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Indication of Minimum Inhibitory Concentration of β-Lactam Antimicrobials for the Primary Extraction of IMP-Producing *Enterobacteriaceae*

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Carbapenem-resistant *Enterobacteriaceae* (CRE), often called "nightmare bacteria," are a world-wide medical concern. Carbapenem resistance is due to various biochemical or microbiological mechanisms present in Gram-negative bacilli. Among these, carbapenemase production is considered the most important clinically. Carbapenemase-encoding genes are often harbored in extrachromosomal DNA, such as plasmids, which can be disseminated through conjugal transfer to other bacterial strains. Although clinical isolation of carbapenemase-producing *Enterobacteriaceae* (CPE) is rare in Japan, IMP-type-CPE (IMP-CPE) strains, which have caused several outbreaks in medical institutions, are the most frequently isolated. A central issue associated with the detection of IMP-CPE is that their minimum inhibitory concentration (MIC) of carbapenems is often low in vitro, although most of them develop carbapenem resistance in vivo (1,2). Therefore, early and accurate detection of IMP-CPE is critical for both infection control and treatment of associated infectious diseases. In Japan, administrative criteria are in place for reporting cases of clinically isolated CRE (meropenem-MIC ≥ 2 μg/mL, or both imipenem-MIC ≥ 2 μg/mL and cefmetazole-MIC ≥ 32 μg/mL). However, there is no indication regarding how to detect CPE in clinical situations. Therefore, medical institutions are required to employ additional phenotypic methods, such as the carbapenem inactivation method (CIM) (3,4), or genetic tests using polymerase chain reaction (PCR) to detect CPE clinically. However, it is difficult for general hospitals to conduct these tests for all clinically isolated *Enterobacteriaceae*. Thus, primary indications to suspect CPE, especially IMP-CPE, are urgently needed.

We evaluated the correlation between MICs of β-lactam antimicrobials and IMP production, using 998 *Enterobacteriaceae* strains, including 459 IMP-CPE, stored in our laboratory (Department of Microbiology and Infectious Diseases, Nara Medical University, Nara, Japan and Department of Infection Control, Fukushima Medical University, Fukushima, Japan). These bacterial species were identified via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, Bruker Dalitonics K.K., Kanagawa, Japan) (5). Carbapenemase-encoding genes were identified through PCR and sequencing of products (6,7). The *Enterobacteriaceae* identified were as follows: 551 *Escherichia coli* including 279 IMP producers, 364 *Klebsiella pneumoniae* including 127 IMP producers and 83 *Enterobacter* spp. including 53 IMP producers (Table 1). Carbapenemase producers other than IMP producers were not detected. Subsequently, the MICs of tazobactam/piperacillin, meropenem, imipenem, ceftazidime, cefepime, cefmetazole, and aztreonam for the tested strains were determined using the agar dilution method as indicated by the Clinical and Laboratory Standards Institute (8). We also investigated whether the tested IMP-CPE strains coincided with the Japanese CRE criteria.

Correlation between the MICs of β-lactam antimicrobials and carbapenemase production in the tested *Enterobacteriaceae* strains is shown (Table 1). Among the β-lactam antimicrobials tested, meropenem, cefepime, and cefmetazole were found to be suitable for use as indicator antimicrobials for primary extraction of IMP-CPEs. When the cut-off for MIC was set to 0.25 μg/mL meropenem, 96.1% (441/459) of IMP-CPEs were extracted, and 78.3% (422/539) of non-CPEs were excluded. Similarly, the cut-off, set to 0.125 μg/mL meropenem-MIC, extracted 99.6% (457/459) of IMP-CPEs and excluded 71.1% (383/539) of non-CPEs. With respect to β-lactam antimicrobials other than carbapenems, the cut-off, set to 16 μg/mL cefmetazole-MIC, extracted 99.8% (405/406) of IMP-producing *E. coli* and *K. pneumonia*e, and excluded 69.5% (354/509) of non-carbapenemase-producing *E. coli* and *K. pneumonia*e. When the cut-off was set to 2 or 4 μg/mL cefepime-MIC, 100% (53/53) or 98.1% (52/53) of IMP-producing *Enterobacter* spp. were extracted, and 70.0% (21/30) or 73.3% (22/30) of non-carbapenemase-producing *Enterobacter* spp. were excluded, respectively. In addition, the coincidence rate between the tested strains and Japanese CRE criteria is shown (Table 1). Tested IMP-CPE strains that met the criteria were 17.0% (78/459), while 81.8% (441/539) of the tested non-CPE strains were excluded using the criteria (Table 1). Additionally, the MIC distributions of meropenem, cefmetazole, and cefepime for the tested *Enterobacteriaceae* strains are shown, displaying the basis for the cut-off set for our indication (Table 2).

