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Note

Detection of carbapenemases with a newly developed commercial assay using Matrix Assisted Laser Desorption Ionization-Time of Flight

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ABSTRACT

This study evaluated the performance of the MBT STAR-Carba kit (Bruker Daltonics), to detect carbapenemase producing Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. in comparison with the RAPIDEC® CARBA NP test (BioMerieux). MBT STAR-Carba allowed the detection of carbapenemases in Enterobacteriaceae and *P. aeruginosa*.

1. Introduction

Carbapenemase-producing bacteria are a threat to modern medicine (Glasner et al., 2013). Phenotypic methods for the detection of carbapenemases like the RAPIDEC® CARBA NP (Tamma et al., 2017; Dortet et al., 2015) and Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) (Burckhardt and Zimmermann, 2011; Hrabák et al., 2011, 2012; Johansson et al., 2014; Oviaño et al., 2017) based methods has been proven useful for the detection and characterization (Johansson et al., 2014) of carbapenemases. However, the MALDI-TOF based methods have so far been based on laborious in-house methods (Burckhardt and Zimmermann, 2011; Hrabák et al., 2011, 2012; Johansson et al., 2014; Oviaño et al., 2017) although simplified by the automated STAR BL Software (Bruker Daltonic) (Oviaño et al., 2017). In the present study, we evaluated the first commercial test for carbapenemase detection using MALDI-TOF, the MBT STAR-Carba (Bruker Daltonics), and compared the performance to the RAPIDEC® CARBA NP (BioMerieux).

A collection of 55 clinical isolates of carbapenemase-positive and -negative *Klebsiella pneumoniae* (n = 24), *Escherichia coli* (n = 13), *Acinetobacter baumannii* (n = 8), *Pseudomonas aeruginosa* (n = 7), *Enterobacter cloacae* (n = 2) and *Proteus mirabilis* (n = 1) with reduced susceptibility to carbapenems (meropenem non-wild type) was included (Table 1). All isolates had been collected as part of the reference analysis for carbapenemase-production at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance. Presence or absence of carbapenemase genes were determined by PCR for bla_{KPC}, bla_{IMI}, bla_{VIM}, bla_{NDM}, bla_{IMP}, bla_{GIM}, bla_{SPM}, bla_{SIM}, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, and $bla_{OXA\text{-}58\text{-}like}$ (Naas et al., 2008; Mendes et al., 2007; Swayne et al., 2011; Poirel et al., 2011; Woodford et al., 2006). MICs were determined by broth microdilution using Sensititre microtiter plates (TREK Diagnostic Systems, Thermo Fischer Scientific). The distribution of carbapenemase genes and MIC-values is presented in Table 1. Twelve of the isolates were genetically devoid of carbapenemase genes (5 *K. pneumoniae,* 4 *E. coli* and 3 *P. aeruginosa*). Isolates were stored frozen (−70 °C) and retrieved on blood agar overnight before analysis. The RAPIDEC® CARBA NP test (Biomérieux, Marc L' Étoile, France) was performed according to the manufacturer's instructions. In brief, the test was read after 30 min. If no reaction could be visualized, or if a borderline result was obtained, the tests were incubated for another 90 min.

The research use only version of the now IVD approved MBT STAR-Carba kit (Bruker Daltonics, Bremen, Germany) was used according to the manufacturer's instructions. One to five bacterial colonies were separately mixed with the MBT STAR-Carba antibiotic solution (Bruker Daltonics) to a theoretical concentration of 3–6 × 10^8 CFU/mL in plastic tubes (included in the kit) and vortexed for 5 (± 1)s. The samples and controls (*E. coli* ATCC 25922 (negative control) and *K. pneumoniae* CCUG 56233, expressing KPC-2 (positive control)) were incubated with agitation (800–900 rpm) at 35 °C in ambient air for 30

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Table 1
The distribution of species and carbapenemases and the results of the MBT STAR-Carba kit and the RAPIDEC® CARBA NP test (P = positive, E = Equivocal, N = Negative).

