The Open Microbiology Journal

Content list available at: https://openmicrobiologyjournal.com

RESEARCH ARTICLE

Performance of QMAC-dRAST™ (Direct Rapid Antimicrobial Susceptibility Testing) - a Newcomer in Phenotypic Automatic AST

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Abstract:

Objective:

QMAC-dRAST™ is a phenotypic automatized Antibiotic Susceptibility Testing (AST) system based on microfluidic chip technology enabling observation of changes in a single bacterial cell under antibiotic treatment conditions. The 96 wells plate with dried antibiotics comprises 19 and 17 antibiotics for the Gram-Negatives (GNs) and Gram-Positives (GPs), respectively. Categorical (Sensitive, Intermediate or Resistant) results were compared to results obtained by our laboratory standard susceptibility testing procedure and given as Categorical Agreement (CA).

Methods:

In a 3-month period (2019/2020), blood cultures detected positive were included. Excluded were known off-panel strains of QMAC-dRAST™, such as Gram-positive bacilli, *Streptococcus* and *Candida* species. Percentages of CA (CA, %) between QMAC-dRAST™ and routine testing methods used in the laboratory (EUCAST disc diffusion and/or etest/Broth Micro Dilution MIC), were calculated.

Results:

255 positive blood cultures from as many patients were examined. Of the positive blood culture strains, 144 were GNs, and 111 were GPs. An overall combined CA,% of 96.3 (2410 of 2502 determinations) was obtained, and discrepancies were noted in 92 of 2502 test results (3.7%). The percentage of very major errors (VMEs) was 0.7% for GNs and 2.2% for GPs. For 87% of blood culture specimens examined, susceptibility reports were available within 6-7 hours.

Conclusion:

The high CA,% for as well GNs as GPs are promising. The presented time to report data obtained by QMAC-dRAST™ in this study being of 3-8 hours for blood culture specimens examined strongly support a further possible improvement in the workflow for handling blood stream infections.

Keywords: Quantamatrix-direct, Rapid, Antimicrobial Susceptibility Testing (QMAC-dRAST™), Blood Stream Infection, Antibiotic Susceptibility Testing (AST), Phenotypic AST testing.

Article History	Received: September 22, 2020	Revised: January 31, 2021	Accepted: February 04, 2021

1. INTRODUCTION

In a recent review, estimates of the total burden of Blood Stream Infection (BSI) from population-based studies from North America and Europe were summarized [1]. The BSI incidence ranged between 113 and 220 per 100,000 population-based on reports from eight countries [2]. The serious prognosis for many of these infections and the increasing emergence of antimicrobial resistance making the demands for quick and accurate diagnosis of involved pathogens and their susceptibility to applicable antimicrobial agents exigent [3]. Approximately 25,000 people in Europe and 23,000 people in the United States die every year because of infection caused by antibiotic-resistant bacteria [4].

Within clinical microbiology, much focus is on accurate identification and susceptibility testing, automatization, speed and economy. Introduction of Matrix-assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry

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(MALDI-ToF MS) made a revolution with respect to establishing accurate, rapid and cheap pathogen identification [5]. Rapidly growing micro organisms often may be convincing identified after 3-6 hour growth on agar plates. A great focus is on fast Antimicrobial Susceptibility Testing (AST) in bloodstream infections in order to help clinicians being able to give the most optimized antimicrobial treatment. QMAC-dRAST™ (Quanta Matrix Inc., Seoul, Republic of Korea) is a phenotypic AST system based on microfluidic chip technology that enables the observation of changes in a single bacterial cell under antibiotic treatment conditions, with a total turn around time of only 6 h from Gram stain reporting of recognized positive blood cultures [6]. The positive blood culture sample is Gram stained prior to loading the instrument for choosing the right antibiotic panel. On the instrument blood, culture material is automatically mixed with agarose and inoculated into a 96 wells plate with dried antibiotic agents. Each well is followed over time by microscopic detection of changes in bacterial colony formation in the agarose. The performance of this technique has been evaluated in recent publications showing promising categorical (Sensitive, Intermediate or Resistant) agreements (CAs) with standard methods [6 - 10]. The patented 96 wells plate with dried antibiotics comprises, 19 and 17 antibiotics for the Gram Negatives (GNs) and Gram Positives (GPs), respectively. The size of an instrument is a minor refrigerator (width 59 cm, depth 76.5 cm and 109 cm high, weighting 180 kg), fully automated with random access of up to 12 samples (Quantamatrix.com).

