Original article

Clinical usefulness of the QMAC-dRAST system for AmpC β-lactamase-producing *Enterobacterales* in Korea

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Abstract

Background: Rapid antimicrobial susceptibility testing (RAST) is important for the appropriate treatment of bloodstream infections. The QMAC-dRAST system (QuantaMatrix Inc., Korea) can directly perform RAST using positive blood culture samples with microscopic imaging. This study aimed to evaluate the performance of the QMAC-dRAST system for AmpC-β-lactamase-producing *Enterobacterales.*

Methods: Eighty isolates (20 *Morganella morganii*, 20 *Serratia marcescens*, 10 *Klebsiella aerogenes*, 10 *Enterobacter cloacae*, and 20 *Citrobacter freundii*) and 14 antimicrobial agents were included in the antimicrobial susceptibility testing (AST). The performance of the QMAC-dRAST system was evaluated by simulating the clinical blood culturing process. We conducted a comparative evaluation of the QMAC-dRAST and Vitek 2 systems (bioMérieux Inc., France). Broth microdilution tests were performed as the reference method to resolve any discrepancies in the AST results between the two systems.

Results: For 20 *M. morganii* and 20 *S. marcescens*, the categorical agreement (CA) between the QMAC-dRAST and Vitek 2 systems increased from 55.4% to 83.8% after AST algorithm optimization. Moreover, the discrepancy rates decreased as follows: from 19.1% to 5.4% very major errors (VME), from 38.3% to 4.3% major errors (ME), and from 14.6% to 12.1% minor errors (mE) for the QMAC-dRAST system compared to the Vitek 2 system. For all 80 tested isolates, the QMAC-dRAST system showed 93.0% CA, 1.7% VME, 2.3% ME, and 4.9% mE.

Conclusion: The QMAC-dRAST system was comparable to the Vitek 2 system after AST algorithm optimization for AmpC β-lactamase-producers, which are major pathogens and require time to express the enzyme. However, further modifications of the AST algorithm are still warranted.

Keywords: Antimicrobial susceptibility testing, AmpC β-lactamase, Bacteremia

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Introduction

The ever-increasing number of antibiotic-resistant bacteria poses a worldwide public health threat [1,2]. Rapid antimicrobial susceptibility testings (RASTs) provide the tools for choosing appropriate antibiotics for prompt and effective treatment of bloodstream infections, thereby decreasing morbidity and mortality [3-5]. There are several commercial phenotypic antimicrobial susceptibility testings (ASTs) for identifying pathogens in blood, such as Vitek 2 (bioMérieux Inc., Marcy l'Etoile, France), the MicroScan system (Siemens Healthcare, Sacramento, CA, USA), and the Phoenix system (BD Diagnostic Systems, Sparks, MD, USA) [6-8]. However, these AST methods rely on the growth of blood cultures, which takes more than a day, thus delaying the treatment of patients with the most effective antibiotics [9].

We evaluated an automated, direct & RAST (dRAST) system called QMAC-dRAST (QuantaMatrix Inc., Seoul, Korea). This system uses micropatterned plastic microchips with a nutrient agarose well containing patient blood samples atop a layer of dried antibiotic at different concentrations [10]. The system analyzes bacterial microcolony growth over time directly in the wells with different antibiotics that diffuse into the agarose layer with the sample [6,10,11]. The bacterial growth is measured by time-lapse microscopic imaging in 6 hours [6,10,11]. Several studies have evaluated the clinical performance of QMAC-dRAST [12-15], however, performance data on QMAC-dRAST in AmpC β-lactamase-producing *Enterobacterales* are still insufficient.