Species	Carbapenemase	No. of isolates	MIC range (mg/L)		Test results (no.)					
					STAR_BL_ CARBA kit			RAPIDEC® CARBA NP		
			MEM	IMI	P	E	N	P	E	N
A. baumannii (n = 8)	OXA-23-like	6	32 ≥ 32	32 ≥ 32	3		3	1	2	3
	OXA-24-like	1	> 32	> 32			1			1
	OXA-58-like	1	32	32	1					1
E. coli (n = 13)	NDM	3	2–32	4–32	3			3		
	IMP	1	1	0.5	1			1		
	OXA-48-like	5	0.5–2	0.5–1	5				3	2
	Negative	4	1–4	0.5–1			4			4
E. cloacae (n = 2)	IMI	1	1	16	1			1		
	KPC	1	8	8	1			1		
K. pneumoniae (n = 24)	KPC	7	1 ≥ 32	2–32	7			7		
	NDM[a]	5	8–32	8 ≥ 32	5			5		
	VIM	3	2 ≥ 32	4 ≥ 32	3			3		
	OXA-48-like	4	0.5–32	0.5–32	4					4
	Negative	5	0.25–8	0.5–2			5			5
P. mirabilis (n = 1)	NDM	1	2	4	1			1		
P. aeruginosa (n = 7)	VIM	2	32	> 32	2			1		1
	IMP	1	> 32	> 32	1			1		
	NDM	1	> 32	> 32	1			1		
	Negative	3	8–32	4–32			3			3

[a] Includes one isolate harboring both NDM-1 and OXA-181.

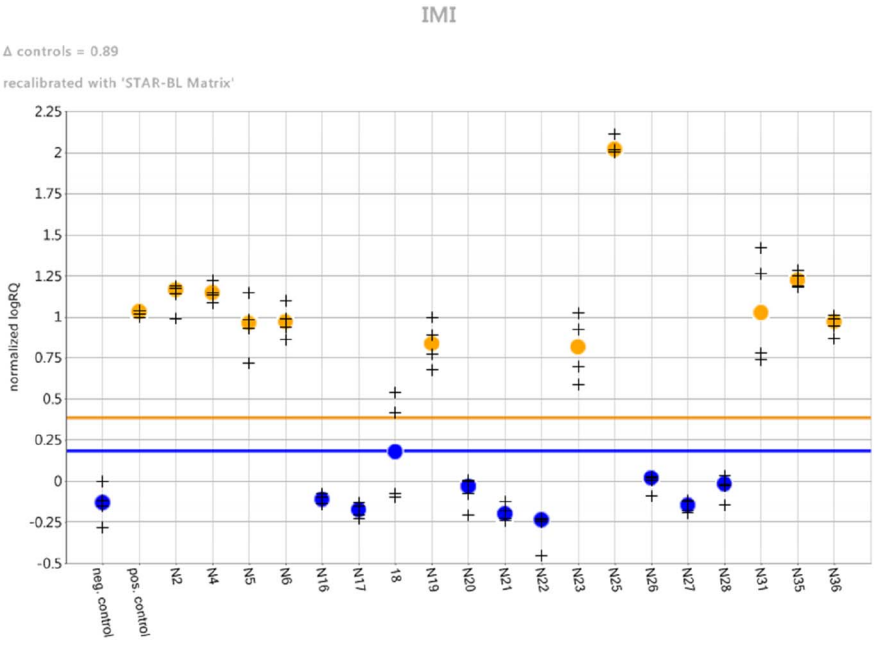

Fig. 1. An example of the presentation of the results in the MBT STAR BL software of the MBT STAR-Carba test results (1a). The mean of the normalized logRQ value for each isolate (dot) and the variation (crosses) are plotted together with the horizontal lines representing the limit for positive and negative results.