In this study, AST results for 255 recognized positive blood culture bottles from as many patients obtained by QMAC-dRAST™ were compared to results obtained by the standard procedures performed in the laboratory in order to be able to comment on CAs, error rates, time to result and commenting on laboratory flow.

2. MATERIALS AND METHODS

2.1. Study Population

This prospective study was conducted in a Danish Regional Department of Clinical Microbiology performing microbiological service to a region (Region Zealand) with 800,000 inhabitants and six hospitals with a 2353 bed capacity. On a yearly basis, 140,000 blood cultures are examined. In a 3-month period (2019/2020), blood cultures detected positive in the morning were included. Only the first positive blood culture was included for each patient. Cases, where the pathogen was not identifiable *via* MALDI-ToF MS analysis, were excluded as was known off-panel strains of QMAC-dRAST™, such as Gram-Positive bacilli, *Streptococcus* and *Candida* species. No polymicrobial cases were included.

2.2. Laboratory Procedures

Besides being routinely processed, the blood cultures detected positive were examined in a separate set up in order to compare CA between QMAC-dRAST™ and routine testing methods used in the laboratory (EUCAST recommendations, see below). Evaluation of categorical disagreements included Minimal Inhibitory Concentration (MIC) determinations (etest and Broth Micro Dilution (BMD)).

During the study period the BACT/ALERT® VIRTUO® system (bioMerieuxInc., Marcy l'Etoile, France) was in use. Each blood culture set consisted of two FA Plus bottles and one FN Plus bottle. Positive blood cultures were Gram stained prior to analysis. Taxon identification by MALDI-ToF MS was performed by use of MBT Compass software version 4.1 containing 6903 MSP´s (Bruker Daltonics). 5% horse-blood-agar plates were streaked with material from blood cultures detected positive and after approximately 3-6 hours incubation in 5% CO_2-enriched atmosphere growth sufficient for MALDI-ToF MS examination was present. MALDI-ToF MS examination was performed as recommended by the manufacturer for direct examination of bacterial growth. The standard criteria for taxon confirmation used in the laboratory were applied. Briefly, identification results were considered reliable at the genus level when the score value was ≥ 1.7 and at the species level when the score value was ≥ 2.0 or ≥ 1.7 and the score value difference to the next best taxon match was ≥ 0.3.

QMAC–dRAST™ testing: A GP or GN panel was chosen for further AST according to the Gram stain result of positive blood culture. Briefly, after the identification of Gram negative and positive bacteria by direct smear examination about 300 µl of the culture was taken from the blood culture bottle, using a syringe, and added to a test tube. The test tubes and other kit components were placed in the QMAC-dRAST™ instrument and the following testing was done fully automated. The test was set up and performed according to the instructions given by the instrument. GN AST was performed using the panel card for the GN bacteria, including examining for susceptibility to amikacin, gentamicin, amoxicillin/clavulanic acid, ampicillin, ampicillin/ sulbactam, piperacillin/tazobactam, aztreonam, cefotaxime, ceftazidime, cefepime, ertapenem, imipenem, meropenem, colistin, trimethoprim/ sulfametho-xazole, and an ESBL test. For GP bacteria, the GP card included testing for susceptibility to penicillin, ampicillin, oxacillin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, inducible clinda-mycin resistance, gentamicin, streptomycin, rifampicin, trimethoprim/sulfamethoxazole, vancomycin and linezolid. Cefoxitin screening was also performed in order to detect MRSA and methicillin-resistant Coagulase Negative Staphylococci (CoNS). MIC results were interpreted in accordance with EUCAST recommendations. A well testing for inducible clindamycin resistance was included in the GP panel.

