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Note

Evaluation of the modified carbapenem inactivation method and sodium mercaptoacetate-combination method for the detection of metallo-β-lactamase production by carbapenemase-producing *Enterobacteriaceae*

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ABSTRACT

We evaluated the effectiveness of carbapenem inactivation method (CIM) and modified CIM (mCIM). Our results indicated that mCIM with 4 h incubation improved sensitivity and specificity for detecting carbapenemase-producing *Enterobacteriaceae* compared to CIM. Additionally, we developed a sodium mercaptoacetate-combination method (SMA-mCIM) to detect metallo-β-lactamase (MBL) with high sensitivity and specificity.

To date, numerous β-lactam antibiotics have been developed and used for treating various bacterial infections. These antibiotics have broad-spectrum effects against gram-negative and positive pathogens. In particular, carbapenems are used as last resort treatment of antimicrobial-resistant pathogens. Nevertheless, the increase of plasmid-mediated β-lactam-resistant pathogens, including extended-spectrum β-lactamase (ESBL), AmpC, and carbapenemase producers, is a major concern worldwide. Among the β-lactam-resistant pathogens, carbapenemase-producing *Enterobacteriaceae* (CPE), which inactivate carbapenems, have become a global concern (Patel and Bonomo, 2013). The World Health Organization (WHO) currently regards antimicrobial resistance as a critical issue; thus, member nations are actively seeking mitigation strategies. Therefore, highly sensitive and specific methods for CPE detection in clinical laboratory settings are critical. A highly sensitive, specific, and cost-effective carbapenem inactivation method (CIM) was developed in 2015 (van der Zwaluw et al., 2015) and its utility has been reported (Tijet et al., 2016; Yamada et al., 2016). Additionally, the Clinical and Laboratory Standards Institute has planned to adopt the CIM, with some modification, for the detection of carbapenemase-producers. In the modified CIM (mCIM), the detection sensitivity was improved by extending the incubation time. However, detailed information on the modified method is currently lacking. Thus, in this study, we evaluated the utility of mCIM to detect CPE.

In Japan, the prevalence of metallo-β-lactamase (MBL) producers is higher than that of other carbapenemase producers. Therefore, an effective MBL detection method is critical. Previous reports demonstrated that thiol compounds including sodium-mercaptoacetate (SMA) were useful for the detection of MBL-producing isolates (Goto et al., 1997; Arakawa et al., 2000). Therefore, in addition to evaluating the utility of mCIM, we examined the effectiveness of mCIM with sodium mercaptoacetate (SMA-mCIM) for MBL detection.

Fifty-seven *Enterobacteriaceae* isolates, including 23 non-carbapenemase producers (ESBL and AmpC producers) and 34 carbapenemase producers (16 IMP, nine NDM, six OXA-48-like, two KPC, and one OXA-181 and NDM producers), were evaluated in this study (Table 1). The minimum inhibitory concentrations (MICs) of two carbapenems (meropenem and ertapenem) were determined using Etest (SYSMEX bioMérieux CO., Ltd., Tokyo, Japan) according to the manufacturer's instructions. To determine the *bla* genotype, PCR and sequencing were performed as previously described (Dallenne et al., 2010; Szabó et al., 2005; Notake et al., 2013).

Abbreviations: CPE, carbapenemase-producing *Enterobacteriaceae*; CIM, carbapenem inactivation method; mCIM, modified carbapenem inactivation method; MBL, metallo-β-lactamase; SMA, sodium mercaptoacetate; CLSI, Clinical and Laboratory Standards Institute.

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Table 1
The minimum inhibitory concentration (MIC) of meropenem and ertapenem for each carbapenemase-producing *Enterobacteriaceae* (CPE) evaluated in the study.

