Short Communication

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Comparison between detection power of MBT STAR-Carba test and KBM CIM Tris II for carbapenemase-producing bacteria

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Abstract

Objectives: The rapid detection of carbapenemase-producing bacteria is clinically important for selecting appropriate antimicrobial therapy. Recently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry was used to detect carbapenemase activity.
Methods: In this study, we evaluated the detection power of MBT STAR-Carba test on identifying carbapenemase-producing bacteria isolated in Kobe city, Japan, compared with that of the KBM CIM Tris II kit using the modified procedure parameters. The obtained results were expressed as normalized logRQ values indicating a measure of hydrolysis efficiency.
Results: The MBT STAR-Carba test rapidly detected not only major carbapenemases, such as IMP-1 and IMP-6 that are most prevalent in Japan, but also GES-type and OXA-51-like carbapenemases, which are difficult to detect by reaction with inhibitors or KBM CIM Tris II by extending the incubation time.
Conclusions: The MBT STAR-Carba test will be beneficial in rapid identification of carbapenemases in clinical settings and environmental investigations.

Keywords: β-lactamases; carbapenemase; MALDI-TOF MS

Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a valuable analytical technique for microbial identification owing to its speed and simplicity [1]. MALDI-TOF MS has recently been utilized for detecting carbapenemase activity [2]. The commercially available MBT STAR-Carba test can identify carbapenemase activity by detecting the loss of native carbapenems and/or production of carbapenem hydrolysis products after incubation with carbapenem molecules. Carbapenemases are classified into three groups according to the Ambler classification system: (i) Ambler class A includes KPC and some GES variants, (ii) Ambler class B consists of metallo-β-lactamases such as VIM, IMP, and NDM, and (iii) Ambler class D includes OXA-48 [3]. While studies have confirmed the effectiveness of the MBT STAR-Carba test, the predominant types of carbapenemases differ between countries and regions [4–6]. This study evaluated the MBT STAR-Carba test on carbapenemase-producing bacteria isolated from Kobe City, Japan, and compared its performance with the KBM CIM Tris II kit (Kohjin Bio Co., Ltd., Saitama, Japan), which has a shorter reaction time than the modified carbapenem inactivation method (mCIM), and can be used for *Acinetobacter* and *Pseudomonas* species as well as *Enterobacterales,* and assessed its detection capabilities using modified procedure parameters [7].

Materials and methods

Bacterial strains

We analyzed 33 carbapenemase-producing *Enterobacterales* (CPE) strains (IMP-type: 15 strains, NDM-type: 5 strains, KPC-type: 7 strains, GES-type: 5 strains, and OXA-48-type: 1 strain), 22 non-CPE strains, and 2 Multidrug-Resistant *Acinetobacter* (MDRA) strains (Table 1). The strain was identified using a MALDI Biotyper (Bruker Daltonik GmbH, Bremen,

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Table 1: Information of the investigated strains on type of β-lactamase, minimum inhibitory concentration, and KBM CIM Tris II.