In addition to QMAC-dRAST™ testing, positive blood cultures were processed using standard setups in the laboratory following EUCAST disc diffusion recommendations and for vancomycin MIC determinations by etest. Thereby the following result comparisons could be made: *Entero bacteriaceae* (No. of tests =10): gentamicin, amoxicillin/ clavulanic acid, ampicillin, piperacillin/tazobactam, cefotaxime, ceftazidime, imipenem, meropenem, ciprofloxacin, trimethoprim/sulfamethoxazole and also ESBL testing for strains of *E. coli*, *Klebsiella* sp., and *Proteus mirabilis.Ps. aeruginosa* (No. of tests =7): Gentamicin, piperacillin/

tazobactam, ceftazidime, imipenem, meropenem, ciprofloxacin and trimethoprim/ sulfamethoxazole. *Acinetobacter* species (No. of tests =7): Gentamicin, piperacillin/ tazobactam, ceftazidime, imipenem, meropenem, trimethoprim/ sulfamethoxazole and an ESBL test. *Staphylococcus* species (No. of tests = 8, in addition, penicillin and cefoxitin for strains of *Staphylococcus aureus* and inducible clindamycin resistance for erythromycin-resistant strains): Penicillin (*S. aureus*), oxacillin, erythromycin, clindamycin, inducible clindamycin resistance (erythromycin-resistant strains), gentamicin, rifampicin, vancomycin, linezolid and cefoxitin (*S. aureus*). *Enterococcus* sp. (No. of tests =4): ampicillin, gentamicin-high, vancomycin and linezolid. If discrepancies, MIC testing was performed according to EUCAST recommendations for etesting and BMD. For piperacillin/tazobactam BMD testing, the MIC-strip Piperacillin-Tazobactam from Merlin Diagnostika GmbH was used.

2.3. Performance Evaluation of Tests

The terms for the AST accuracy evaluation were defined as follows: Categorical Agreement (CA), comparison of categorical result (Sensitive, Intermediate or Resistant) obtained with QMAC-dRAST™ and with EUCAST disc diffusion and/or etest/BMD MIC. Very major error (VME), false susceptibility of QMAC-dRAST™ compared to EUCAST disc diffusion and/or etest/BMD MIC. Major Error (ME), false resistance of QMAC-dRAST™ compared to EUCAST disc diffusion and/or etest/BMD MIC. Minor error (mE), intermediate susceptibility to QMAC-dRAST™ and susceptible or resistant according to EUCAST disc diffusion and/or etest/BMD MIC, or *vice versa*.

For all blood culture specimen runs, data measuring time from loading the instrument to available susceptibility report were extracted from the instrument.

3. RESULTS

3.1. Blood Culture Specimens Included

During the study period, 255 positive blood cultures from as many patients were examined. All were monobacterial infections. All strains were reliably identified by MALDI-ToF MS to the species level except two strains (strains belonging to the genera *Proteus* and *Acinetobacter*). GNs comprised 144 of the positive blood culture strains; 111 were GPs.

3.2. Taxons and Numbers of Strains Included No. of Comparable Tests for Each Taxon and Susceptibility Patterns Obtained on GNs and GPs by QMAC-dRAST Examination

Data on GNs and GPs included and no. of comparable tests are presented in Table **1**. *E. coli* and *Klebsiella* species dominating among the GNs and *S. aureus*, CNS and enterococci among the GPs. Respectively, 1530 and 972 comparable results were obtained. In Tables **2** and **3** susceptibility patterns obtained on GNs and GPs by QMAC-dRAST examinations are given, except for enterococci, being comparable with the standard setup in our laboratory. In general, strains showed relatively high susceptibility to many antibiotics. Exceptions were ampicillin and amoxicillin/ clavulanic acid for the Gram-negatives, penicillin for *S. aureus* and for CoNS, more resistant phenotypes were typically seen. ESBL production was detected in six strains, methicillin resistance in one *S. aureus* strain. Among the 14 enterococcal strains, VanA, VanB and high-level gentamicin resistance in each one *E. faecium* strain were detected as well as resistance to ampicillin and linezolid in respectively eight and two strains.

Table 1. Gram-Negatives and Gram Positives examined, no. of strains and comparable results (per strain and in total).

Organisms Identified	No. of Strains	Comparable Results	
-	-	Per Strain	In Total
-	-	-	-
Gram Negatives	-	-	-
-	-	-	-
Eschericia coli	82	10	820
Klebsiella pneumoniae	24	10	240
Klebsiella oxytoca	10	10	100
Pseudomonas aeruginosa	7	7	49
Serratia marcescens	5	10	50
Citrobacter freundii	3	10	30
Enterobacter cloacae	5	10	50
Acinetobacter sp.	1	6	6
Proteus mirabilis (3) + sp. (1)	5	10	50
Citrobacter koseri	2	10	20
(ESBL tests)	-	-	115
-	-	-	-
In Total	144	-	1530
-	-	-	-
Gram Positives	-	-	-
-	-	-	-

(Table 1) contd.....

