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Original Article

Evaluation of the modified carbapenem inactivation method for the detection of carbapenemase-producing *Enterobacteriaceae*

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ABSTRACT

Carbapenemase-producing *Enterobacteriaceae* (CPE) are increasing worldwide. Rapid and accurate detection of CPE is necessary for appropriate antimicrobial treatment and hospital infection control. However, CPE contains some strains that are difficult to detect depending on genotype and MIC value of carbapenem, and a detection method has not been established. The recently reported modified carbapenem inactivation method (mCIM) has been developed in CLSI M100-S27 as a phenotypic technique for detecting carbapenemase activity. In the present study, we examined mCIM as a new CPE detection method using 207 *Enterobacteriaceae* isolates in comparison with the three existing screening methods of modified Hodge test, Carba NP test and carbapenem inactivation method and evaluated its performance. Consequently, both the sensitivity and specificity of mCIM were 100%, indicating better results than the conventional screening methods. The mCIM is a useful tool for microbiology laboratories due to its simplicity, clear criteria, cost-effectiveness and availability at any laboratory.

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Abbreviations: CIM, carbapenem inactivation method; CNP, Carba NP test; CPE, carbapenemase-producing *Enterobacteriaceae*; ESBLs, extended-spectrum β-lactamases; IPM, imipenem; mCIM, modified carbapenem inactivation method; MEPM, meropenem; MHA, Mueller Hinton agar; MHT, modified Hodge test; MICs, minimum inhibitory concentrations; TSB, trypticase soy broth.

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1. Introduction

Carbapenems are a commonly used primary therapeutic option for serious infections caused by Gram-negative bacilli such as *Enterobacteriaceae* and are often considered agents of last resort. Carbapenemase-producing *Enterobacteriaceae* (CPE) are being increasingly reported worldwide [1]. The emergence and spread of recent CPE are a significant clinical and public health concern [2]. The detection of CPE is very important therapeutically and is necessary for nosocomial infection control. Present detection methods of CPE include the Carba NP test (CNP) using one of the biochemical method [1,3] and the modified Hodge test (MHT). The recently reported carbapenem inactivation method (CIM) was developed as a phenotypic technique for detecting carbapenemase activity [4]. It was reported to be highly sensitive and specific for CPE detection. In addition, it does not require specific equipment and is cost-effective. CLSI M100-S27 announced the modified CIM test (mCIM) with improved incubation conditions in 2017 [5]. This method demonstrated both a sensitivity and specificity of ≥99% for the detection of carbapenemase-producing isolates. Furthermore, the CLSI working group has published details of the evaluation of the mCIM as an original paper [6]. However, only some major carbapenemase genotypes have been studied in the CLSI study, and examination of the most common IMP type in Japan is not considered sufficient. Therefore, we conducted a study aimed at evaluating mCIM as a CPE detection method using clinical isolates actually detected from multiple medical institutions in Japan.

2. Materials and methods

For the target strains, clinical isolates collected by participating facilities to the Study of Bacterial Resistance in the Kinki Region of Japan for the period from 2000 to 2016 were used. The 207 *Enterobacteriaceae* isolates, including 100 non-CPE (extended-spectrum β-lactamases [ESBLs] and AmpC producers, including 26 non-CPE Carbapenem Resistant *Enterobacteriaceae*) and 107 CPE (96 IMP, 2 VIM, 1 KPC, 1 NDM, 6 GES and 1 IMP and GES producer) that were collected from multiple medical institutions in the Kinki region of Japan were used in this investigation. Several ATCC and NCTC strains were used as reference strains for testing as CPE (1 VIM, 1 KPC, 3 NDM, and 1 OXA-48 producers) and non-CPE to examine other carbapenemase genes. Confirmation of resistance mechanisms was determined by a PCR method and sequence analysis. The carbapenemase genotype of strains were investigated using PCR methods, with 14 sets of primers for the detection of IMP-1, IMP-2, VIM, NDM, KPC, GES and OXA-48 carbapenemases, as previously reported [7–11]. The amplified products were sequenced in an automated DNA sequencer (ABI 3100, Applied Biosystems, Foster City, CA). In the non-CPE group, PCR results were negative for carbapenemase, and strains suspected of over-expression of chromosomal AmpC or ESBLs and/or plasmid-mediated AmpC-producing strains were used. The plasmid mediated AmpC and ESBL were detected using previously reported multiplex PCR [12–15]. The minimum inhibitory concentrations (MICs) were determined by a dry plate Eiken (Eiken Chemical, Tokyo). As quality control strains for each carbapenemase confirmation test, the *Klebsiella pneumoniae* ATCC BAA-1705 strain was used as a positive control and the *K. pneumoniae* ATCC BAA-1706 strain was used as a negative control. Each phenotypic tests were performed using a stock strain. Stock strains were stored at −80 °C using Difco skim milk (BD, Tokyo, Japan) or microbank (Iwaki, Tokyo). The pre-culture was cultured at 35 °C for 18–24 h using a sheep blood agar as a test strain.

