two or more species grow in a mixture of blood cultures. Fourth, because the strains were randomly collected, it was difficult to test combinations of various categories.

Despite these limitations, the RAST method shortens the TAT by more than one day; therefore, if applied properly according to laboratory conditions, AST results can be reported faster. Hence, EUCAST-RAST can be used for AST of positive blood cultures for certain bacterial species. However, the general application of EUCAST-RAST is limited to clinical microbiology laboratories, and further improvements are warranted.

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AUTHOR CONTRIBUTIONS

Conceptualization: Park JM and Yong D; formal analysis and data curation: Park JM and Kwon M; writing–original draft preparation: Park JM and Kwon M; writing–review and editing: Park JM, Kwon M, Hong KH, Lee H, and Yong D; supervision: Yong D. All authors read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. Goto M and Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect 2013;19:501-9.
2. Martinez RM and Wolk DM. Bloodstream infections. Microbiol Spectr 2016;4:653-89.
3. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for sepsis: for the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:762-74.
4. Cecconi M, Evans L, Levy M, Rhodes A. Sepsis and septic shock. Lancet 2018;392:75-87.
5. Ferrer R, Artigas A, Suarez D, Palencia E, Levy MM, Arenzana A, et al. Effectiveness of treatments for severe sepsis: a prospective, multicenter, observational study. Am J Respir Crit Care Med 2009;180:861-6.
6. Ferrer R, Martin-Loeches I, Phillips G, Osborn TM, Townsend S, Dellinger RP, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. Crit Care Med 2014;42:1749-55.
7. Puskarich MA, Trzeciak S, Shapiro NI, Arnold RC, Horton JM, Studnek JR, et al. Association between timing of antibiotic administration and mortality from septic shock in patients treated with a quantitative resuscitation protocol. Crit Care Med 2011;39:2066-71.
8. Vilella AL and Seifert CF. Timing and appropriateness of initial antibiotic

therapy in newly presenting septic patients. Am J Emerg Med 2014;32:7-13.
9. Sterling SA, Miller WR, Pryor J, Puskarich MA, Jones AE. The impact of timing of antibiotics on outcomes in severe sepsis and septic shock: a systematic review and meta-analysis. Crit Care Med 2015;43:1907-15.
10. Munita JM and Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr 2016;4:481-511.
11. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 2017;17:153-63.
12. Denis B, Lafaurie M, Donay JL, Fontaine JP, Oksenhendler E, Raffoux E, et al. Prevalence, risk factors, and impact on clinical outcome of extended-spectrum beta-lactamase-producing *Escherichia coli* bacteraemia: a five-year study. Int J Infect Dis 2015;39:1-6.
13. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791-8.
14. Wilson ML, Weinstein MP, Reller LB. Laboratory detection of bacteremia and fungemia. In: Manual of clinical microbiology, 2015:15-28.
15. Yu SH, Lee JH, Kim MC, Choi SH, Chung JW, Lee MK. Ten-year prevalence trends of phenotypically identified community-associated methicillin-resistant *Staphylococcus aureus* strains in clinical specimens. Ann Lab Med 2021;41:386-93.
16. Turnidge JD. Susceptibility test methods: general considerations. In: Jorgensen JH, Carroll KC, et al. eds. Manual of clinical microbiology. Hoboken: Wiley, 2015:1246-52.
17. Jorgensen JH and Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. In: Jorgensen JH, Carroll KC, et al. eds. Manual of clinical microbiology. Hoboken: Wiley, 2015:1253-73.
18. Karlowsky JA and Richter SS. Antimicrobial susceptibility testing systems. In: Jorgensen JH, Carroll KC, et al. eds. Manual of clinical microbiology. Hoboken: Wiley, 2015:1274-85.
19. Bauer A. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966;45:149-58.
20. CLSI. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2022.
21. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. version 12.0. Växjö, Sweden: European Committee on Antimicrobial Susceptibility Testing, 2022.
22. Kahlmeter G, Giske CG, Kirn TJ, Sharp SE. Point-counterpoint: differences between the European committee on antimicrobial susceptibility testing and Clinical and Laboratory Standards Institute recommendations for reporting antimicrobial susceptibility results. J Clin Microbiol 2019;57:e01129-19.
23. European Committee on Antimicrobial Susceptibility Testing. Methodology-EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles version 3.0. Växjö, Sweden: European Committee on Antimicrobial Susceptibility Testing, 2022.
24. European Committee on Antimicrobial Susceptibility Testing. Zone diameter breakpoint tables for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles version 5.1. Växjö, Sweden: European Committee on Antimicrobial Susceptibility Testing, 2022.
25. Kim D, Yoon EJ, Hong JS, Choi MH, Kim HS, Kim YR, et al. Major bloodstream infection-causing bacterial pathogens and their antimicrobial resistance in South Korea, 2017-2019: phase I report from Kor-GLASS. Front Microbiol 2022;12:799084.
26. CLSI. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2019.
27. European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST version 12.0. Växjö, Sweden: European Committee on Antimicrobial Susceptibility Testing, 2022.
28. U.S. Food and Drug Administration. Class II special controls guidance document: antimicrobial susceptibility test (AST) systems. Rockville, MD: Food and Drug Administration, 2009.

https://doi.org/10.3343/alm.2023.43.5.443