(Enterobacteriaceae and *P. aeruginosa*) and 60 min (*Acinetobacter* spp.) followed by a centrifugation for 2 min at 10–12000 × *g*. 1 μL of the supernatant was applied in duplicates on a MALDI Steel target plate (Bruker Daltonics). For calibration 1 μL of MBT STAR ACS-standard (Bruker Daltonics) was spotted in the first position per run. All spots were overlaid with 1 μL of the MBT STAR-Carba Matrix (Bruker Daltonics). The target plate was analyzed in a Microflex LT masspectrometer (Bruker Daltonics) using the MBT STAR BL module of the MBTcompass software (Bruker Daltonics). Four spectra were acquired for each isolate and normalized. The software calculated the difference in intensity (AUC) for the specific peak of imipenem (*m/z* 300) and compared the result of the tested isolates with the negative (no hydrolysis) and the positive (hydrolysis) controls resulting in a "Normalized logRQ value" for each spectra. After the controls were accepted by the software, the median logRQ-value was calculated for each isolate and compared to the predefined limits of positive (> 0.4), equivocal (0.2–0.4) or negative test < 0.2 (Fig. 1). The software interpreted the result as positive, equivocal or negative and the results were presented as a graph (Fig. 1). Inspection of individual spectra in the mass range of interest could be performed in the software.

All results are presented in detail in Table 1. Both the RAPIDEC® CARBA NP test and the MBT STAR-Carba correctly assigned all the carbapenemase-negative isolates (n = 12) as negative. The MBT STAR-Carba correctly assigned all carbapenemase-positive Enterobacteriaceae and *P. aeruginosa* isolates (Table 1) while four of the eight carbapenemase-positive *A. baumannii* isolates, three with $bla_{\text{OXA-23-like}}$ and one with $bla_{\text{OXA-24-like}}$ enzymes, tested negative (Table 1). All these four isolates had an MIC for imipenem of > 32 mg/L. With the RAPIDEC® CARBA NP test a positive test result was obtained for 26/43 (60%) of the carbapenemase-positive isolates. With the exception of

one *A. baumannii* isolate with $bla_{\text{OXA-23-like}}$, all isolates with an OXA-carbapenemase tested negative. One *P. aeruginosa* isolate with bla_{VIM} also tested negative. Using genotypic data as the reference both methods displayed 100% specificity while the sensitivity were significantly higher (39/43; 91%) for the MBT STAR Carba compared to RAPIDEC® CARBA NP 31/43; 72% ($p < 0.05$ McNemar) or 26/43; 60% ($p < 0.001$ McNemar). In terms of user friendliness both assays were easy to use. The time to result varied between 1 h 15 min–2 h 40 min for the RAPIDEC® CARBA NP test depending on incubation time and 50 min–2 h 20 min for the MBT STAR-Carba. The hands-on time was similar for both methods (15 min for 1 sample). The software of the MBT STAR-Carba was easy to use and provided an easy report including both the species ID and the test result.

Both methods were able to detect carbapenemase-production in isolates harboring class A and B carbapenemases. However, the RAPIDEC® CARBA NP, detected only one out of 18 isolates producing OXA-carbapenemases. These results are in line with previous results of the inhouse Carba NP test first developed (Dortet et al., 2012; Osterblad et al., 2014). However, evaluations available so far of the RAPIDEC® CARBA NP test have shown better results (Osterblad et al., 2016, Kabir et al., 2016). The RAPIDEC® CARBA NP test also classified one *P. aeruginosa* with VIM-4 as negative. Previous in-house developed MALDI-based methods have also reported lack of sensitivity with respect to the detection of OXA-48-like carbapenemases (Hrabák et al., 2012; Johansson et al., 2014). The addition of bicarbonate has however increased the detection of OXA-48 (Papagiannitsis et al., 2015). In the MBT STAR-Carba, bicarbonate is added which could explain the higher number of positive results seen here. Still, four OXA-carbapenemase-positive isolates tested false-negative, all *A. baumannii* with OXA-23 or OXA-24 and high MICs for imipenem. Extended incubation time (2 h) did not improve the results. The limited number of carbapenemase-negative isolates investigated here limits the generalizability of the high specificity observed.

In conclusion, the MBT STAR-Carba kit and the RAPIDEC® CARBA NP showed a high sensitivity and specificity in the detection of class A and class B carbapenemases. However, both tests reported false-negative results on isolates harboring OXA-carbapenemases, with the MBT STAR-Carba showing higher sensitivity.

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Conflicts of interest

None.

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