AmpC β-lactamases are enzymes that inactivate cephalosporin antibiotics by cleaving their β-lactam ring. The genes for these enzymes, which are found on the chromosomes of many *Enterobacterales*, are induced by β-lactam antibiotics, leading to resistance to broad-spectrum cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone [16]. *Enterobacterales* with the ability to make inducible AmpC β-lactamases, such as *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and *Morganella morganii* are major causes of bloodstream infections. However, resistance to antimicrobial agents has increased in these species and the development of new drugs is insufficient to ensure adequate treatments [17,18]. In a landmark study by Chow et al.[19], 19% (6 of 31) of the *Enterobacterales* strains isolated from patients with bacteremia were resistant to broad-spectrum cephalosporins. According to a multicenter study in Korea, the proportion of AmpC derepressed strains of *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* was 27.5%-47.3%, which is much higher than has been reported in European studies (11%-34%) [20,21]. Therefore, it is necessary to evaluate the effectiveness of the QMAC-dRAST system for AmpC β-lactamase-producing *Enterobacterales*.

In our preliminary research for the performance of QMAC-dRAST, *M. morganii* and *S. marcescens* showed many discrepant results in AST compared to Vitek 2 for the following antimicrobial agents: aztreonam, cefepime, cefotaxime, ceftazidime, imipenem, piperacillin/tazobactam. Therefore, we updated the system partially for these bacterial species-antibiotics combinations. Then, we compared multiple AST results of QMAC-dRAST for several AmpC β-lactamase-producing strains with results from the Vitek 2 system. We improved and evaluated the performance of QMAC-dRAST for AmpC β-lactamase-producing *Enterobacterales* in the clinical laboratory.

Materials and methods

Sample collection

This study was conducted at Severance Hospital, Yonsei University College of Medicine, South Korea, from April 2018 to March 2019. The performance of QMAC-dRAST was assessed using 80 *Enterobacterales* isolates: 20 *M. morganii*, 20 *S. marcescens,* 10 *Klebsiella aerogenes,* 10 *E. cloacae,* and 20 *C. freundii.* All isolates were collected from blood samples of patients hospitalized at Severance Hospital from January 2016 to June 2018 and identified by the Vitek 2 identification system and MALDI TOF M/S (Bruker Daltonics Inc., Billerica, MA, USA).

Bacterial spiking protocol

All 80 isolates, which had been inoculated in 15% glycerol stock and stored at -70℃, were cultured on blood agar. For the QMAC-dRAST method, each bacterial colony was dispersed in 1.0 mL of 0.9% saline and adjusted to approximately 1.5×10^8 CFU/mL (0.5 McFarland suspension). Mixtures were serially diluted to a final concentration of approximately 1.0×10^3 CFU/mL. A 1.0-mL sample of the final dilution was inoculated into a Bact/Alert FA blood culture bottle containing 5.0 mL of sheep blood and was incubated in the Bact/Alert 3D system (bioMérieux Inc., Marcy l'Etoile, France). After the Bact/Alert 3D system detected the blood culture bottle as positive, a 350-μL sample from the bottle was transferred to a glass tube and tested directly in the QMAC-dRAST system.

In the first test, the old interpretation algorithm of AST results (AST algorithm) was applied. Bacterial growth within the agarose matrix only was measured in the old AST algorithm. In the final test, the AST algorithm for *M. morganii* and *S. marcescens* in aztreonam, cefepime, cefotaxime ceftazidime, imipenem, piperacillin/tazobactam was changed to observe bacterial growth not only within the agarose matrix but also outside the agarose matrix.

AST using the Vitek 2 system and the broth microdilution (BMD)

The performance of QMAC-dRAST was evaluated in comparison with that of Vitek 2. We conducted ASTs using QMAC-dRAST in parallel with Vitek 2 using 14 kinds of antibiotics. Discrepant AST results showing a different susceptibility category (resistant, susceptible, or intermediate) between two systems were adjudicated by BMD tests as the reference method to determine the correct results. The Vitek 2 ASTs were performed according to the manufacturer's guidelines and BMD tests were carried out according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [22].

Quality control

The three CLSI standard strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumonia* ATCC 700603) were used for quality control for the QMAC-dRAST system and BMD tests. For the Vitek 2 system, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used.

