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Evaluation of the QMAC-dRAST System Version 2.5 for Rapid Antimicrobial Susceptibility Testing of Gram-Negative Bacteria From Positive Blood Culture Broth and Subcultured Colony Isolates

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Keywords: antimicrobial resistance | antimicrobial susceptibility testing | bloodstream infections | broth microdilution | Gram-negative | positive blood culture broth | QMAC-dRAST | VITEK 2

ABSTRACT

Background: Rapid antimicrobial susceptibility testing (AST) for bloodstream infections (BSIs) facilitates the optimization of antimicrobial therapy, preventing antimicrobial resistance and improving patient outcomes. QMAC-dRAST (QuantaMatrix Inc., Korea) is a rapid AST platform based on microfluidic chip technology that performs AST directly using positive blood culture broth (PBCB). This study evaluated the performance of QMAC-dRAST for Gram-negative bacteria using PBCB and subcultured colony isolates, comparing it with that of VITEK 2 (bioMérieux, France) using broth microdilution (BMD) as the reference method.

Methods: We included 141 Gram-negative blood culture isolates from patients with BSI and 12 carbapenemase-producing clinical isolates of Enterobacterales spiked into blood culture bottles. QMAC-dRAST performance was evaluated using PBCB and colony isolates, whereas VITEK 2 and BMD were tested only on colony isolates.

Results: For PBCB, QMAC-dRAST achieved 92.1% categorical agreement (CA), 95.3% essential agreement (EA), with 1.8% very major errors (VMEs), 3.5% major errors (MEs), and 5.2% minor errors (mEs). With colony isolates, it exhibited 92.5% CA and 95.1% EA, with 2.0% VMEs, 3.2% MEs, and 4.8% mEs. VITEK 2 showed 94.1% CA and 96.0% EA, with 4.3% VMEs, 0.4% MEs, and 4.3% mEs. QMAC-dRAST yielded elevated error rates for specific antimicrobial agents, with high VMEs for carbapenems and aminoglycosides. The median time to result for QMAC-dRAST was 5.9 h for PBCB samples and 6.1 h for subcultured colony isolates.

Conclusions: The QMAC-dRAST system demonstrated considerable strengths and comparable performance to the VITEK 2 system; however, challenges were discerned with specific antimicrobial agents, underlining a necessity for improvement.

Tae Yeul Kim and Minhee Kang contributed equally to this work.

1 | Introduction

Sepsis is a major cause of morbidity and mortality worldwide [1–3]. Early administration of broad-spectrum antibiotics is crucial for sepsis management; however, prolonged use contributes to the emergence and spread of antimicrobial resistance (AMR) and increases the risk of *Clostridioides difficile* infection [4, 5]. Accordingly, once the causative pathogen and its antimicrobial susceptibility are known, de-escalation of empirical broad-spectrum antibiotics to targeted therapy is recommended [6]. However, the slow turnaround time of conventional antimicrobial susceptibility testing (AST) methods (approximately 48–72 h from the time a blood culture turns positive) is a major bottleneck that delays the de-escalation of empirical broad-spectrum antibiotics to targeted antibiotics [7, 8]. To minimize such delays, rapid AST methods that provide results within a few hours of blood culture positivity have been developed, a few of which have been approved by regulatory agencies [9, 10].

QMAC-dRAST (QuantaMatrix, Seoul, Korea) is a rapid AST system that performs AST directly on positive blood culture broth (PBCB) based on microfluidic chip technology within 6 h [7]. This system immobilizes bacterial cells using microfluidic agarose channel technology and monitors their growth under different concentrations of antibiotics using time-lapse microscopy. Recently, a new version of this system, QMAC-dRAST v2.5, has been developed and has received a Conformité Européenne In Vitro Diagnostic (CE-IVD) marking. This version differs from its non-commercialized prototype, QMAC-dRAST v2.0, by introducing several advanced features. These include automated reading and interpretation of AST results after 4, 5, and 6 h (which reduces reporting time) and an internal structure designed to minimize cross-contamination. However, studies addressing the performance of this latest version are lacking. Therefore, this study aimed to evaluate the performance of QMAC-dRAST for Gram-negative bacteria (GNB) from PBCB and subcultured colony isolates and compare it with that of VITEK 2 (bioMérieux, Marcy l'Etoile, France), using broth microdilution (BMD) as the reference method.