Organisms Identified	No. of Strains	Comparable Results	
-	-	Per Strain	In Total
Staphylococcus aureus	40	10	400
Staphylococcus lugdunensis	1	8	8
Staphylococcus epidermidis	31	8	248
Staphylococcus hominis	17	8	136
Staphylococcus capitis	3	8	24
Staphylococcus warneri	2	8	16
Staphylococcus haemolyticus	3	8	24
Enterococcus faecalis	3	4	12
Enterococcus faecium	9	4	36
Enterococcus gallinarum	2	4	8
(cefoxitin)	-	-	40
(CLI inducible resistance)	-	-	20
-	-	-	-
In Total	111	-	972

Table 2. Susceptibility patterns obtained on 144 Gram Negatives (*Enterobacteriaceae*: n = 136, non-*Enterobacteriaceae*: n = 8) by QMAC-dRAST™ examination. Only test results comparable with the laboratory routine testing are included.

Antimicrobial Agent	Susceptibility		
-	Sensitive	Intermediate	Resistant
-	-	-	-
Amoxicillin/clavulanic acid	76	0	60
Ampicillin	53	0	83
Piperacillin/tazobactam	125	6	12
Cefotaxime	126	1	9
Ceftazidime	133	0	13
Imipenem	136	3	5
Meropenem	142	1	1
Gentamicin	137	0	7
Ciprofloxacin	127	3	14
Trimethoprim/sulfamethoxazole	113	1	23
ESBL production	109	-	6

Table 3. Susceptibility patterns obtained on 97 Gram positives (*Staphylococcus aureus*: n = 41, coagulase-negative staphylococci: n = 56) by QMAC-dRAST™ examination. Only test results comparable with the laboratory routine testing are included.

Antimicrobial Agent	*Staphylococcus aureus**			Coagulase Negative Staphylococci		
-	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
-	-	-	-	-	-	-
Penicillin	9	0	32	ND	ND	ND
Ampicillin	ND	ND	ND	ND	ND	ND
Oxacillin	41	0	0	26	0	31
Erythromycin	39	0	2	29	3	25
Clindamycin	40	0	1	44	3	10
Gentamicin	41	0	0	41	0	16
Rifampicin	40	0	1	52	3	2
Vancomycin	41	0	0	54	0	3
Linezolid	40	0	1	55	0	2
Fusidic acid	39	0	2	23	0	34
Cefoxitin screen	40	0	1	ND	ND	ND

ND: Not done; *One *Staphylococcus lugdunensis* included.

3.3. Discrepancy and Categorical Agreement Data

Data are given in Table **4**. All in all, discrepancies were found in approximately 4% of comparable data when looking on as well GNs as GPs. The percentage of VMEs were 0.7% for GNs and 2.2% for GPs. Among the GNs most frequent discrepancies were detected when comparing results for piperacillin/tazobactam, ceftazidime, cefotaxime, imipenem and ciprofloxacin. Of the 12 discrepancies noticed when comparing data for piperacillin/tazobactam susceptibility four were VMEs of which one was one dilution step from the breakpoint. Additional two VME`s were noticed, one with imipenem on a *Ps. aeruginosa* strain (one dilution step from the breakpoint) and one with cefotaxime on a *E. coli* strain (QMAC-dRAST™ MIC ≤1 µg/ml and etest MIC of 4 µg/ml). Among the Gram positives most frequent discrepancies were noticed when comparing results for penicillin, erythromycin, clindamycin and fusidic acid. Two VME discrepancies were noticed when testing *S. aureus* for penicillin susceptibility; in both cases, the QMAC-dRAST™ MIC was one dilution step below their breakpoint MIC. In 11 tests, QMAC-dRAST™ MIC`s were in the range 0.25-0.5 µg/ml and interpreted as resistant in contrast to results obtained with clover leaf testing [11]. For two VME`s seen when examining for erythromycin susceptibility, MIC differences were ≥ two dilution steps from the EUCAST breakpoint recommendation. Eleven of 13 testing results with discrepancy when examining susceptibility to clindamycin were VME`s; two when testing *S. aureus* strains and nine when examining CoNS strains. In nine testings these discrepancies were based on not detecting inducible clindamicin resistance by the QMAC-dRAST™ setup (QMAC-dRAST™ MIC values were one dilution step from the recommended breakpoint). In one testing, QMAC-dRAST™ detected in accordance with the routine method inducible clindamycin resistance. The four remaining VMEs were among *E. coli* strains and all related to susceptibility testing of ampicillin and amoxicillin/clavulanic acid; for all, MICs were within one MIC dilution step from the breakpoints given by EUCAST.