Data analysis

Based on the AST results of Vitek 2, isolates were categorized as resistant (R), susceptible (S), or intermediate (I) according to the manufacturer's instructions. Using Vitek 2 as the comparator, the concordance in results was determined using categorical agreement (CA, i.e., agreement of the results between the test method and the comparator) and essential agreement (EA, i.e., agreement within ±1 two-fold dilution of the test method under evaluation with the comparator minimum inhibitory concentration determination). The discrepancy rates for the detection of antimicrobial susceptibility were classified as very major errors (VME, i.e., S with test method vs. R with comparator), major errors (ME, i.e., R with test method vs. S with comparator), and minor errors (mE, i.e., I with test method vs. R or S with comparator or vice versa) [23]. Data were presented as numbers with percentages for categorical variables.

Results

Before and after QMAC-dRAST algorithm optimization

Initially, ASTs using the QMAC-dRAST system were conducted for 20 isolates of *M. morganii* and 20 isolates of *S. marcescens* using six antibiotics: aztreonam, cefepime, cefotaxime, ceftazidime, imipenem, and piperacillin/tazobactam. In case of ME results of these isolate-antimicrobial agent combinations, the actual wells of the QMAC-dRAST chip and their microscopic images were inspected. Then the AST algorithm for the combinations was updated based on the findings of the inspection. After the AST algorithm was improved, we conducted the final test. Susceptibility tests with the six antibiotics were performed again for 20 *M. morganii* and 20 *S. marcescens*.

Using six antimicrobial agents described above, we compared AST results between QMAC-dRAST and Vitek 2 for 20 *M. morganii* and 20 *S. marcescens* isolates before and after optimization of the AST algorithm for the two bacterial species. For all isolates, the agreement rates before optimization were 55.4% of CA, 19.1% of VME, 38.3% of ME, and after optimization were 83.8% of CA, 5.4% of VME, 4.3% of ME. For *M. morganii*, agreement rates before optimization were 67.5% of CA, 25.8% of VME, 17.9% of ME, and after were 79.2% of CA, 0% of VME, 7.1% of ME. Agreement rates of *S. marcescens* before optimization were 42.5% of CA, 0% of VME, 55.6% of ME, and after were 88.3% of CA, 16.7% of VME, 1.9% of ME (Table 1).

Table 1. Comparison of agreement & error rates between QMAC-dRAST and Vitek 2* before and after AST algorithm optimization

Bacterial species	No. of AST results	Before algorithm optimization (%)				After algorithm optimization (%)			
		CA	VME	ME	mE	CA	VME	ME	mE
M. morganii	120	67.5	25.8	17.9	17.5	79.2	0	7.1	15.8
S. marcescens	120	42.5	0	55.6	11.7	88.3	16.7	1.9	8.3
Total	240	55.4	19.1	38.3	14.6	83.8	5.4	4.3	12.1

*For results showing discrepancies between QMAC-dRAST and Vitek 2, broth microdilution test results were used as the reference AST results to resolve discrepancies.

Abbreviations: AST, antimicrobial susceptibility testing; CA, categorical agreement; VME, very major error; ME, major error; mE, minor error.

The performance of the QMAC-dRAST system for all samples

To fully evaluate the performance of QMAC-dRAST for AmpC β-lactamase-producing *Enterobacterales*, we performed the final ASTs for all 80 isolates using both QMAC-dRAST and Vitek 2 with 14 antibiotics: amikacin, amoxicillin/clavulanate, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, imipenem, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole. Among 1,120 total AST results, 121 showed discrepancies between QMAC-dRAST and Vitek 2. Therefore, BMD tests were performed for these samples to resolve discrepancies. The CA between QMAC-dRAST and BMD tests was 40.5% (49/121), lower than that between Vitek 2 and BMD tests, which was 48.8% (59/121). The VME between QMAC-dRAST and BMD tests was 3.3% (4/121) and that between Vitek 2 and BMD tests was 16.5% (20/121). In contrast, the ME between QMAC-dRAST and BMD tests was 14.0% (17/121), higher than that between Vitek 2 and BMD tests at 1.7% (2/121) (Table 2).

The CAs between QMAC-dRAST and Vitek 2 for each bacterial species were all above 90%. Only *S. marcescens* and *C. freundii* showed VMEs, but *M. morganii, K. aerogenes,* and *E. cloacae* showed relatively high ME rates (Table 3). Among antimicrobial agents, imipenem and piperacillin/tazobactam showed relatively high VME rates (Table 4). The CA between QMAC-dRAST and Vitek 2 for all 1,120 AST results was 93.0%, and the discrepancy rates were 1.7% for VME, 2.3% for ME, and 4.9% for mE (Table 3).