2 | Materials and Methods

2.1 | Study Design

This prospective study was conducted at Samsung Medical Center, a tertiary care hospital in Seoul, South Korea. We examined 153 GNB isolates, which included 141 blood culture isolates obtained from patients with bloodstream infections between April and November 2020 and 12 blood culture samples artificially spiked with carbapenemase-producing Enterobacterales (CPE) clinical isolates. Specimens containing Gram-positive organisms, fungi, or multiple organisms were excluded. This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB No. SMC 2016-01-102). The requirement for informed consent was waived.

2.2 | Species Identification and AST

Blood culture bottles (BacT/ALERT FA Plus and FN Plus; bioMérieux) were incubated in a fully automated blood culture system (BacT/ALERT VIRTUO; bioMérieux) for up to 5 days. Organisms were identified using VITEK-MS (bioMérieux). The minimum inhibitory concentrations (MICs) were determined using four different methods: QMAC-dRAST 2.5 (software version 1.2.5) with two types of inocula (PBCB and subcultured colony isolates), VITEK 2, and the BMD method. Following the detection of positive blood culture, PBCB was promptly removed from the culture system and consistently utilized directly in the QMAC-dRAST system upon bacterial identification. This was followed by subculturing on a blood agar plate and incubation overnight at 35°C. Subcultured colony isolates were used for the QMAC-dRAST, VITEK 2, and BMD tests, which were performed in parallel. The CLSI M100-Ed33 breakpoints were used to interpret the results (the breakpoints for the antimicrobial agent–microorganism combinations are described in Table S1) [11].

2.3 | AST Using QMAC-dRAST

Sample preparation involved retrieving 1 mL of the suspension from PBCB with a sterile syringe and transferring it into 5 mL polystyrene tubes without additional processing. The sample tubes were then inserted into the designated sample slots along with the prepackaged QMAC-dRAST consumables, as prompted by the QMAC-dRAST instrument. An appropriate number of Gram-negative AST panels was then inserted into the instrument side panel. The antibiotics included in the QMAC-dRAST kit (the Clinical and Laboratory Standards Institute [CLSI] GN panels) used in this study are listed in Table S2. Extended-spectrum beta-lactamases (ESBL) were detected using QMAC-dRAST only for *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Upon initiation of the test, the QMAC-dRAST instrument automatically prepares, incubates, acquires time-lapse images, and interprets the AST results for each sample. AST was also performed using the QMAC-dRAST system by spiking CPE clinical isolates into blood culture bottles. The bacterial spiking protocol and list are provided in Table S3.

2.4 | AST Using the VITEK 2 System and the CLSI BMD Method

Colony isolates were tested for AST using the VITEK 2 system with AST-N224 or AST-N225 cards, following the manufacturer's protocol. ESBLs were detected by the VITEK 2 system only for *E. coli*, *K. oxytoca*, and *K. pneumoniae*. Furthermore, AST using BMD with colony isolates was performed as recommended by the CLSI guideline (Appendix S1) [12].

2.5 | Data Analysis

Results for antimicrobial agents present in both the QMAC-dRAST and VITEK 2 panels were included in the data analysis. Colistin was excluded because CLSI-EUCAST only recommended BMD as an acceptable method [13]. Cefazolin was excluded from the analysis because the MIC range of VITEK 2 could not distinguish between intermediate and susceptible isolates. Categorical agreement (CA), essential agreement (EA), rates of very major errors (VMEs), major errors (MEs), and minor errors (mEs) were assessed. CA was defined

as the total number of tested isolates that yielded the same categorical interpretation as those in the reference method. EA was defined as obtaining a MIC within one doubling dilution of the reference method. VMEs were defined as the number of isolates interpreted as resistant by the reference method but susceptible by the test method. MEs were defined as the number of isolates interpreted as susceptible by the reference method but resistant by the test method. mEs were defined as the number of isolates identified as intermediate by one test and as susceptible or resistant by the other.

The validity criteria used in this study, based on CLSI document M15, were as follows: CA ≥ 90%, VME ≤ 3%, and ME ≤ 3% when compared to the reference method [14].

2.6 | Time to Result

Time to result (TTR) was measured between T0 and Tr. Point T0 corresponds to the beginning of the QMAC-dRAST process, that is, when all samples and reagents were correctly positioned in the device and the start button was pressed. Tr corresponds to the time when the AST results were available. An independent *t*-test was used for the comparison of TTR for both inocula. A p value of <0.05 was considered statistically significant.