The results of this study indicate that Japanese CRE criteria may not be suitable for clinically suspecting

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Table 1. Correlation between the minimum inhibitory concentrations (MICs) of β-lactam antimicrobials and IMP production in the tested *Enterobacteriaceae* strains

MIC cut-off line (μg/mL)		CPE showing MIC not less than cut-off line (Sensitivity)						Non-CPE showing MIC less than cut-off line (Specificity)					
		E. coli		*K. pneumoniae*		*Enterobacter* spp.		*E. coli*		*K. pneumoniae*		*Enterobacter* spp.	
Tazobactam/	4	94/279	33.7%	101/127	79.5%	51/53	96.2%	220/272	80.9%	11/237	4.6%	15/30	50.0%
Piperacillin	2	202/279	72.4%	124/127	97.6%	53/53	100%	116/272	42.6%	4/237	1.7%	3/30	10.0%
Meropenem	0.25	264/279	94.6%	126/127	99.2%	51/53	96.2%	219/272	80.5%	185/237	78.1%	18/30	60.0%
	0.125	279/279	100%	127/127	100%	51/53	96.2%	193/272	71.0%	173/237	73.0%	17/30	56.7%
Imipenem	0.25	158/279	56.6%	55/127	43.3%	50/53	94.3%	151/272	55.5%	132/237	55.7%	2/30	6.7%
	0.125	275/279	98.6%	106/127	83.5%	53/53	100%	19/272	7.0%	17/237	7.2%	1/30	3.3%
Ceftazidime	128	69/279	24.7%	48/127	37.8%	52/53	98.1%	231/272	84.9%	99/237	41.8%	17/30	56.7%
	64	108/279	38.7%	74/127	58.3%	53/53	100%	198/272	72.8%	79/237	33.3%	16/30	53.3%
	32	193/279	69.2%	116/127	91.3%	53/53	100%	163/272	59.9%	56/237	23.6%	13/30	43.3%
Cefepime	4	250/279	89.6%	109/127	85.8%	52/53	98.1%	144/272	52.9%	56/237	23.6%	22/30	73.3%
	2	263/279	94.3%	121/127	95.3%	53/53	100%	74/272	27.2%	45/237	19.0%	21/30	70.0%
Cefmetazole	16	279/279	100%	126/127	99.2%	53/53	100%	187/272	68.8%	167/237	70.5%	0/30	0%
	8	279/279	100%	127/127	100%	53/53	100%	175/272	64.3%	141/237	59.5%	0/30	0%
Aztreonam	16	117/279	41.9%	37/127	29.1%	7/53	13.2%	188/272	69.1%	76/237	32.1%	25/30	83.3%
	8	220/279	78.9%	68/127	53.5%	10/53	18.9%	139/272	51.1%	65/237	27.4%	23/30	76.7%
Japanese CRE criteria Meropenem ≥ 2 or both Imipenem ≥ 2 and Cefmetazole ≥ 32		41/279	14.7%	32/127	25.2%	5/53	9.4%	231/272	84.9%	206/237	86.9%	4/30	13.3%

Table 2. The distribution of minimum inhibitory concentrations (MICs) of meropenem, cefmetazole, and cefepime against the tested *Enterobacteriaceae* strains