Table 2. The number of agreement & error categories with the BMD test for isolates showing discrepancies between QMAC-dRAST and Vitek 2

Bacterial species	No. of AST results	No. of susceptibility category in BMD			No. of agreement & error categories									
					QMAC-dRAST vs. BMD					Vitek 2 vs. BMD				
		R	I	S	EA	CA	VME	ME	mE	EA	CA	VME	ME	mE
M. morganii	40	15	11	14	23	14	0	6	20	29	16	8	0	16
S. marcescens	24	6	3	15	18	8	0	4	12	22	15	2	0	7
K. aerogenes	10	1	1	8	3	3	0	3	4	9	7	1	0	2
E. cloacae	24	6	9	9	20	14	0	3	7	19	9	3	0	12
C. freundii	23	17	0	6	17	10	4	1	8	15	12	6	2	3
Total	121	45	24	52	81	49	4	17	51	94	59	20	2	40

Abbreviations: BMD, broth microdilution: AST, antimicrobial susceptibility testing; R, resistant; S, susceptible; I, intermediate; CA, categorical agreement; EA, essential agreement; VME, very major error; ME, major error; mE, minor error.

Table 3. The number of AST results and agreement & error rates between QMAC-dRAST and Vitek 2* for each bacterial species

Bacterial species	No. of AST results	No. of susceptibility category in Vitek 2†			EA	CA	VME	ME	mE
		R	I	S	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
M. morganii	280	88	12	180	257 (91.8)	252 (90.0)	0	6 (3.3)	22 (7.9)
S. marcescens	280	78	5	197	264 (94.3)	261 (93.2)	2 (2.6)	4 (2.0)	13 (4.6)
K. aerogenes	140	41	2	97	133 (95.0)	132 (94.3)	0	3 (3.1)	5 (3.6)
E. cloacae	140	63	9	68	136 (97.1)	130 (92.9)	0	3 (4.4)	7 (5.0)
C. freundii	280	86	0	194	274 (97.9)	267 (95.4)	4 (4.7)	1 (0.5)	8 (2.9)
Total	1,120	356	28	736	1,064 (95.0)	1,042 (93.0)	6 (1.7)	17 (2.3)	55 (4.9)

*Discrepant results between QMAC-dRAST and Vitek 2 were resolved using the BMD test.

†Several Vitek 2 AST results that exhibited a different susceptibility category from results of QMAC-dRAST were replaced with BMD test results.

Abbreviations: AST, antimicrobial susceptibility testing; R, resistant; I, intermediate; S, susceptible; EA, essential agreement; CA, categorical agreement; VME, very major error; ME, major error; mE, minor error.

Table 4. Agreement & error rates between QMAC-dRAST and Vitek 2* for each antimicrobial agent

Antimicrobial agents	Total No. of tested isolates	EA (%)	CA (%)	VME (%)	ME (%)	mE (%)
Amikacin	80	100.0	100.0	0	0	0
Amoxicillin/Clavulanate	80	96.3	91.3	3.8	0	5.0
Ampicillin	80	98.8	98.8	0	50.0	0
Aztreonam	80	85.0	91.3	0	8.2	2.5
Cefazolin	80	100.0	98.8	0	0	1.3
Cefepime	80	93.8	91.3	0	2.9	6.3
Cefotaxime	80	92.5	87.5	0	6.0	8.8
Ceftazidime	80	88.8	93.8	0	3.6	3.8
Ciprofloxacin	80	97.5	93.8	0	1.4	5.0
Ertapenem	80	100.0	93.8	0	0	5.0
Gentamicin	80	98.8	98.8	0	1.3	0
Imipenem	80	95.0	83.8	13.3	1.8	12.5
Piperacillin/Tazobactam	80	85.0	81.3	8.3	0	17.5
Trimethoprim/Sulfamethoxazole	80	98.8	98.8	0	1.4	0
Total	1,120	95.0	93.0	1.7	2.3	4.9

*For results showing discrepancies between QMAC-dRAST and Vitek 2, BMD tests were used as the reference method to resolve discrepancies.
Abbreviations: AST, antimicrobial susceptibility testing; EA, essential agreement; CA, categorical agreement; VME, very major error; ME, major error; mE, minor error.