Strain classification		Strain ID	Species	Source (nosocomial infection cases)		β-Lactamase content	MICs, μg/mL for		KBM CIM Tris II kit	Hydrolytic activity level [normalized logRQ]		
							MPM	IPM		30 min	1 h	2 h
CPE	IMP-1 (n=5)	EBXX	*E. cloacae* complex	Accuracy control		IMP-1	3	2	+	0.97	NT	NT
		KA0003	*E. coli*	Patient		IMP-1, CTX-M-2 group	4	0.25	+	0.85	NT	NT
		KA0034	*K. aerogenes*	Patient		IMP-1, SHV group	3	2	+	1.08	NT	NT
		KA0061	*K. pneumoniae*	Patient		IMP-1, SHV group	12	0.5	+	0.96	NT	NT
		KA0062	*K. pneumoniae*	Patient		IMP-1, SHV group	2	0.5	+	1.09	NT	NT
	IMP-6 (n=10)	MGEP	*K. pneumoniae*	Accuracy control		IMP-6, CTX-M-2 group, SHV group	2	0.19	+	0.95	NT	NT
		KA0027	*K. pneumoniae*	Patient		IMP-6, SHV group	1.5	0.5	+	0.96	NT	NT
		KA0033	*K. pneumoniae*	Patient		IMP-6, SHV group, CTX-M-2 group	>32	0.25	+	1.06	NT	NT
		KA0041	*K. pneumoniae*	Patient		IMP-6, SHV group, CTX-M-2 group, CTX-M-9 group	8	0.25	+	1.07	NT	NT
		KA0059	*K. pneumoniae*	Patient		IMP-6, SHV group, CTX-M-2 group	>32	0.25	+	0.84	NT	NT
		KA0060	*E. coli*	Patient		IMP-6, TEM group CTX-M-2 group	8	0.125	+	0.94	NT	NT
		KA0064	*K. oxytoca*	Patient		IMP-6, CTX-M-2 group	>32	0.094	+	0.92	NT	NT
		KA0126	*E. coli*	Patient		IMP-6, CTX-M-2 group	>32	0.25	+	0.79	NT	NT
		KA0128	*E. coli*	Patient		IMP-6, TEM group, CTX-M-2 group	6	0.14	+	0.9	NT	NT
		KA0133	*K. pneumoniae*	Patient		IMP-6, TEM group, SHV group, CTX-M-1 group, CTX-M-2 group	4	0.19	+	0.91	NT	NT
	NDM-1 (n=3)	KA0007	*E. cloacae* complex	Patient		NDM-1	6	>32	+	1.13	NT	NT
		KA0125	*E. cloacae* complex	Patient		NDM-1	>32	>32	+	1.18	NT	NT
		KA0127	*P. mirabilis*	Patient		NDM-1, TEM group	>32	>32	+	1.22	NT	NT
	NDM-5 (n=2)	SZYA	*E. coli*	Accuracy control		NDM-5, CTX-M-15	>32	>32	+	1.01	NT	NT
		KA0042	*E. coli*	Patient		NDM-5, TEM group	1.5	3	+	0.86	NT	NT
	KPC-2 (n=7)	PXYG	*K. pneumoniae*	Accuracy control		KPC-2, CTX-M-65	>32	>32	+	0.99	NT	NT
		KA0091	*E. kobei*	Patient	(Case 1)	KPC-2, CTX-M-9 group	>32	>32	+	1.13	NT	NT
		KA0096	*E. kobei*	Environment	(Case 1)	KPC-2, CTX-M-9 group, EBC group	>32	>32	+	1.11	NT	NT
		KA0098	*E. kobei*	Environment	(Case 1)	KPC-2, CTX-M-9 group, TEM group	>32	>32	+	1.15	NT	NT
		KA0101	*E. kobei*	Environment	(Case 1)	KPC-2, EBC group	12	>32	+	1.13	NT	NT
		KA0102	*Kosakonia* sp.	Environment	(Case 1)	KPC-2	1.5	6	+	1.03	NT	NT
		KA0106	*E. coli*	Patient	(Case 1)	KPC-2	3	3	+	1.04	NT	NT
	GES-5 (n=3)	KA0044 (2020-O-9)	*S. marcescens*	Patient	(Case 2)	GES-5	>32	>32	–	0.08	NT	1.28
		KA0048 (2020-O-14-1)	*S. marcescens*	Patient	(Case 2)	GES-5	>32	>32	–	1.17	NT	1.27
		KA0054 (2020-O-25)	*S. marcescens*	Patient	(Case 2)	GES-5	>32	>32	–	1.03	NT	1.22
	GES-24 (n=2)	WWTPB1	*R. ornithinolytica*	Environment		GES-24	>32	>32	–	0.06	NT	1.17
		WWTPB2	*K. pneumoniae*	Environment		GES-24	>32	>32	–	0.15	NT	1.19
	OXA-181 (n=1)	RMZA	*E. coli*	Accuracy control		OXA-181	1	3	+	0.95	NT	NT

Table 1: (continued)