The MHT was performed according to CLSI M100-S27 [5]. Mueller Hinton agar (MHA; BD) and a 10-μg meropenem (MEPM; BD) disk were used to confirm growth enhancement of *E. coli* ATCC 25922. The CNP method was also performed according to CLSI M100-S27 [5]. One-hundred microliters of the bacterial suspensions was added to 100 μL of phenol red reagent added by IMP and incubated for 2 h at 35 °C. The results were positive for changes to yellow, dark yellow and light orange and negative for changes to red or red-orange.

The CIM test was performed according to the original method [4]. In the present study, the MEPM disk by CIM was checked at 6 h of incubation time to confirm formation of an inhibition zone diameter. The mCIM method was performed according to CLSI M100-S27 [5]. First, a 1-μL loopful of test isolate was suspended in 2 mL of trypticase soy broth (TSB; BD), and a MEPM disk was added into the suspension, which was then incubated for 4 h at 35 °C. Second, a bacterial suspension of *E. coli* ATCC 25922 adjusted to McFarland standard no. 0.5 was inoculated on MHA for the routine disk diffusion procedure. The incubated disk was transferred from the TSB suspension onto the MHA and incubated for 16–20 h at 35 °C. The results were judged to be positive for inhibition zone diameters of 6–15 mm, negative for ≥19 mm and indeterminate for 16–18 mm.

3. Results

The results of each method for the test isolates are shown in Table 1 for CPE and Table 2 for non-CPE. The sensitivity of each test method to CPE was 100% for MHT, 89.7% for CNP, 95.3% for CIM and 100% for mCIM. The specificity was 94.0% for MHT and 100% for CNP, CIM and mCIM. Table 3 shows the results of testing with ATCC and NCTC strains. The sensitivity of each test to these strains was 50% for MHT and 100% for CNP, CIM and mCIM, and specificity was 100% for all methods.

4. Discussion

The CIM is a method for detecting carbapenemase reported by van der Zwaluw et al., in 2015 [4] that not only exhibits high sensitivity and specificity but has also been evaluated as a cost-effective method with high practicality. The mCIM is a new detection method announced by CLSI with further improvement such as that for incubation conditions [5]. In the present study, mCIM showed excellent results with a sensitivity and specificity of 100% for the detection of CPE. This excellent result was equal to that in the evaluation report of Yamada et al. [16] and CLSI report [5], and mCIM is considered to be superior to the conventional MHT, CNP and CIM.

Although MHT is a conventional detection method, its detection sensitivity is low in NDM, and the specificity is inferior due to the presence of false positives [17]. The detection sensitivity of MHT in this study was 100%, but this is considered to be related to the low detection ratio of NDM in Japan. In this study using ATCC and NCTC strains, MHT was not able to detect all NDM types, so we believe that this method cannot be used for suspected cases of NDM as reported. For strains with membrane mutations in AmpC over-expression, false positives are generated [18], so MHT is inferior in specificity as compared with mCIM.

CNP has been reported to show false negatives in GES and OXA-48 [19]. In the present study, CNP showed false negatives with GES-4- and several IMP-6- and NDM-1-producing *Proteus mirabilis* strains. As in previous reports, the detection sensitivity was inferior compared to that of mCIM as genotypes and strains with low MICs of carbapenem tend to be falsely negative. Although CNP has been suggested to show false negatives in some bacterial species such as

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Table 1
Results of mCIM, modified Hodge test, Carba NP test and CIM in 107 carbapenemase-producing *Enterobacteriaceae* clinical isolates.