3 | Results

A total of 123 Enterobacterales were tested: 46 *E. coli*, 44 *K. pneumoniae*, 11 *Enterobacter cloacae*, 6 *Klebsiella aerogenes*, 5 *P. mirabilis*, 5 *Serratia marcescens*, 4 *Citrobacter freundii*, 1 *Citrobacter braakii*, and 1 *K. oxytoca*. Furthermore, a total of 30 nonfermenting GNBs were tested: 19 *Pseudomonas aeruginosa*, 7 *Acinetobacter baumanii*, 2 *Acinetobacter nosocomialis*, 1 *Acinetobacter gyllenbergii*, and 1 *Acinetobacter pittii*.

AST results were available for all 153 isolates tested against 17 antimicrobial agents, including ESBL, resulting in 1993 AST measurements available for evaluation. The overall performance of the three test methods—the QMAC-dRAST system with PBCB, QMAC-dRAST with colony isolates, and the VITEK 2 system—was compared with that of BMD. The overall CA and VME rates for the QMAC-dRAST system with PBCB/colony isolates relative to the BMD test were >90% and <3%, respectively, which satisfied the target criteria for both inocula types (Table 1). However, the ME rates exceeded 3% for both. The VITEK 2 system showed acceptable CA and MEs, but a high VME rate exceeding 3% (Table 1).

The distribution of agreements and errors for each method by individual antimicrobial agents for Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp. is provided in Table 2. Additionally, the overall distribution of agreements, errors, and diagnostic accuracies for each method with respect to individual antimicrobial agents is presented in Tables S4–S7. The QMAC-dRAST system with PBCB yielded results comparable to those with colony isolates. The CA for QMAC-dRAST was <90% for piperacillin–tazobactam, ceftazidime, cefepime, meropenem, and aztreonam for both types of inocula, whereas the EA was below 90% only for piperacillin–tazobactam and meropenem for both inocula types. Moreover, high error rates were noted in QMAC-dRAST for specific antimicrobial agents as follows: (1) high VME rates for piperacillin–tazobactam, ertapenem, imipenem, amikacin, and gentamicin; (2) high ME rates for trimethoprim–sulfamethoxazole and most β-lactam agents. For the VITEK 2 system, the CAs and EAs exceeded 90% for all agents, except cefepime. The VME rates for VITEK 2 exceeded 3% for most β-lactam agents (cephalosporins, carbapenems, and aztreonam), amikacin, and trimethoprim–sulfamethoxazole. However, the ME rates for each antimicrobial agent were all <3%. The performance of each method stratified by Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp. strains is represented in Tables S8–S10. ESBL screening results were compared across *E. coli*, *K. oxytoca*, and *K. pneumoniae* isolates ($n = 91$). In QMAC-dRAST, 96.7% (29/30) of ESBL screens were positive for both inocula, with no false positives detected among non-ESBL-producing isolates (0/61). In the VITEK 2 system, 100.0% (29/30) of ESBL screens were positive, though 6.6% (4/61) false positives were identified among non-ESBL-producing isolates. All the false-positive ESBL screens occurred with *K. pneumoniae*.

The overall median TTR using QMAC-dRAST was 5.9 h (interquartile range [IQR], 5.3–6.4 h) and 6.1 h (IQR, 5.8–6.5 h) for PBCB samples and subcultured colony isolates, respectively. The median TTRs for Enterobacterales, *P. aeruginosa*, and *Acinetobacter* spp. strains are listed in Table 3. According to our analysis, the median TTR for PBCB and colony isolates varies significantly depending on the organism, except for *Acinetobacter* spp. However, no significant differences were noted between the time length and the vast majority of antimicrobial agents (Table 3).

4 | Discussion

Although rapid phenotypic AST methods have not demonstrated a significant reduction in mortality in most studies [15, 16], they have the potential to shorten the time to optimal therapy and improve antibiotic stewardship in patients with bloodstream

TABLE 1 | Overall performance of the QMAC-dRAST and VITEK 2 systems compared with the BMD method for GNB (number of isolates = 153).

Method	Categorical agreement (%)	Essential agreement (%)	Errors (%)		
			VME	ME	mE
QMAC-dRAST system with positive blood culture broth	92.1 (1751/1902)	95.3 (1813/1902)	1.8 (11/611)	3.5 (42/1217)	5.2 (98/1902)
QMAC-dRAST system with colony isolates	92.5 (1760/1902)	95.1 (1809/1902)	2.0 (12/611)	3.2 (39/1217)	4.8 (91/1902)
VITEK 2 system	94.1 (1789/1902)	96.0 (1825/1902)	4.3 (26/611)	0.4 (5/1217)	4.3 (82/1902)

Abbreviations: BMD, broth microdilution; GNB, Gram-negative bacteria; ME, major error; mE, minor error; VME, very major error.