Antimicrobial		Distribution of MIC													
Meropenem-MIC (μg/mL)		≤ 0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	> 256
E. coli (*n*)	IMP	—	15	82	81	59	29	8	4	—	1	—	—	—	—
	non-CPE	193	26	23	4	9	6	3	6	1	1	—	—	—	—
K. pneumoniae (*n*)	IMP	—	1	5	40	50	20	5	2	2	1	1	—	—	—
	non-CPE	173	12	9	5	10	8	11	6	2	—	1	—	—	—
Enterobacter spp. (*n*)	IMP	2	0	6	14	17	6	6	1	—	—	1	—	—	—
	non-CPE	17	1	2	4	2	1	2	1	—	—	—	—	—	—
Cefmetazole-MIC (μg/mL)		≤ 0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	> 256
E. coli (*n*)	IMP	—	—	—	—	—	—	—	—	23	55	70	62	42	27
	non-CPE	—	—	2	17	101	39	16	12	8	24	34	6	2	11
K. pneumoniae (*n*)	IMP	—	—	—	—	—	—	—	1	3	11	32	37	8	35
	non-CPE	—	—	—	5	33	61	42	26	13	6	12	14	8	17
Cefepime-MIC (μg/mL)		≤ 0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	> 256
Enterobacter spp. (*n*)	IMP	—	—	—	—	—	1	3	32	10	6	1	—	—	—
	non-CPE	10	5	3	1	2	1	4	3	1	—	—	—	—	—

IMP-CPE, while IMP-CPEs are extracted using the indication MICs of meropenem, cefepime, and cefmetazole. When the indication MIC of meropenem was used, IMP-CPEs were extracted regardless of their species identity. On the other hand, cefmetazole-MIC was suited for extracting IMP-producing *E. coli* and *K. pneumoniae*, whereas, the MIC of cefepime was suited for extracting IMP-producing *Enterobacter* spp. Substrate specificity of AmpC cephalosporinases, which are often produced by *Enterobacter* spp. chromosomally, may be related to these results. Although results revealed that the meropenem-MIC was most suited to extract IMP-CPEs first, this is not always adoptable in medical institutions because low ranges of meropenem-MICs, such as 0.25 or 0.125 μg/mL, cannot be necessarily measured clinically. On the other hand, the MICs of cefmetazole and cefepime are commonly measured for isolating *Enterobacteriaceae*. Hence, primary extraction of IMP-CPEs using those MICs under clinical conditions may be reasonable. Therefore, the following protocol is suggested; *E. coli* and *K. pneumonia*e strains showing cefmetazole-MIC of 16 μg/mL and above and *Enterobacter* spp. showing cefepime-MIC of 4 μg/mL and above may be first extracted as potential IMP producers. Although genetic tests or phenotypic methods, such as CIM, are needed to firmly identify CPEs, it is beneficial to exclude many, if not all, non-CPEs first, in order to narrow down strains that are to be subjected to second stage

inspection. We believe that this may save time and costs, because using our indications to exclude most non-CPEs first, would eliminate the need to conduct unnecessary tests for those non-CPEs. CPEs other than IMP-CPEs, such as KPC-type, NDM-type, OXA-48-type, VIM-type and GES-type producers, were not evaluated in this study, as those were intended to be subjects for the next study. We also confirmed that Japanese CRE criteria are not suited for extraction of IMP-CPEs. Although these criteria are not intended for screening CPEs, but for reporting potential cases for clinical CRE isolation, they are being used for screening CRE, including IMP-CPE, in several Japanese medical institutions. As a result, more than a few IMP-CPEs are considered to have been clinically overlooked in Japan. Thus, an accurate indication for clinical suspecting IMP-CPE is required, as such an indication may help prevent IMP-CPE from spreading. Implementation of primary IMP-CPE extraction using our indication by hospital laboratories followed by secondary detection using genetic tests or phenotypic methods such as the CIM, may enable efficient detection of IMP-CPE, thereby leading to improved infection control and appropriate treatment of associated infectious diseases.

Conflict of interest None to declare.

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