Strain classification		Strain ID	Species	Source (nosocomial infection cases)		β-Lactamase content	MICs, μg/mL for		KBM CIM Tris II kit	Hydrolytic activity level [normalized logRQ]		
							MPM	IPM		30 min	1 h	2 h
Non-CPE	ESBL (n=20)	KA0008	*E. coli*	Patient		CTX-M-1 group, TEM group	6	1	–	−0.06	NT	−0.04
		KA0011	*E. coli*	Patient		CTX-M-9 group	1	1	–	−0.12	NT	−0.03
		KA0012	*E. coli*	Patient		CTX-M-1 group, TEM group	0.032	0.125	–	−0.12	NT	−0.12
		KA0013	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	6	1	–	−0.11	NT	−0.11
		KA0018	*K. pneumoniae*	Patient		SHV group	0.38	0.19	–	−0.06	NT	0.01
		KA0019	*E. coli*	Patient		CTX-M-1 group, TEM group	3	1	–	−0.03	NT	0.12
		KA0020	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	1	0.5	–	−0.17	NT	−0.07
		KA0022	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	1	0.5	–	−0.02	NT	−0.01
		KA0023	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	3	1	–	−0.08	NT	−0.15
		KA0040	*E. cloacae* complex	Patient		SHV group	4	4	–	−0.03	NT	0
		KA0045	*E. coli*	Patient		CTX-M-1 group, TEM group	4	1.5	–	0.03	NT	0.09
		KA0055	*K. pneumoniae*	Patient		CTX-M-2 group, SHV group	2	0.5	–	0.05	NT	0.15
		KA0063	*E. coli*	Patient		CTX-M-1 group	0.5	0.25	–	−0.07	NT	0.06
		KA0072	*E. coli*	Patient		CTX-M-1 group, TEM group	3	1	–	−0.01	NT	0
		KA0079	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	6	1.5	–	−0.01	NT	0.15
		KA0089	*K. pneumoniae*	Patient		CTX-M-1 group, SHV group, TEM group	2	0.75	–	−0.08	NT	−0.11
		KA0104	*E. coli*	Patient		SHV group, TEM group	2	0.75	–	−0.02	NT	0.1
		KA0105	*E. cloacae complex*	Patient		TEM group, DHA group	0.75	0.5	–	−0.1	NT	−0.09
		KA0132	*E. coli*	Patient		CTX-M-1 group	2	0.38	–	−0.09	NT	0.05
		KA0134	*P. mirabilis*	Patient		CTX-M-2 group	0.094	3	–	−0.06	NT	−0.05
	Non-ESBL (n=2)	KA0129	*E. cloacae complex*	Patient		EBC group	0.023	0.38	–	0.03	NT	NT
		KA0130	*K. aerogenes*	Patient		Not detected	0.023	0.19	–	−0.12	NT	NT
MDRA		MDRA1	*A. baumannii*	Patient	(Case 3)	OXA-66	3	3	–	NT	0.07	1.26
		MDRA2	*A. baumannii*	Patient	(Case 3)	OXA-66	3	3	–	NT	0.06	1.09

NT, not test; MICs, the minimal inhibitory concentraions; mCIM, the modified carbapenem inactivation method; IMP, imipenem; MPM, meropenem.

Germany), and the minimal inhibitory concentrations of imipenem and meropenem were determined by using the E-test (bioMérieux Japan Ltd., Tokyo, Japan), following the manufacturer's guidelines. The presence of β-lactamase was confirmed by multiplex PCR, and the carbapenemase type was identified by sequencing or whole-genome sequencing [8–13].

KBM CIM Tris II assay

The KBM CIM Tris II (Kohjin Bio Co., Ltd., Saitama, Japan) assay was performed according to the manufacturer's instructions. Briefly, using a 5 µL loop, pre-cultured bacteria was suspended in 0.5 M Tris-HCl buffer (pH 7.6) (Kohjin Bio Co., Ltd.) after which a 5 µg meropenem disc was added. After incubation at 35 °C for 1 h, the meropenem disc was removed and placed on Mueller-Hinton agar medium seeded with meropenem-sensitive *Escherichia coli* ATCC25922. After incubation of the plate at 35 °C for 18 h, the size of the inhibition zone around the disc was measured. Carbapenamase activity was regarded as positive if the diameter of the inhibition zone was 6–14 mm or 15–18 mm with satellite colonies, and as negative if the diameter of the inhibition zone was greater than 15 mm without satellite colonies.