Discussion

AmpC β-lactamase, extended-spectrum β-lactamase, and carbapenemase are major reasons for antimicrobial resistance [24]. A study in Korea evaluated 732 patients with infections due to *Enterobacter* spp., *S. marcescens, C. freundii, or M. morganii* which have chromosomally encoded AmpC β-lactamases. Among 732 patients, 14 (1.9%) patients had bacteria that demonstrated antimicrobial resistance during antimicrobial therapy [25]. Another study included 46 patients who were initially infected with cephalosporin-susceptible *Enterobacter* spp. that became resistant to the antibiotic. Compared to 113 matched control patients who were infected with persistently susceptible isolates of the same organism, the first group of patients showed higher mortality, had a longer hospital stay, and had higher hospital costs [26].

Conventional AST systems such as Vitek 2, Phoenix, or Microscan are now widely used in the clinical setting, but they require 8–20 hours to produce results, excluding the time for isolation and growth of the bacteria [27]. In the QMAC-dRAST system, minimum inhibitory concentration (MIC) is determined by observing microcolony formation from a single bacterium without subculturing bacteria from blood culture bottles [10]. A QMAC-dRAST chip consists of 96 test wells. Each test well has a micropatterned chamber for an agarose matrix and a sub-well containing dried antibiotics. A sample of bacteria mixed with agarose is inoculated into the chamber. For multiplex ASTs, different concentrations of several antibiotics are dried in each well of the chip. The antibiotics are rehydrated by adding cation-adjusted Mueller-Hinton broth (CAMHB, BD Biosciences, CA, USA) and diffuse into the agarose matrix [10,11]. In the QMAC-dRAST instrument, bacterial microcolonies forming in the agarose matrix are imaged every hour and quantified by in-house software for automated image analysis to determine bacterial growth at each antibiotic concentration. This growth information is used to determine the MIC and the bacterial antibiotic susceptibility can be reported [10].

The QMAC-dRAST system uses bright-field microscopy. Bacteria located in the upper region of the agarose matrix can migrate outside the agarose matrix and grow in the CAMHB. This bacterial growth blocks the illumination and reduces the brightness of the image (Fig. 1). However, the old AST algorithm measured bacterial growth only within the agarose matrix as originally designed, regardless of bacterial growth outside the agarose matrix. For instance, if some wells with different concentrations of the same antibiotic showed similar bacterial growth each other in the agarose matrix but different bacterial growth outside the agarose matrix, the brightness of images of each well would be different from each other. However, in the old AST algorithm, bacterial growth is considered to have occurred in all these wells. As a result, high ME (38.3%) for *M. morganii* and *S. marcescens* was observed in the first test before AST algorithm improvement (Table 1). To address the discrepancy, we modified the old AST algorithm so that the new AST algorithm did not ignore the bacterial growth outside the agarose matrix. In the new AST algorithm, the brightness of the images is calculated numerically by its own standard. It was considered that actual bacterial growth had occurred only in the wells with reduced brightness of the images (Fig. 1). This new AST algorithm applied only to *M. morganii* and *S. marcescens* when testing aztreonam, cefepime, cefotaxime, ceftazidime, imipenem, and piperacillin/tazobactam. Otherwise, the old AST algorithm is still applied.