TABLE 2 | Agreement and error distribution for each method stratified by antimicrobial agents for Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter* species.

Organism/ Antimicrobial agent	No. of isolates	CLSI BMD			QMAC-dRAST using PBCB					QMAC-dRAST using colony isolates					VITEK 2				
		S	I	R	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE
Enterobacterales ($n = 123$)																			
β-Lactams																			
Ampicillin	123	11	0	112	99.2%	99.2%	0.0%	9.1%	0.0%	100.0%	99.2%	0.0%	0.0%	0.0%	99.2%	94.3%	0.9%	0.0%	0.0%
					(122/123)	(122/123)	(0/112)	(1/11)	(0/123)	(123/123)	(122/123)	(0/112)	(0/11)	(0/123)	(122/123)	(116/123)	(1/112)	(0/11)	(0/123)
Amoxicillin–clavulanate	123	57	17	49	87.0%	100.0%	0.0%	0.0%	13.0%	90.2%	100.0%	0.0%	0.0%	9.8%	90.2%	96.7%	0.0%	1.8%	8.9%
					(107/123)	(123/123)	(0/49)	(0/57)	(16/123)	(111/123)	(123/123)	(0/49)	(0/57)	(12/123)	(111/123)	(119/123)	(0/49)	(1/57)	(11/123)
Piperacillin–tazobactam	123	85	9	29	90.2%	92.7%	6.9%	3.5%	5.7%	92.7%	95.1%	6.9%	0.0%	5.7%	89.4%	95.1%	0.0%	0.0%	10.6%
					(111/123)	(114/123)	(2/29)	(3/85)	(7/123)	(114/123)	(117/123)	(2/29)	(0/85)	(7/123)	(110/123)	(117/123)	(0/29)	(0/85)	(13/123)
Cefotaxime	123	66	1	56	95.9%	95.1%	0.0%	4.5%	1.6%	98.4%	95.9%	0.0%	0.0%	1.6%	98.4%	95.1%	3.6%	0.0%	0.0%
					(118/123)	(117/123)	(0/56)	(3/66)	(2/123)	(121/123)	(118/123)	(0/56)	(0/66)	(2/123)	(121/123)	(117/123)	(2/56)	(0/66)	(0/123)
Ceftazidime	123	77	1	45	93.5%	95.9%	0.0%	5.2%	3.3%	95.1%	98.4%	2.2%	1.3%	3.3%	95.1%	96.7%	4.4%	0.0%	3.3%
					(115/123)	(118/123)	(0/45)	(4/77)	(4/123)	(117/123)	(121/123)	(1/45)	(1/77)	(4/123)	(117/123)	(119/123)	(2/45)	(0/77)	(4/123)
Cefepime	123	74	11	38	88.6%	95.9%	0.0%	5.4%	8.1%	91.1%	96.7%	2.6%	2.7%	6.5%	81.3%	84.6%	15.8%	1.4%	13.0%
					(109/123)	(118/123)	(0/38)	(4/74)	(10/123)	(112/123)	(119/123)	(1/38)	(2/74)	(8/123)	(100/123)	(104/123)	(6/38)	(1/74)	(16/123)