3.4. Data on Time to Instrument Reporting on Susceptibility Pattern

For 87% of blood culture specimens examined, susceptibility reports were available within 6-7 hours and all reports available within 8 hours. Results for strains of *E. coli*, *Klebsiella* species and *S. aureus*, being the most often detected taxons, were ready in 5-6 hours after blood culture material being loaded into the instrument.

4. DISCUSSION

The QMAC-dRAST™ set up tested in this study is a newcomer in AST testing of blood cultures detected positive. It is based on single-cell morphological analysis that can determine antimicrobial susceptibility by automatically analyzing and categorizing morphological changes in single bacterial cells under various antimicrobial conditions [12]. Determining factors for optimal antimicrobial treatment are correct bacterial identification, reliable performance and interpretation of AST results and the speed with which these informations can be obtained [13]. Combining the accuracy and speed of MALDI-ToF MS identifications with the accuracy and speed of QMAC-dRAST™ has proven to holdthe potential of improving the existing examination flow [7 - 10].

Reports from high-income countries have documented key pathogens to have been and remain being *S. aureus*, *E. coli*, *Klebsiella* species, *Ps. aeruginosa*, enterococci, streptococci and CoNS [14]. The spectrum of organisms differs for community-acquired and healthcare associated (community and hospital onset) infections. *Ps. aeruginosa* and staphylococci are clearly associated with healthcare institutions, where as among community-acquired cases there are more typically infections due to *Streptococcus pneumoniae* and other streptococci, and due to *E. coli* [1].

The robustness and accuracy of MALDI-ToF MS identifications have been documented for the majority of BSI pathogens irrespective of being the setup from Bruker or from Bio Merieux, though especially non-hemolytic streptococci, including pneumococci, still are challenging with respect to species identification. Directly from recognized positive blood cultures the Sepsityper kit (Bruker Daltonics) has been used also in connection with testing the QMAC-dRAST™ strategy [7, 8]. In only 18 of 346 (5.2%) incidences of monobacterial infections and in 7 of 38 (18.4%) incidences of polymicrobial infection no identifications were obtained [8], being comparable to results from previous studies [5]. Identifications have to be added to the instrument for finalizing the AST report in order to adjust for expert rules. In our laboratory, we streak positive blood culture material on agar-plates and incubates 3-6 hours making it possible, in by far the most cases, to have identifications and these being ready before AST testing is ready for reporting, which is a prerequisite for interpretation of obtained data by the included expert system based on EUCAST breakpoint recommendations. This is in agreement with the study by Sekercioglu *et al*. [15], on 1351 positive blood cultures,where a Columbia agar plate with 5% sheep blood was inoculated with one drop from the blood culture broth. After a 5-hour incubation period, a colony from the culture plate was submitted to MALDI-ToF MS. When manufacturer-recommended score values were taken into account, MALDI-ToF MS correctly identified 98.4% of the isolates to the species level with a score of > 2.0, 89.1% with a score between 1.7 and 2.0, and 75.4% with a score of < 1.7.

In the first report from 2014 by Choi et al [12] they compared 189 clinical hospital samples, including extended-spectrum β-lactamase–positive *E. coli* and *K. pneumoniae*, imipenem-resistant *Ps. aeruginosa*, methicillin-resistant *S. aureus*, and vancomycin-resistant enterococci with BMD testing. A CA of 91.5% with 6.51% mEs 2.56% MEs, and 1.49% VMEs were obtained. Since more studies have been added [7 - 10]. In Table **5** published studies comparing data obtained by QMAC-dRAST™ and standard AST methods are given. Four additional studies, including our study have included as well GNs as GPs [6 - 8], one study focused on GNs [10] and one on staphylococci and enterococci [9]. In the study by Grohs *et al*. and in our study QMAC-dRAST™ data

Table 4. No. and percentages of discrepancies and categorical agreements (CAs) of comparable results obtained by QMAC-dRAST™ and current standard methods (See Materials & Methods 2.3).

