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Simple Screening for Carbapenemase-Producing *Enterobacteriaceae* by Moxalactam Susceptibility Testing

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The increase in carbapenemase producing *Enterobacteriaceae* (CPE) is a serious concern worldwide (1–7). However, not all CPE isolates show reduced susceptibility to carbapenems (6, 8–11). Some CPE isolates also produce other beta-lactamases, such as extended-spectrum and/or AmpC-type beta-lactamases (12–14). For these reasons, screening for CPE by antibiotic susceptibility testing is challenging. The specific phenotypic detection methods for CPE currently in use include the carbapenem inactivation method (CIM) (15), the Carba NP test (15, 16), and the Cica-beta test (17). The CIM is based on the disk diffusion method. The Carba NP and Cica-beta tests are able to identify some beta-lactamase classes by using specific inhibitors. However, specific inhibitors that work against OXA-48 group class D carbapenem-hydrolyzing beta-lactamases are not available (18, 19). A screening technique for CPE before a second confirmatory assay by CIM, Carba NP test, Cica-beta test, or genetic detection test by PCR would be useful. Here, we demonstrate the efficiency of a simple screening technique for CPE using moxalactam.

Nonduplicate isolates including CPE and non-CPE were identified and characterized at Toho University (Table 1). The types of beta-lactamase genes were confirmed by PCR amplification and DNA sequencing. All isolates were stored in a freezer at −80°C until use. Antibiotic susceptibility testing was performed by the Clinical and Laboratory Standards Institute-recommended microdilution method (M07-A10) (20). Customized frozen plates for microdilution testing were purchased from Eiken Chemical Co., Ltd. (Tokyo, Japan). The Clinical and Laboratory Standards Institute interpretative criteria in document M100-S25 (21) were applied. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains for antibiotic susceptibility testing.

The positive predictive values (PPVs) of CPE detection by using CLSI resistance criteria for imipenem, meropenem, ceftazidime, and moxalactam were 93.5, 96.3, 74.8, and 93.7%, respectively. The negative predictive values (NPVs) of CPE detection by using the nonsusceptibility criteria for imipenem, meropenem, ceftazidime, and moxalactam were 50.7, 50.0, 80.4, and 72.9%, respectively. The NPV increased from 72.9% to 81.5% when the criterion for moxalactam (≥16 mg/liter) was used, but the PPV decreased from 93.7% to 90.4% (Table 2). Five false-positive results were observed in

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TABLE 1 Antibiotic activities of imipenem, meropenem, ceftazidime, and moxalactam against members of the family *Enterobacteriaceae*