Ertapenem	123	97	6	20	91.9%	99.2%	5.0%	0.0%	7.3%	91.9%	98.4%	10.0%	0.0%	6.5%	95.9%	98.4%	5.0%	0.0%	3.3%
					(113/123)	(122/123)	(1/20)	(0/97)	(9/123)	(113/123)	(121/123)	(2/20)	(0/97)	(8/123)	(118/123)	(121/123)	(1/20)	(0/97)	(4/123)
Imipenem	123	99	6	18	90.2%	92.7%	11.1%	2.0%	6.5%	91.1%	94.3%	11.1%	1.0%	6.5%	91.9%	96.7%	5.6%	0.0%	7.3%
					(111/123)	(114/123)	(2/18)	(2/99)	(8/123)	(112/123)	(116/123)	(2/18)	(1/99)	(8/123)	(113/123)	(119/123)	(1/18)	(0/99)	(9/123)
Aztreonam	123	76	4	43	92.7%	94.3%	2.3%	6.6%	2.4%	94.3%	96.7%	2.3%	2.6%	3.3%	94.3%	95.1%	7.0%	0.0%	3.3%
					(114/123)	(116/123)	(1/43)	(5/76)	(3/123)	(116/123)	(119/123)	(1/43)	(2/76)	(4/123)	(116/123)	(117/123)	(3/43)	(0/76)	(4/123)
Fluoroquinolones																			
Ciprofloxacin	123	69	3	51	97.6%	96.7%	0.0%	0.0%	2.4%	97.6%	97.6%	0.0%	0.0%	2.4%	95.1%	98.4%	0.0%	2.9%	3.3%
					(120/123)	(119/123)	(0/51)	(0/69)	(3/123)	(120/123)	(120/123)	(0/51)	(0/69)	(3/123)	(117/123)	(121/123)	(0/51)	(2/69)	(4/123)
Aminoglycosides																			
Amikacin	123	120	1	2	98.4%	98.4%	50.0%	0.0%	0.8%	98.4%	98.4%	50.0%	0.0%	0.8%	99.2%	99.2%	0.0%	0.0%	0.8%
					(121/123)	(121/123)	(1/2)	(0/120)	(1/123)	(121/123)	(121/123)	(1/2)	(0/120)	(1/123)	(122/123)	(122/123)	(0/2)	(0/120)	(1/123)
Gentamicin	123	96	0	27	95.1%	98.4%	7.4%	0.0%	3.3%	93.5%	98.4%	7.4%	0.0%	4.9%	100.0%	100.0%	0.0%	0.0%	0.0%
					(117/123)	(121/123)	(2/27)	(0/96)	(4/123)	(115/123)	(121/123)	(2/27)	(0/96)	(6/123)	(123/123)	(123/123)	(0/27)	(0/96)	(0/123)
Sulfonamides																			
Trimethoprim–sulfamethoxazole	123	75	0	48	95.9%	97.6%	0.0%	6.7%	0.0%	97.6%	98.4%	0.0%	4.0%	0.0%	96.7%	97.6%	6.3%	1.3%	0.0%
					(118/123)	(120/123)	(0/48)	(5/75)	(0/123)	(120/123)	(121/123)	(0/48)	(3/75)	(0/123)	(119/123)	(120/123)	(3/48)	(1/75)	(0/123)