MBT STAR-Carba assay

The MBT STAR-Carba test was performed using the MBT STAR-Carba IVD Kit (Bruker Daltonik) according to the manufacturer's instructions. The MBT STAR-Carba test was performed with all bacterial concentrations standardized as the suspension density affects activity [5]. For each sample and controls, 1 mL of the bacterial solution adjusted to McFarland standard 7 using turbidity measure (DEN-1B Wakenbtech Co., Ltd., Japan) was centrifuged at 16,000×*g* for 10 min. The bacterial sediment was suspended in the antibiotic solution (MBT STAR-Carba Antibiotic Reagent dissolved in 50 µL MBT STAR Buffer), transferred to tube containing imipenem supplied in the kit, and then incubated for 30 min (60 min for *Acinetobacter* spp.) at 35 ± 2 °C with shaking (900 rpm) using a Thermomixer MSC-100 (Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China) according to the manual. For strains with GES-type and MDRA strains that could not be detected using the manual incubation time, the incubation time was extended to 2 h. The reaction mixture was centrifuged and 1 µL of the supernatant was spotted in duplicate onto the MALDI target. Dried spots were overlaid with MBT STAR Matrix and were analyzed on the MALDI Biotyper smart system (Bruker Daltonik GmbH) with the MBT STAR-BL IVD module. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC BAA-1705 were used as the negative control strain (β-lactam non-hydrolyzing) and positive control strain (β-lactam hydrolyzing), respectively. The obtained results were expressed as normalized logRQ values, meaning the calculated logarithm of the ratio of the summed signal intensities of the hydrolyzed forms to the summed signal intensities of the non-hydrolyzed forms of the antibiotic, normalized using the respective positive (hydrolyzing) and negative (non-hydrolyzing) control strains. The normalized logRQ value is understood to be a measure of hydrolysis efficiency, with values <0.2 being determined as not hydrolyzed, values >0.4 being hydrolyzed, and values between 0.2 and 0.4 being unclear results [5].

Results and discussion

All strains, except those harboring $bla_{\text{GES-5}}$, $bla_{\text{GES-24}}$, or $bla_{\text{OXA-66}}$ tested positive for carbapenemase using the KBM CIM Tris II kit (Table 1). For strains that were not active for 30 min, the incubation time was extended, and the respective positive (hydrolyzing) and negative (non-hydrolyzing) control strains at 30 min were used for the calculation. IMP-1, IMP-6, NDM-1, NDM-5, KPC-2, and OXA-181 were detected after an incubation time of 30 min in the MBT STAR-Carba test (Figure 1). IMP-1 shows high degradation activity for imipenem and meropenem, while IMP-6 shows higher degradation activity for meropenem, but not for imipenem, owing to the different susceptibilities of IMP-1 producers to carbapenem antibiotics [14]. Nonetheless, the average logRQ values on IMP-1 and IMP-6 were 0.99 and 0.94, respectively, suggesting no difference in hydrolyzing activity in the MBT STAR-Carba test. Among the GES-type β-lactamases, those with a replacement of glycine at position 170 with an asparagine or serine (such as GES-5 and GES-24) exhibit carbapenem-hydrolyzing activity [15]. Three GES-5 producing *Serratia marcescens* strains caused an intensive care unit outbreak that was negative for KBM CIM Tris II and harbored bla_{GES} according to PCR screening [13]. MBT STAR-Carba activity showed differences among the three strains after 30 min of incubation (Table 1). Two strains (KA0048 and KA0054) tested positive at 30 min. Meanwhile, MBT STAR-Carba activity of the KA0044 strain was negative at 30 min but turned positive after 2 h of incubation (Figure 1). GES-24 producing *R. ornithinolytica* and *K. pneumoniae* strains were negative for KBM CIM Tris II. However, MBT STAR-Carba activity of these strains increased after 2 h. There was no change in the activities of the strains used as negative or