Species	β-Lactamase content		No. of strains	MICs (mg/L)		Modified Hodge test with MEPM	Carba NP test	CIM test with MEPM	mCIM test (mm)
	Carbapenemase	ESBLs/PABL		IPM	MEPM				
C. freundii (2)	IMP-1		1	4	16	+	+	+	+(6)
	VIM-2		1	2	≤0.5	+	+	−	+(6)
E. aerogenes (1)	IMP-6	CTX-M-2	1	≤0.5	>16	+	+	+	+(6)
E. cloacae (5)	IMP-1		3	4–8	8	+	+	+	+(6)
	IMP-1, GES-4		1	8	16	+	+	+	+(6)
	VIM-2		1	1	1	+	+	+	+(6)
E. coli (18)	IMP-6		1	2	>16	+	+	+	+(6)
	IMP-6	CTX-M-2	14	≤0.5	2 to >16	+	pos (12); neg (2)	+	+(6)
	IMP-6	CTX-M-2, CTX-M-14	2	≤0.5	8	+	+	+	+(6)
	IMP-6	CTX-M-2, CTX-M-27	1	≤0.5	>16	+	+	+	+(6)
K. oxytoca (7)	IMP-1		2	2–4	8–16	+	+	+	+(6)
	IMP-6	CTX-M-2	2	≤0.5	>16	+	+	+	+(6)
	IMP-34	CTX-M-2	3	≤0.5	4 to >16	+	+	+	+(6)
K. pneumoniae (54)	GES-4		4	≤0.5 to 1	≤0.5 to 1	+	−	−	+(6–15)
	GES-4	CTX-M15	2	≤0.5 to 1	1	+	−	pos (1); neg (1)	+(6)
	IMP-1		1	≤0.5	4	+	+	+	+(6)
	IMP-6		3	≤0.5	>16	+	+	+	+(6)
	IMP-6	CTX-M-2	40	≤0.5 to 2	≤0.5 to >16	+	pos (38); neg (2)	+	+(6)
	IMP-6	CTX-M-2, DHA	1	≤0.5	>16	+	+	+	+(6)
	IMP-8		1	8	8	+	+	+	+(6)
	IMP-34		1	1	2	+	+	+	+(6)
	KPC-2	CTX-M-14	1	>16	>16	+	+	+	+(6)
M. morganii (1)	IMP-1		1	8	16	+	+	+	+(6)
P. mirabilis (1)	NDM-1	CTX-M-65	1	8	4	+	−	+	+(6)
P. rettgeri (1)	IMP-1		1	16	16	+	+	+	+(6)
S. marcescens (17)	IMP-1		16	16 to >16	16 to >16	+	+	+	+(6)
	IMP-6		1	>16	>16	+	+	+	+(6)

CIM: carbapenem inactivation method, ESBLs: extended-spectrum β-lactamases, IPM: imipenem, mCIM: modified carbapenem inactivation method, MEPM: meropenem, MICs: minimum inhibitory concentrations, PABL: plasmid-mediated AmpC β-lactamase, pos: positive, neg: negative.

Table 2
Results of mCIM, modified Hodge test, Carba NP test and CIM in 100 non-carbapenemase-producing *Enterobacteriaceae* clinical isolates.

Species	β-Lactamase content		No. of strains	MICs (mg/L)		Modified Hodge test with MEPM	Carba NP test	CIM test with MEPM	mCIM test (mm)
	Carbapenemase	ESBLs/PABL		IPM	MEPM				
E. aerogenes (8)			8	2–8	≤0.5 to 4	pos (1); neg (7)	−	−	−(21–23)
E. cloacae (11)			11	2–8	≤0.5 to 2	pos (4); neg (7)	−	−	−(20–22)
E. coli (52)		CTX-M-1	5	≤0.5	≤0.5	−	−	−	−(22–24)
		CTX-M-2	3	≤0.5	≤0.5	−	−	−	−(21–23)
		CTX-M-9	6	≤0.5 to 2	≤0.5 to 1	−	−	−	−(20–24)
		SHV-12	8	≤0.5	≤0.5	−	−	−	−(21–24)
		TEM-20	1	≤0.5	≤0.5	−	−	−	−(22)
		TEM-52	1	≤0.5	≤0.5	−	−	−	−(22)
		CMY	9	≤0.5	≤0.5	−	−	−	−(21–24)
		DHA	3	≤0.5	≤0.5	−	−	−	−(21–24)
		MOX	1	≤0.5	≤0.5	−	−	−	−(24)
		CMY, CTX-M-1	5	≤0.5	≤0.5	−	−	−	−(21–24)
		CMY, CTX-M-2	2	≤0.5	≤0.5	−	−	−	−(21–23)
		CMY, CTX-M-9	2	≤0.5	≤0.5	−	−	−	−(23–24)
		CMY, CTX-M-1, CTX-M-9	1	≤0.5	≤0.5	−	−	−	−(24)
		DHA, CTX-M-9	1	≤0.5	≤0.5	−	−	−	−(22)
K. oxytoca (5)		CTX-M-1	1	≤0.5	≤0.5	−	−	−	−(22)
		CTX-M-9	1	≤0.5	≤0.5	−	−	−	−(22)
		CMY	1	≤0.5	≤0.5	−	−	−	−(22)
		DHA	1	≤0.5	≤0.5	−	−	−	−(21)
		MOX	1	≤0.5	≤0.5	−	−	−	−(21)
K. pneumoniae (22)		CTX-M-1	2	≤0.5	≤0.5	−	−	−	−(22)
		CTX-M-2	2	≤0.5	≤0.5	−	−	−	−(22–23)
		CTX-M-9	3	≤0.5	≤0.5	−	−	−	−(22–23)
		CMY	3	≤0.5	≤0.5	−	−	−	−(21–23)
		DHA	8	≤0.5 to 4	≤0.5 to 4	−	−	−	−(21–24)
		MOX	1	≤0.5	≤0.5	−	−	−	−(23)
		DHA, CTX-M-9	2	≤0.5	≤0.5	−	−	−	−(22–24)
P. mirabilis (5)		CTX-M-2	3	≤0.5	≤0.5	−	−	−	−(21–24)
		CMY	2	≤0.5	≤0.5	−	−	−	−(21)
S. marcescens (2)			2	4	1–2	pos (1); neg (1)	−	−	−(22–23)