Ambler class	Species	Carbapenemase genes	Other *bla*-genes			MICs (mg/L)		mCIM		
			TEM/SHV	CTX-M	P-AmpC	Meropenem	Ertapenem	mCIM (mm)		mCIM result[a]
								Meropenem	Ertapenem	
Class A	*Klebsiella pneumoniae*	KPC-2	SHV-11	CTX-M-15		8	16	0	0	+
		KPC-3	SHV-11, TEM-1			>32	>32	0	0	+
Class B	*Citrobacter freundii*	IMP-1	SHV-12	CTX-M-9		2	6	13	11	+
		IMP-1				8	4	0	0	+
		IMP-1, IMP-19				8	2	0	0	+
	Enterobacter cloacae	IMP-1				4	2	0	0	+
		IMP-1	SHV-12	CTX-M-9		>32	>32	0	0	+
		IMP-1				4	4	0	0	+
		IMP-1				1	1	0	0	+
		IMP-19				2	2	0	0	+
		IMP-19				2	4	0	0	+
		IMP-19				0.5	1	0	0	+
	Escherichia coli	IMP-1				1	2	0	0	+
		IMP-19				0.5	1	0	0	+
		NDM-1	TEM-1			8	8	0	0	+
		NDM-1				8	8	0	0	+
		NDM-1	TEM-1	CTX-M-55		32	>32	0	0	+
		NDM-1		CTX-M-15	CMY-42	>32	>32	14	16	+
		NDM-5	TEM-1		CMY-42	32	>32	0	0	+
		NDM-5			CMY-42	>32	>32	0	0	+
		NDM-5		CTX-M-15		>32	>32	0	0	+
	Klebsiella oxytoca	IMP-1				2	4	0	0	+
	Klebsiella pneumoniae	IMP-19	SHV-1			1	2	0	0	+
		IMP-19	SHV-27			0.25	0.25	0	0	+
		NDM-1	SHV-1			16	8	0	0	+
		NDM-1	TEM-1, SHV-11	CTX-M-15	CMY-6	>32	>32	0	0	+
	Serratia marcescens	IMP-11	TEM-1	CTX-M-9	CMY-48	1	1	0	0	+
Class D	*Escherichia coli*	OXA-48	TEM-1	CTX-M-55		1	4	0	0	+
	Klebsiella pneumoniae	OXA-48	TEM-1	CTX-M-15		>32	>32	0	0	+
		OXA-48				0.5	0.5	0	0	+
		OXA-48	TEM-1	CTX-M-15		0.25	0.5	0	0	+
		OXA-48				0.5	1	0	0	+
		OXA-181, NDM-1	TEM-1	CTX-M-15	CMY-4	>32	>32	0	18	+
		OXA-181	TEM-1	CTX-M-15		>32	>32	10	19	+
non-CPE	*Citrobacter freundii*	ND				0.032	0.125	19	20	-
	Enterobacter aerpgenes	ND				>32	>32	21	22	-
		ND				16	>32	21	23	-
	Enterobacter cloacae	ND				2	8	19	19	-
		ND		CTX-M-9		16	>32	20	22	-
		ND				0.064	0.125	21	20	-
	Escherichia coli	ND	SHV-12			0.032	0.125	22	20	-
		ND			CMY-2	0.125	0.5	22	22	-
		ND			CMY-2	0.125	0.5	21	21	-
		ND	TEM-1	CTX-M-14		0.032	0.064	20	24	-
		ND		CTX-M-15		0.032	0.016	19	20	-
		ND		CTX-M-15		0.032	0.032	19	20	-
		ND	TEM-1	CTX-M-14	CMY-2	0.032	0.064	20	22	-
		ND	SHV-12			0.032	0.032	21	18	-
		ND		CTX-M-2		0.064	0.125	21	18	-
		ND		CTX-M-15		0.032	0.012	20	21	-
	Klebsiella pneumoniae	ND	TEM-1	CTX-M-3		0.064	0.032	19	20	-
		ND	SHV-1, TEM-1	CTX-M-14		0.032	0.125	20	22	-
		ND	SHV-26, TEM-1			0.032	0.012	20	20	-
		ND			DHA-1	4	32	19	21	-
		ND			DHA-1	0.064	0.25	20	21	-
	Proteus mirabilis	ND		CTX-M-2		0.064	0.016	19	21	-
	Serratia marcescens	ND	TEM-1			4	16	20	20	-

[a] For mCIM using meropenem disk, inhibition-zone diameters of <15 mm were considered positive results.
ND: not detected.