Enzyme(s) produced (no. of isolates)	No. of isolates of:								Antibiotic	MIC (mg/liter)			%S/%R[a]
	Escherichia coli	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Salmonella* sp.	*Enterobacter* sp.	*Citrobacter* sp.	*Proteus mirabilis*	*Morganella morganii*		Range	MIC50	MIC90	
Carbapenemases													
IMP type (44)	0	0	1	0	43	0	0	0	Imipenem	0.25 to 8	0.5	2	81.8/6.8
									Meropenem	≤0.12 to 8	0.5	2	77.3/4.5
									Ceftazidime	32 to >256	128	>256	0.0/100
									Moxalactam	32 to >256	256	>256	0.0/93.2
IMP and CTX-M types (19)	7	5	4	0	3	0	0	0	Imipenem	≤0.12 to 2	0.25	1	94.7/0.0
									Meropenem	≤0.12 to 8	1	4	63.2/26.3
									Ceftazidime	4 to 128	32	64	5.3/89.5
									Moxalactam	16 to >256	256	>256	0.0/84.2
NDM-1 (11)	4	6	0	0	1	0	0	0	Imipenem	2 to 64	8	64	0.0/90.9
									Meropenem	2 to 64	16	64	0.0/90.9
									Ceftazidime	>256	>256	>256	0.0/100
									Moxalactam	>256	>256	>256	0.0/100
KPC type (12)	3	4	0	0	5	0	0	0	Imipenem	0.25 to 32	4	8	16.7/58.3
									Meropenem	≤0.12 to 32	1	8	58.3/25
									Ceftazidime	4 to 256	64	256	16.7/66.7
									Moxalactam	1 to 32	2	32	75/0.0
GES-4 (3)	0	1	0	0	2	0	0	0	Imipenem	16 to 64			
									Meropenem	16 to 64			
									Ceftazidime	4 to >256			
									Moxalactam	16 to >256			
OXA-48 (11)	3	6	0	0	1	1	0	0	Imipenem	0.5 to 128	4	16	18.2/54.5
									Meropenem	0.25 to 128	0.5	32	63.6/27.3
									Ceftazidime	0.25 to 256	64	256	27.3/72.7
									Moxalactam	2 to >256	8	>256	54.5/36.4
Total (100)	17	22	5	0	55	1	0	0	Imipenem	≤0.12 to 128	1	16	58.0/29.0
									Meropenem	≤0.12 to 128	1	16	60.0/26.0
									Ceftazidime	0.25 to >256	128	>256	8.0/89.0
									Moxalactam	1 to >256	128	>256	15.0/74.0
Noncarbapenemases													
CTX-M type (57)	57	0	0	0	0	0	0	0	Imipenem	≤0.12 to 0.5	≤0.12	0.25	100/0.0
									Meropenem	≤0.12	≤0.12	≤0.12	100/0.0
									Ceftazidime	0.5 to 256	4	64	57.9/24.6
									Moxalactam	≤0.12 to 16	0.25	1	98.2/0.0
Chromosomal AmpC (7)	0	0	0	0	5	1	0	1	Imipenem	≤0.12 to 2			
									Meropenem	≤0.12 to 0.25			
									Ceftazidime	0.25 to 256			
									Moxalactam	≤0.12 to 64			
External AmpC (11)	5	4	0	1	0	0	1	0	Imipenem	≤0.12 to 64	0.25	4	81.8/18.2
									Meropenem	≤0.12 to 16	≤0.12	≤0.12	90.9/9.1
									Ceftazidime	1 to >256	64	256	9.1/90.9
									Moxalactam	≤0.12 to >256	4	>256	63.6/18.2
Total (75)	62	4	0	1	5	1	1	1	Imipenem	≤0.12 to 64	≤0.12	0.25	96.0/2.7
									Meropenem	≤0.12 to 16	≤0.12	≤0.12	98.7/1.3
									Ceftazidime	0.25 to >256	8	128	46.5/40
									Moxalactam	≤0.12 to >256	0.25	16	88.0/6.7

[a]S, susceptible; R, resistant.

TABLE 2 Results of screening of carbapenemase-producing members of the family *Enterobacteriaceae* by interpretation criteria[a]

Antibiotic	% PPV[b]	% NPV[c]
Imipenem	93.5 (29/31)	50.7 (73/144)
Meropenem	96.3 (26/27)	50.0 (74/148)
Ceftazidime	74.8 (89/119)	80.4 (45/56)
Moxalactam	93.7 (74/79)	72.9 (70/96)
Moxalactam (≥16 mg/liter)	90.4 (85/94)	81.5 (66/81)
Ceftazidime[d]	67.0 (61/91)	95.7 (45/47)

[a]The interpretation criteria used were those in reference 21, except for moxalactam (≥16 mg/liter).
[b]The values in parentheses are the number of carbapenem producers/number of resistant isolates.
[c]The values in parentheses are the number of non-carbapenem producers/number of susceptible and nonsusceptible isolates.
[d]IMP-type enzyme producers, $n = 63$.

AmpC producers, and 26 false-negative results were observed in 12 KPC-type, 7 OXA-type, 6 IMP-type, and 1 GES-4-like enzyme-producing members of the family *Enterobacteriaceae*.

A limitation of this study is that we were unable to test a comprehensive range of CPE isolates because of a limited number of KPC-type, OXA-48, OXA-181, NDM-type, VIM-type, and VEB-type enzyme-producing CPE isolates. In Japan, the major carbapenemase is of the IMP type. Further testing to assess performance with chromosomal or acquired AmpC-producing *Enterobacteriaceae* isolates is in progress.

In conclusion, moxalactam at ≥16 mg/liter may be a useful, cheap, and simple primary screening method for detecting CPE in the clinical laboratory but requires follow-up confirmatory testing.

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We have no conflicts of interest to declare.

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