(Continues)

TABLE 2 | (Continued)

Organism/ Antimicrobial agent	No. of isolates	CLSI BMD			QMAC-dRAST using PBCB					QMAC-dRAST using colony isolates					VITEK 2				
		S	I	R	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE
ESBL (for *K. pneumoniae*, *K. oxytoca*, *E. coli*)																			
ESBL	91	61	0	30	99.0%	—	3.3%	0.0%	—	99.0%	—			—	94.5%	—	0.0%	6.6%	—
					(90/91)	—	(1/30)	(0/61)	—	(90/91)	—	(1/30)	(0/61)	—	(87/91)	—	(0/30)	(4/61)	—
Pseudomonas aeruginosa ($n = 19$)																			
β-Lactams																			
Piperacillin–tazobactam	19	17	1	1	63.2%	68.4%	0.0%	11.8%	26.3%	47.4%	47.4%	0.0%	17.6%	36.8%	89.5%	94.7%	0.0%	0.0%	10.5%
					(12/19)	(13/19)	(0/1)	(2/17)	(5/19)	(9/19)	(9/19)	(0/1)	(3/17)	(7/19)	(17/19)	(18/19)	(0/1)	(0/17)	(2/19)
Cefepime	19	16	3	0	68.4%	73.7%	0.0%	25.0%	10.5%	57.9%	57.9%	0.0%	43.8%	5.3%	89.5%	94.7%	0.0%	0.0%	10.5%
					(13/19)	(14/19)	(0/0)	(4/16)	(2/19)	(11/19)	(11/19)	(0/0)	(7/16)	(1/19)	(17/19)	(18/19)	(0/0)	(0/16)	(2/19)
Ceftazidime	19	17	1	1	68.4%	68.4%	100.0%	17.6%	10.5%	57.9%	63.2%	0.0%	29.4%	15.8%	94.7%	100.0%	0.0%	0.0%	5.3%
					(13/19)	(13/19)	(1/1)	(3/17)	(2/19)	(11/19)	(12/19)	(0/1)	(5/17)	(3/19)	(18/19)	(19/19)	(0/1)	(0/17)	(1/19)
Imipenem	19	16	1	2	84.2%	94.7%	50.0%	0.0%	10.5%	89.5%	100.0%	0.0%	0.0%	10.5%	89.5%	94.7%	50.0%	0.0%	5.3%
					(16/19)	(18/19)	(1/2)	(0/16)	(2/19)	(17/19)	(19/19)	(0/2)	(0/16)	(2/19)	(17/19)	(18/19)	(1/2)	(0/16)	(1/19)
Meropenem	19	16	1	2	63.2%	78.9%	0.0%	12.5%	26.3%	52.6%	63.2%	0.0%	25.0%	26.3%	89.5%	89.5%	50.0%	0.0%	5.3%
					(12/19)	(15/19)	(0/2)	(2/16)	(5/19)	(10/19)	(12/19)	(0/2)	(4/16)	(5/19)	(17/19)	(17/19)	(1/2)	(0/16)	(1/19)
Aztreonam	19	15	1	3	52.6%	84.2%	0.0%	0.0%	47.4%	52.6%	68.4%	0.0%	33.3%	21.1%	78.9%	89.5%	0.0%	0.0%	21.1%
					(10/19)	(16/19)	(0/3)	(0/15)	(9/19)	(10/19)	(13/19)	(0/3)	(5/15)	(4/19)	(15/19)	(17/19)	(0/3)	(0/15)	(4/19)
Fluoroquinolones																			
Ciprofloxacin	19	16	1	2	94.7%	89.5%	0.0%	0.0%	5.3%	94.7%	84.2%	0.0%	0.0%	5.3%	89.5%	100.0%	0.0%	0.0%	10.5%
					(18/19)	(17/19)	(0/2)	(0/16)	(1/19)	(18/19)	(16/19)	(0/2)	(0/16)	(1/19)	(17/19)	(19/19)	(0/2)	(0/16)	(2/19)
Aminoglycosides																			
Amikacin	19	19	0	0	94.7%	94.7%	0.0%	0.0%	5.3%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(18/19)	(18/19)	(0/0)	(0/19)	(1/19)	(19/19)	(19/19)	(0/0)	(0/19)	(0/19)	(19/19)	(19/19)	(0/0)	(0/19)	(0/19)
Gentamicin	19	19	0	0	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(19/19)	(19/19)	(0/0)	(0/19)	(0/19)	(19/19)	(19/19)	(0/0)	(0/19)	(0/19)	(19/19)	(19/19)	(0/0)	(0/19)	(0/19)
Acinetobacter species ($n = 11$)																			
β-Lactams																			
Ampicillin–sulbactam	11	5	1	5	90.9%	100.0%	0.0%	0.0%	9.1%	81.8%	90.9%	0.0%	20.0%	9.1%	90.9%	100.0%	0.0%	0.0%	9.1%
					(10/11)	(11/11)	(0/5)	(0/5)	(1/11)	(9/11)	(10/11)	(0/5)	(1/5)	(1/11)	(10/11)	(11/11)	(0/5)	(0/5)	(1/11)
Piperacillin–tazobactam	11	5	0	6	81.8%	81.8%	0.0%	20.0%	9.1%	63.6%	63.6%	0.0%	20.0%	27.3%	100.0%	100.0%	0.0%	0.0%	0.0%
					(9/11)	(9/11)	(0/6)	(1/5)	(1/11)	(7/11)	(7/11)	(0/6)	(1/5)	(3/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)
Cefepime	11	5	0	6	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)

(Continues)

TABLE 2 | (Continued)