As we expected, ME rates of QMAC-dRAST for *M. morganii* and *S. marcescens* decreased after AST

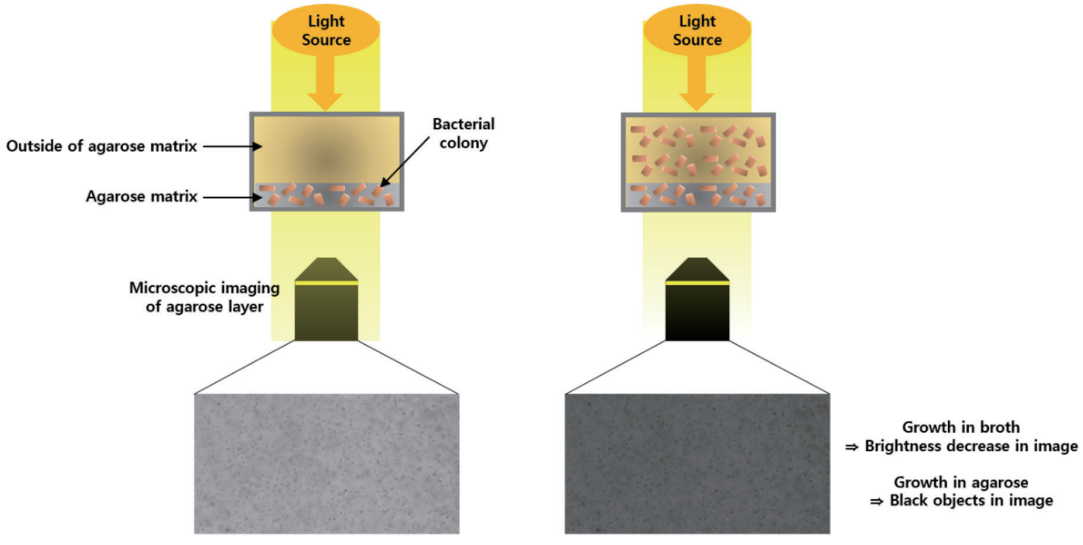

	Image feature used for decision	Decision result		Decision result
Old Algorithm	Objects	Growth		Growth
New Algorithm	Objects + brightness	Inhibition		Growth

Fig. 1. Differences between the old & new AST algorithm of the QMAC-dRAST. The new AST algorithm judges that bacterial growth is inhibited if there is no decrease in the brightness of the image. In the new AST algorithm, bacterial growth is considered to have occurred actually only when the brightness of the image decreased. AST, antimicrobial susceptibility testing.

algorithm optimization (Table 1). However, the VME rate of QMAC-dRAST for *M. morganii* was changed from 25.8% to zero (Table 1). Therefore, we reviewed our data and found that only 2 out of 20 *M. morganii* isolates yielded VMEs (4 VMEs each) in the first test. In 4 out of the 8 isolate-antimicrobial agent combinations, susceptibility categories of Vitek 2 or BMD results were changed in the final test compared to the first test. In the other 4 combinations, susceptibility categories of QMAC-dRAST results were changed. MIC values of those four QMAC-dRAST results were increased in the final test, which was unexpected considering the mechanism of the new AST algorithm. However, we could not figure out the cause of these conflicting results and further studies are required.

Except for VME (1.7%), the overall performance of the QMAC-dRAST with AmpC β-lactamase-producing *Enterobacterales* met the criteria for AST in the FDA guidelines (e.g. CA ≥ 90%, VME ≤ 1.5%, ME ≤ 3%) [23] (Table 3). However, our study has some limitations. Firstly, BMD tests were performed only for samples that showed discrepancies between QMAC-dRAST and Vitek 2. In further studies, it is desirable to conduct BMD tests in all samples. Secondly, more bacterial species and samples may be required to validate our findings. Finally, further modifications based on the characteristics of each of the bacteria are still needed to improve the performance of the QMAC-dRAST.

In conclusion, we updated the AST algorithm used in the commercially available in-house software of QMAC-dRAST; the AST algorithm has been improved by considering the brightness of the images of each well of the QMAC-dRAST chip. The performance of the QMAC-dRAST automated system can likely be improved by further modifications of the AST algorithm and this system will show a more acceptable level of agreement for AmpC β-lactamase-producing *Enterobacterales*.

Ethics statement

This study was approved by the Severance Hospital Institutional Review Board, Seoul, Korea (IRB No.1-2017-0079).

Conflicts of interest

Jung-Hyun Byun is currently an Assistant Editor and Dongeun Yong is an editorial board member of the *Annals of Clinical Microbiology*. However, they were not involved in the review process of this article.

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