



# 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 ( $\geq 16$  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:								MIC (mg/liter)					
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>	<i>Salmonella</i> sp.	<i>Enterobacter</i> sp.	<i>Citrobacter</i> sp.	<i>Proteus mirabilis</i>	<i>Morganella morganii</i>	Antibiotic	Range	MIC50	MIC90	%S/R <sup>a</sup>	
<b>Carbapenemases</b>														
IMP type (44)	0	0	1	0	43	0	0	0	0	0	0	0	0	81.8/6.8
									Imipenem	0.25 to 8	0.5	2	2	77.3/4.5
									Meropenem	≤0.12 to 8	0.5	2	2	0.0/100
									Ceftazidime	32 to >256	128	>256	>256	0.0/93.2
									Moxalactam	32 to >256	256	>256	>256	94.7/0.0
IMP and CTX-M types (19)	7	5	4	0	3	0	0	0	0	0	0	0	0	63.2/26.3
									Meropenem	≤0.12 to 2	0.25	1	1	5.3/89.5
									Ceftazidime	≤0.12 to 8	1	4	4	0.0/84.2
									Moxalactam	4 to 128	32	64	64	0.0/90.9
NDM-1 (11)	4	6	0	0	1	0	0	0	0	0	0	0	0	0.0/90.9
									Imipenem	16 to >256	256	>256	>256	0.0/100
									Meropenem	2 to 64	8	64	64	0.0/100
									Ceftazidime	2 to 64	16	64	64	0.0/100
									Moxalactam	>256	>256	>256	>256	0.0/100
KPC type (12)	3	4	0	0	5	0	0	0	0	0	0	0	0	16.7/58.3
									Imipenem	0.25 to 32	4	4	8	16.7/58.3
									Meropenem	≤0.12 to 32	1	8	8	58.3/25
									Ceftazidime	4 to 256	1	64	256	16.7/66.7
									Moxalactam	1 to 32	2	2	32	75/0.0
GES-4 (3)	0	1	0	0	2	0	0	0	0	0	0	0	0	0.0/100
									Imipenem	16 to 64	16	64	64	0.0/100
									Meropenem	16 to 64	16	64	64	0.0/100
									Ceftazidime	4 to >256	4	16	16	0.0/100
									Moxalactam	16 to >256	16	64	64	0.0/100
OXA-48 (11)	3	6	0	0	1	1	0	0	0	0	0	0	0	18.2/54.5
									Imipenem	0.5 to 128	4	16	16	63.6/27.3
									Meropenem	0.25 to 128	0.5	32	32	27.3/72.7
									Ceftazidime	0.25 to 256	64	256	256	54.5/36.4
									Moxalactam	2 to >256	8	>256	>256	58.0/29.0
Total (100)	17	22	5	0	55	1	0	0	0	0	0	0	0	60.0/26.0
									Imipenem	≤0.12 to 128	1	16	16	8.0/89.0
									Meropenem	≤0.12 to 128	1	16	16	15.0/74.0
									Ceftazidime	0.25 to >256	128	>256	>256	15.0/74.0
									Moxalactam	1 to >256	128	>256	>256	15.0/74.0
<b>Noncarbapenemases</b>														
CTX-M type (57)	57	0	0	0	0	0	0	0	0	0	0	0	0	100/0.0
									Imipenem	≤0.12 to 0.5	≤0.12	0.25	0.25	100/0.0
									Meropenem	≤0.12	≤0.12	≤0.12	≤0.12	100/0.0
									Ceftazidime	0.5 to 256	4	64	64	57.9/24.6
									Moxalactam	≤0.12 to 16	0.25	1	1	98.2/0.0
Chromosomal AmpC (7)	0	0	0	0	5	1	0	0	1	0	0	0	0	81.8/18.2
									Imipenem	≤0.12 to 2	0.25	4	4	90.9/9.1
									Meropenem	≤0.12 to 0.25	0.25	4	4	9.1/90.9
									Ceftazidime	0.25 to 256	0.25	4	4	63.6/18.2
									Moxalactam	≤0.12 to 64	0.25	4	4	96.0/2.7
External AmpC (11)	5	4	0	1	0	0	1	0	0	0	0	0	0	98.7/1.3
									Imipenem	≤0.12 to 16	0.25	4	4	46.5/40
									Meropenem	≤0.12 to 64	0.25	4	4	88.0/6.7
									Ceftazidime	1 to >256	64	256	256	88.0/6.7
									Moxalactam	≤0.12 to >256	0.25	16	16	88.0/6.7
Total (75)	62	4	0	1	5	1	1	1	1	0	0	0	0	81.8/18.2
									Imipenem	≤0.12 to 0.5	≤0.12	0.25	0.25	100/0.0
									Meropenem	≤0.12	≤0.12	≤0.12	≤0.12	100/0.0
									Ceftazidime	0.5 to 256	4	64	64	57.9/24.6
									Moxalactam	≤0.12 to 16	0.25	1	1	98.2/0.0
									Imipenem	≤0.12 to 2	0.25	4	4	81.8/18.2
									Meropenem	≤0.12 to 0.25	0.25	4	4	90.9/9.1
									Ceftazidime	0.25 to 256	0.25	4	4	9.1/90.9
									Moxalactam	≤0.12 to 64	0.25	4	4	63.6/18.2
									Imipenem	≤0.12 to 16	0.25	4	4	96.0/2.7
									Meropenem	≤0.12 to 64	0.25	4	4	98.7/1.3
									Ceftazidime	0.25 to >256	8	128	128	46.5/40
									Moxalactam	≤0.12 to >256	0.25	16	16	88.0/6.7

<sup>a</sup>S, susceptible; R, resistant.

**TABLE 2** Results of screening of carbapenemase-producing members of the family *Enterobacteriaceae* by interpretation criteria<sup>a</sup>

Antibiotic	% PPV <sup>b</sup>	% NPV <sup>c</sup>
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 ( $\geq 16$ mg/liter)	90.4 (85/94)	81.5 (66/81)
Ceftazidime <sup>d</sup>	67.0 (61/91)	95.7 (45/47)

<sup>a</sup>The interpretation criteria used were those in reference 21, except for moxalactam ( $\geq 16$  mg/liter).

<sup>b</sup>The values in parentheses are the number of carbapenem producers/number of resistant isolates.

<sup>c</sup>The values in parentheses are the number of non-carbapenem producers/number of susceptible and nonsusceptible isolates.

<sup>d</sup>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  $\geq 16$  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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