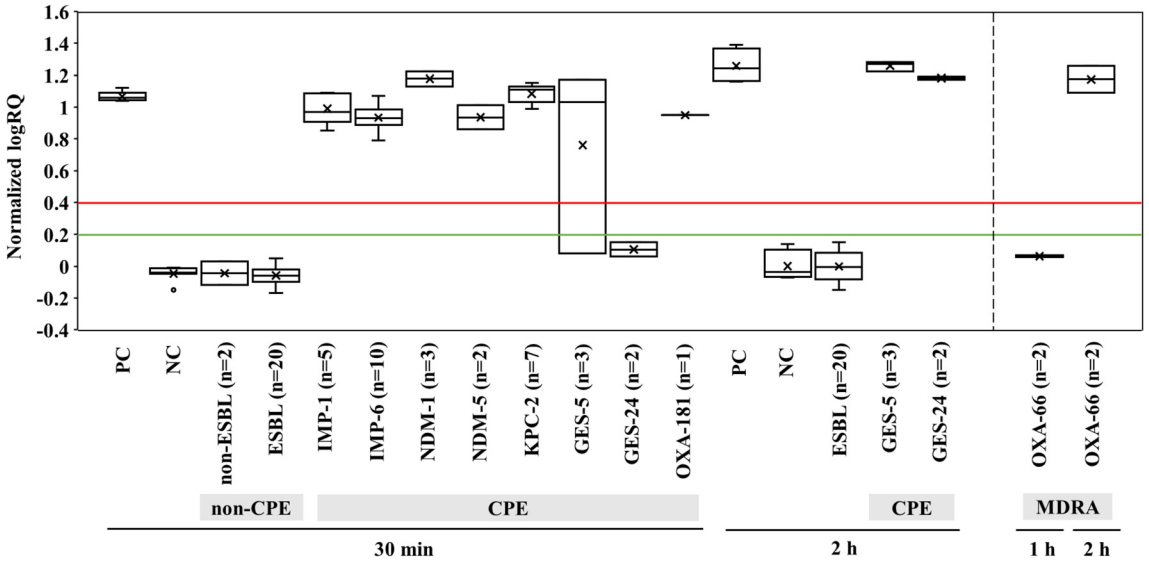

Figure 1: Graph showing the dependence of the determined carbapenemase activity level (normalized logRQ) on type of β-lactamase as well as the incubation time.

positive controls, and ESBL strains when the incubation time was extended (Figure 1). Therefore, some GES-type carbapenemases are difficult to detect by reaction with inhibitors or KBM CIM Tris II, while the MBT STAR-Carba test enabled their detection after incubation for over 30 min.

Two *Acinetobacter baumannii* strains (MDRA1 and MDRA2) isolated from two patients in the same hospital were resistant to ciprofloxacin (>32 µg/mL) and amikacin (>256 µg/mL) and showed low-susceptibility to carbapenems. These strains confirmed the presence of the $bla_{\text{OXA-51-like}}$ variant, $bla_{\text{OXA-66}}$. IS*Aba1* was present upstream of $bla_{\text{OXA-66}}$ in two isolates, leading to $bla_{\text{OXA-66}}$ expression. We detected the induction activity of $bla_{\text{OXA-66}}$ against carbapenems by incubation for 2 h in the MBT STAR-Carba test.

Thus, there were differences in the detection of carbapenemase between the KBM CIM Tris II and MBT STAR-Carba tests. The KBM CIM Tris II and MBT STAR-Carba tests test for carbapenemase activity with meropenem and imipenem, respectively; differences in responsiveness between these tests may be due not only to differences in antimicrobial reactivity, but also to a combination of factors, including bacterial species and expression levels.

In summary, we revealed that the MBT STAR-Carba test can rapidly detect not only major carbapenemases, prevalent IMP-1 and IMP-6 in Japan, but also GES-type and OXA-51-like carbapenemases that are difficult to detect by reaction with inhibitors or KBM CIM Tris II. The MBT STAR-Carba test will help to rapidly investigate the presence of carbapenemases in clinical settings and environmental investigations.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: S.K. and N.N. designed the study methods and wrote the first draft of the manuscript; N.N., S.K., and C.F. performed detection assay, and identification typing. S.K. and N.N. analyzed the data. All the authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
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