CIM: carbapenem inactivation method, ESBLs: extended-spectrum β-lactamases, IPM: imipenem, mCIM: modified carbapenem inactivation method, MEPM: meropenem, MICs: minimum inhibitory concentrations, PABL: plasmid-mediated AmpC β-lactamase, pos: positive, neg: negative.

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Table 3
Results of mCIM, modified Hodge test, Carba NP test and CIM in ATCC and NCTC strains.

Species		β-Lactamase content		No. of strains	MICs (mg/L)		Modified Hodge test with MEPM	Carba NP test	CIM test with MEPM	mCIM test (mm)
		Carbapenemase	ESBLs/PABL		IPM	MEPM				
CPE (6)										
E. coli	ATCC BAA-2469	NDM-1		1	2	4	−	+	+	+(6)
K. pneumoniae	ATCC BAA-2146	NDM-1		1	>16	>16	−	+	+	+(6)
	NCTC 13438	KPC-3		1	>16	>16	+	+	+	+(6)
	NCTC 13440	VIM-1		1	4	0.5	+	+	+	+(6)
	NCTC 13442	OXA-48		1	1	0.5	+	+	+	+(6)
	NCTC 13443	NDM-1		1	>16	>16	−	+	+	+(6)
Non-CPE (5)										
E. coli	ATCC 35218		TEM-1	1	≤0.5	≤0.5	−	−	−	−(23)
	ATCC 700603		SHV-18	1	≤0.5	≤0.5	−	−	−	−(22)
	NCTC 13351		TEM-3	1	≤0.5	≤0.5	−	−	−	−(21)
E. cloacae	ATCC BAA-2146		1	≤0.5	≤0.5	−	−	−	−(23)	
	NCTC 13464		CTX-M-9	1	≤0.5	≤0.5	−	−	−	−(21)

CIM: carbapenem inactivation method, CPE: carbapenemase-producing *Enterobacteriaceae*, ESBLs: extended-spectrum β-lactamases, IPM: imipenem, mCIM: modified carbapenem inactivation method, MEPM: meropenem, MICs: minimum inhibitory concentrations, PABL: plasmid-mediated AmpC β-lactamase.

P. mirabilis [19] as in the present study, the mCIM also shows positive results for these bacterial species and has better sensitivity. However, because CNP is superior in specificity and rapidity, it seemed to be a highly useful detection method in emergent cases.

Although the CIM is an excellent method, there are strains that cannot be detected with some genotypes such as OXA and GES [20]. The CIM also showed some false negatives for strains with GES-4 and VIM-2 that could, however, be detected with mCIM. As the sensitivity to mCIM improves further, it seems that this will become a very useful method for detecting CPE from now on. Because it is recommended that CLSI breakpoints be used with mCIM to determine results, interpretation of the results is easy due to the clear criteria. CPE strains excluding some GES strains did not form inhibition zones, and it was easy to distinguish them from non-CPE strains. Results after 6 h of incubation by CIM may not be easy to distinguish, but because it is possible to generate a report on the same day, it is a useful method in emergent cases. However, caution is necessary as CIM is inferior in its sensitivity to some genotypes compared to mCIM. CLSI research reports that detection sensitivity is not 100% with mCIM because it may be negative for OXA-232-producing *K. pneumoniae*, KPC-4 producing *E. coli* and *E. cloacae* and IMP-4 producing *K. pneumoniae* [5,6]. Because this study used clinical isolates obtained in the Kinki region of Japan, OXA was not included as a test strain because it is rarely detected, although many IMP strains are the most frequently detected strains in Japan. Because the study of OXA is inadequate, we consider this to be a limitation of this study, and it will be a future task. However, mCIM was evaluated to be a highly excellent method for detecting CPE in Japan with its many IMP strains.

In conclusion, because of the high sensitivity and specificity of mCIM compared to the other tested methods of CPE detection, we consider it to be a highly excellent method. In addition, as with the CIM, operation is simple and cost effective, and determination of the results is also clear, so it can be implemented in any laboratory. We believe that mCIM is the best CPE inspection method at the present time.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

Authorship statement

All authors meet the ICMJE authorship criteria. All authors have made substantial contributions to all of the following: the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be submitted.

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