Comparison*	Gram Negatives (GNs)			Gram Positives (GPs)			GNs and GPs		
-	Discrepancies		CAs, %	Discrepancies		CAs, %	Discrepancies		CAs, %
-	No.	%	-	No.	%	-	No.	&	-
VME	10	0.7	99.3	22	2.2	97.8	22	1.3	98.7
ME	21	1.4	98.6	12	1.2	98.8	33	1.3	98.7
mE	22	1.4	98.5	5	0.5	99.5	27	1.1	98.9
-	-	-	-	-	-	-	-	-	-
In total	53/1530	3.5	96.5	39/972	4.0	96.0	92/2502	3.7	96.3

*VME: very major error; ME: major error; mE: minor error.

Table 5. Studies comparing QMAC-dRAST™ data with data obtained by standard AST methods.

No. of Samples	Gram Negatives	Gram Positives	Polymicrobial	CAs*, %	VMEs**, %	MEs, %	mEs, %	Reference
-	-	-	-	-	-	-	-	-
189	106	83	-	91.5	1.5	2.6	6.5	[12]
206	105	101	-	91.1	1.5	2.7	6.7	[6]
359	191	137	31	96.1	3.3	1.1	1.9	[8]
119	67	52	-	-	-	-	-	[7]***
100	100	-	-	92.9	0.8	3.2	3.0	[10]
110	-	110	-	91.5	1.2	4.3	5.4	[9]
255	144	111	-	96.3	1.3	1.3	1.1	This study

*CAs: Categorical Agreements.

**VME: very major error; ME: major error; mE: minor error.

***CAs not given; in 66 of 67 Gram negatives and 49 of 52 Gram positives, respectively, recommended identical antibiotic treatment as guided by BMD (broth micro dilution) testing.

were compared mainly to disc diffusion AST; strains with disagreeing results were additionally examined with etest or BMD testing. In the other studies given in (Table **4**) BMD ASTs were used for comparison. All in all CAs of 91.1-96.3% have been found, with our study being the one with highest CAs,%. Likewise, differences in VMEs, MEs and mEs were registered. Thus, comparisons have shown comparable results though with some differences in obtained VMEs, MEs and mEs. Especially the VMEs have to be careful evaluated with respect to possible improvements. In our study MIC determinations for most of discrepancies were close to the breaking points, thereby finding that the QMAC-dRAST™ setup seems very reliable for detecting susceptibilities for by far the most of the compared antimicrobials.

The genera and species QMAC-dRAST™ has been validated for includes the most frequent isolated GNs and GPs. Genera/species dominating in our study are in agreement with those dominating in the other studies, though strains in our study tends to have a more susceptible pattern. This exemplified in the study by Kim *et al*. [7] on 119 patients where more GNs were ESBL producing (*E. coli* and *Klebsiella* species) and carbapenem resistant (*Ps. aeruginosa* and *Acinetobacter baumannii*) as well as the GPs including more vancomycin and ampicillin resistant enterococci and methicillin resistant *S. aureus*. However, single strains were recognized with these susceptibility traits in our study except that no carbapenemase producing strains were recognized. Most often registered discrepancies, though relatively few, when testing GNs were for susceptibility to piperacillin/tazobactam, ceftazidime, cefotaxime, imipenem and ciprofloxacin. By far the most were within +/- one dilution step and the number of VMEs relatively small. This is close to be in accordance with the study by Kim *et al*. [7] where however no VMEs were observed for GNs with commonly used antibiotics such as beta-lactam/beta-lactamase inhibitors, broad spectrum cephalosporins and carbapenem. Huh *et al*. [9] studied 110 detected positive blood cultures with staphylococci and enterococci. AST was performed directly using the QMAC-dRAST™ and thereafter, colony isolates derived from subculture were used for the QMAC-dRAST™, the VITEK-2 system and BMD. The QMAC-dRAST™ with colony isolates produced more reliable results for staphylococci and enterococci than the direct QMAC-dRAST™ blood cultures detected positive. On colony isolates, the QMAC-dRAST™ system performed comparably to BMD and the VITEK-2 system. However, the QMAC-dRAST™ seems relative robust taking the presumed differences in amount of bacterial cells present into consideration; this in agreement with the setup based on single-cell morphological analysis. When examining GPs, especially discrepancies when examining *S. aureus* strains for penicillin susceptibility and CoNS (and two *S. aureus* strains) for inducible clindamycin resistance were noticed. In both, different methods are applied by QMAC-dRAST™ and our routine for their determination. Regarding penicillin susceptibility, we use a combination of growth zone appearance and clover leaf testing [11], whereas QMAC-dRAST™ interpretation is based on MICs. Inducible clindamycin resistance we detect as recommended by