Organism/ Antimicrobial agent	No. of isolates	CLSI BMD			QMAC-dRAST using PBCB					QMAC-dRAST using colony isolates					VITEK 2				
		S	I	R	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE	CA	EA	VME	ME	mE
Cefotaxime	11	3	3	5	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	90.9%	0.0%	0.0%	0.0%
					(11/11)	(11/11)	(0/5)	(0/3)	(0/11)	(11/11)	(11/11)	(0/5)	(0/3)	(0/11)	(11/11)	(10/11)	(0/5)	(0/3)	(0/11)
Ceftazidime	11	5	1	5	81.8%	90.9%	0.0%	20.0%	9.1%	81.8%	81.8%	0.0%	20.0%	9.1%	100.0%	90.9%	0.0%	0.0%	0.0%
					(9/11)	(10/11)	(0/5)	(1/5)	(1/11)	(9/11)	(9/11)	(0/5)	(1/5)	(1/11)	(11/11)	(10/11)	(0/5)	(0/5)	(0/11)
Imipenem	11	5	0	6	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)
Meropenem	11	5	0	6	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)
Fluoroquinolones																			
Ciprofloxacin	11	5	0	6	100.0%	81.8%	0.0%	0.0%	0.0%	90.9%	72.7%	0.0%	20.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%
					(11/11)	(9/11)	(0/6)	(0/5)	(0/11)	(10/11)	(8/11)	(0/6)	(1/5)	(0/11)	(11/11)	(11/11)	(0/6)	(0/5)	(0/11)
Aminoglycosides																			
Amikacin	11	6	0	5	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	63.6%	63.6%	80.0%	0.0%	0.0%
					(11/11)	(11/11)	(0/5)	(0/6)	(0/11)	(11/11)	(11/11)	(0/5)	(0/6)	(0/11)	(7/11)	(7/11)	(4/5)	(0/6)	(0/11)
Gentamicin	11	6	0	5	100.0%	100.0%	0.0%	0.0%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	90.9%	100.0%	0.0%	0.0%	9.1%
					(11/11)	(11/11)	(0/5)	(0/6)	(0/11)	(11/11)	(11/11)	(0/5)	(0/6)	(0/11)	(10/11)	(11/11)	(0/5)	(0/6)	(1/11)
Tetracyclines																			
Minocycline	11	9	1	1	90.9%	100.0%	0.0%	0.0%	9.1%	100.0%	100.0%	0.0%	0.0%	0.0%	90.9%	100.0%	0.0%	0.0%	9.1%
					(10/11)	(11/11)	(0/1)	(0/9)	(1/11)	(11/11)	(11/11)	(0/1)	(0/9)	(0/11)	(10/11)	(11/11)	(0/1)	(0/9)	(1/11)
Sulfonamides																			
Trimethoprim–sulfamethoxazole	11	5	0	6	81.8%	81.8%	0.0%	40.0%	0.0%	81.8%	81.8%	0.0%	40.0%	0.0%	90.9%	100.0%	16.7%	0.0%	0.0%
					(9/11)	(9/11)	(0/6)	(2/5)	(0/11)	(9/11)	(9/11)	(0/6)	(2/5)	(0/11)	(10/11)	(11/11)	(1/6)	(0/5)	(0/11)
Total	1993	1278	74	641	92.4%	95.3%	1.9%	3.3%	5.2%	92.8%	95.1%	2.0%	3.1%	4.8%	94.1%	96.0%	4.1%	0.7%	4.3%
					(1841/1993)	(1813/1902)	(12/641)	(42/1282)	(98/1902)	(1850/1993)	(1809/1902)	(13/641)	(39/1278)	(91/1902)	(1876/1993)	(1825/1902)	(26/641)	(9/1278)	(82/1902)

Abbreviations: BMD, broth microdilution; CA, categorical agreement; EA, essential agreement; ESBL, extended-spectrum beta-lactamase; I, intermediate; ME, major error; mE, minor error; R, resistant; S, susceptible; VME, very major error.

TABLE 3 | Median time to result in antimicrobial susceptibility testing using QMAC-dRAST.

Organism/ Antimicrobial agent	Time to result (h, median, and IQR)		
	PBCB	Colony isolates	*p*
Organism			
Enterobacterales	5.8 (5.2–6.4)	6.0 (5.8–6.4)	<0.001
Pseudomonas aeruginosa	6.3 (5.7–6.9)	6.7 (6.3–7.2)	<0.001
Acinetobacter spp.	6.0 (5.3–6.8)	5.8 (4.8–6.8)	ns
Antimicrobial agent			
Ampicillin	5.8 (5.2–6.3)	6.0 (5.8–6.3)	ns
Amoxicillin–clavulanate	5.8 (5.2–6.4)	6.0 (5.8–6.4)	ns.
Ampicillin–sulbactam	5.8 (5.2–6.4)	6.0 (5.8–6.4)	ns
Piperacillin–tazobactam	5.9 (5.3–6.7)	6.1 (5.8–6.7)	ns
Cefotaxime	5.8 (5.2–6.3)	6.0 (5.8–6.3)	ns
Ceftazidime	5.8 (5.0–6.4)	6.1 (5.8–6.5)	<0.01
Cefepime	6.2 (5.4–6.8)	6.2 (5.8–6.7)	ns
Ertapenem	5.8 (5.0–6.3)	6.0 (5.8–6.4)	ns
Imipenem	5.9 (5.5–6.4)	6.2 (5.8–6.5)	ns
Meropenem	5.9 (5.3–6.4)	6.1 (5.8–6.5)	ns
Aztreonam	5.9 (5.3–6.6)	6.2 (5.8–6.8)	0.037
Ciprofloxacin	6.0 (5.7–6.8)	6.2 (5.8–6.7)	ns
Amikacin	5.8 (5.2–6.3)	6.0 (5.8–6.4)	0.025
Gentamicin	5.8 (5.0–6.3)	6.0 (5.8–6.4)	0.033
Minocycline	6.6 (4.8–6.9)	5.8 (4.8–6.7)	ns
Trimethoprim–sulfamethoxazole	5.8 (5.2–6.4)	6.0 (5.8–6.4)	ns