EUCAST by demonstrating the D phenomenon, which does not seem as applicable when examining in a well as in QMAC-dRAST™. Whether small colony variants of *S. aureus*, potentially responsible for chronicization of an infection [16], will challenge a method using microscopy as the detection method remains to be settled. When examining positive blood cultures, there is an exigent desire/need for the method also being validated to be used for susceptibility testing of other relevant organisms such as Gram positive rods, streptococci (as well pneumococci as hemolytic and non-hemolytic streptococci), Gram negative cocci (especially *Neisseria meningitidis*), the HACEK group of fastidious GNs, anaerobic bacteria and *Candida* species. Patient outcome is critically influenced by delayed effective therapy, wherefore fast and accurate pathogen diagnostics, including AST, decisively improves the care of patients [17]. Progress in BSI pathogen diagnostics is based on a bundle approach that includes optimization of the pre-analytical parameters, rapid start of incubation, the use of rapid methods, re-organization (e.g. 24/7, transportation service) and a close involvement of antimicrobial stewardship teams [17]. Time to available antibiotic susceptibility report for blood culture specimens detected positive plays a major role in diminishing the time to being able to institute the most optimal antibiotic treatment. The presented time to report data obtained by QMAC-dRAST™ in this study, and those mentioned in (Table **4**), being of 3-8 hours for blood culture specimens examined, strongly support a further possible improvement in the workflow for handling blood stream infections.

Studies have reported on the importance of initial antibiotic coverage, coverage after guidance by Gram stain of recognized positive blood culture smears and improvement of coverage when guided by MALDI-ToF MS identifications [18]. Vlek *et al*. [18] reported on 253 episodes of blood stream infections that MALDI-ToF MS directly performed on positive blood culture broths reduced the time until definitive identification of bacterial species by 28.8 hours and increased the proportion of patients on appropriate antimicrobial therapy within 24 hours by 11.3% to 82%. In the study by Kim *et al*. [8] MALDI-ToF MS identification and QMAC-dRAST™ testing were applied on 359 BSI episodes, where involved pathogens were divided into pathogens having a susceptible or resistant pattern. When reporting based on MALDI-TOF MS results on susceptible/resistant pathogens, optimal targeted treatment was found respectively in 79%/63%, unnecessary broad-spectrum treatment in 16%/5%, suboptimal treatment in 4%/1%, and appropriate antibiotic treatment 100%/68%. Adding QMAC-dRAST™ results to the decision making raised the percentage of optimal antibiotic treatments to 98.2%. The global emergence of antibiotic resistant microorganisms makes it desirable being guided as quickly as possible in relation to antibiotic susceptibility [3]. In that respect phenotypic methods as the QMAC-dRAST™ seems desirable as they illustrate all resistance mechanisms expressed in a micro organism.

From a laboratory flow and technician aspect, the procedure about loading the instrument was very easy and intuitive. The QMAC-dRAST™ is a newcomer on the market and when the data transport from the instrument to a LIMS system will be done automatically the combination of easy loading, the automatized running procedure, easy testing termination, automatic data transport (in addition to automatic alarming when ready for reporting) seems to add important benefits to the current testing availabilities. The saved time from blood culture taken to availably of testing results being present will be able to improve antibiotic treatment of patients.

CONCLUSION

The genera and species QMAC-dRAST has been validated for includes the most relevant GNs and GPs in relation to positive blood culture specimens, though the desire for also being able to test other blood stream infection relevant pathogens, including streptococci, are exigent. The high CA for as well GNs as GPs are promising. The QMAC-dRAST is a newcomer on the market and when the data transport from the instrument to a LIMS system will be done automatically the combination of easy loading, the automatized running procedure, easy testing termination, automatic data transport (in addition to automatic alarming when ready for reporting) seems to add important benefits to the current testing availabilities. The presented time to report data obtained by QMAC-dRAST in this study being of 3-8 hours for blood culture specimens examined strongly support a further possible improvement in the workflow for handling blood stream infections.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Due to nature of the study ethics approval is not required.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available from the corresponding author [J. J] on reasonable request.

FUNDING

None.

CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Setup of the instrument, antibiotic panels and needed media/tablets were supplied by Quantamatrix Inc., Seoul, Republic of Korea.

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