Abbreviations: IQR, interquartile range; ns, not significant.

infections [10]. The QMAC-dRAST system provides rapid results and may be a promising alternative to conventional AST methods [7, 17–21]. Previous versions of QMAC-dRAST required 6 h of incubation, excluding the time for preincubation preparation, imaging, or reporting [18, 22]. The new version (v2.5) of QMAC-dRAST is a random-access automated system in which all these processes are fully automated. In version 2.5, the preincubation time has been reduced to 45 min and the time required to obtain the results has been shortened by using a dynamic decision algorithm based on the speed of bacterial growth within a range of 4–6 h. This feature enables a continuous workflow, enhancing efficiency and throughput in the laboratory setting. In this study, we demonstrated that the median TTR with the QMAC-dRAST v2.5 system for PBCB was <6 h, representing a significant decrease in time compared with the VITEK 2 system [23–25] or the previous version of QMAC-dRAST [18, 22, 26, 27].

This study focused on a parallel performance comparison of QMAC-dRAST, VITEK 2, and BMD. Considering the unpredictable bacterial concentrations in PBCB (ranging from 10^7 to 10^9 CFU/mL) along with the presence of blood cells, standardizing the inoculum size through subcultured colony isolates ensures an unbiased assessment of their individual performances, facilitating more dependable and comparable results. Our findings indicate that QMAC-dRAST performs similarly whether utilizing PBCB or subcultured colony isolates, confirming the system's robustness against variations in inoculum size. Furthermore, the false susceptibility to amikacin displayed by *Acinetobacter baumannii* (CA 42.9%) in VITEK 2 was not demonstrated in QMAC-dRAST (CA 100% for both inocula). However, in comparison with VITEK 2, despite demonstrating notable strengths and comparable performance, the QMAC-dRAST system presents certain challenges. First, high ME rates were noted for cefepime, ceftazidime, and piperacillin–tazobactam, which were most evident in *P. aeruginosa* strains. These results are consistent with performances reported in previous studies [19, 22, 28]. Second, despite CAs exceeding 95%, Enterobacterales exhibited high VME rates for aminoglycosides, specifically amikacin and gentamicin, aligning with previous findings [14, 17, 21]. Aminoglycosides are one of the most effective drugs used for CRE infections, particularly urinary tract infections [29], and addressing high VME rates for these drugs should be a focus in the next version of QMAC-dRAST. Lastly, the QMAC-dRAST system exhibited a less favorable performance with carbapenems, resulting in a lower CA for meropenem, and most errors were classified as VMEs for ertapenem and imipenem. Despite previous studies evaluating the performance of QMAC-dRAST for GNB, such findings have not yet been fully described, likely because these previous studies included minimum to no carbapenem-resistant (CR) GNB [17, 19, 30, 31]. The escalation in the global prevalence of CRGNB, including CPE, poses substantial threats to public health and emphasizes the importance of rapid and accurate detection of carbapenem resistance [32–34]. Consequently, more robust performance evaluations of QMAC-dRAST for specific antimicrobial agents, such as carbapenems, and performance improvement are needed.

However, this study has certain limitations, including the restricted number of resistant phenotypes such as amikacin and carbapenems. The distribution of resistance phenotypes, especially concerning cephalosporin and carbapenem-resistant strains, was not thoroughly addressed. Although blood culture bottles spiked with CPE isolates were included, a more extensive study should be conducted with a sufficient number of positive blood cultures from patients.

In conclusion, the QMAC-dRAST system demonstrated rapid results and performance comparable to that of the VITEK 2 system. Despite a few challenges observed with specific antimicrobial agents, indicating areas for improvement, QMAC-dRAST is poised to become a rapid AST tool in clinical microbiology laboratories.

Author Contributions

Kim T.Y. was responsible for conceptualization, methodology, investigation, writing the original draft, reviewing, and editing. Kang M. was responsible for formal analysis, data curation, writing the original draft, reviewing, and editing. Shim H.J. and Kang O.-K. contributed to the experiment. Huh H.J. and Lee N.Y. supervised the research and provided the samples for the clinical tests. Huh H.J. was responsible for

conceptualization and methodology and contributed to the design of this study. All authors have read, reviewed, and approved the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.