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Rapid Identification of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$ by Multiplex Amplification Refractory Mutation System PCR

Akiyo Nakano, M.S.[1,*], Ryuichi Nakano, Ph.D.[1,*], Yuki Suzuki, M.S.[1], Kyoichi Saito, M.D.[1], Kei Kasahara, M.D.[2], Shiro Endo, M.D.[3], and Hisakazu Yano, M.D.[1]

Department of Microbiology and Infectious Diseases[1] and Center for Infectious Diseases[2], Nara Medical University, Nara, Japan; International University of Health and Welfare[3], Shioya Hospital, Tochigi, Japan

Dear Editor,

Carbapenem-resistant Enterobacteriaceae have acquired carbapenemase genes [1], which differ substantially across countries [2]. Transferable carbapenemase IMP-type metallo-β-lactamases, particularly IMP-1 and IMP-6, are commonly identified in the clinical setting in Japan [3, 4] and exhibit different substrate specificity despite having a difference of only one amino acid (IMP-6: Ser214Gly). IMP-1 producers are more resistant to imipenem than to meropenem, whereas IMP-6 producers are more resistant to meropenem [5]. We previously found that the susceptibility rate of IMP-6-positive *Escherichia coli* was higher for imipenem than for meropenem [3]. Thus, IMP-6-producing isolates may be erroneously categorized as imipenem-susceptible, which could lead to treatment failure in patients.

It is important to distinguish between the genes *bla*$_{IMP-1}$ (encoding IMP-1) and *bla*$_{IMP-6}$ (encoding IMP-6) because of the differences in substrate specificity; these variants can be identified by either DNA sequencing or amplification refractory mutation system (ARMS) PCR [6]. However, these methods are costly and time-consuming, and ARMS PCR requires multiple reaction tubes per sample. Therefore, we developed a simple, rapid multiplex ARMS PCR assay to discriminate between IMP-1 and IMP-6. We designed two sets of Multiplex ARMS PCR primers based on the nucleotide sequences of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$. Primers IMP-f and IMP-r were designed to recognize the conserved sequences of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$, while primers IMP-1f and IMP-6r were designed to recognize specific nucleotide sequences of *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$, respectively [7].

PCR amplification using the primer pair IMP-1f (5′-AAGGCAAAACTGGTTGTTCCTA-3′) and IMP-r (5′-CGACTTGTTAGAAATTTAGTTGC-3′) results in a 120-bp *bla*$_{IMP-1}$ gene-specific fragment, and PCR amplification using the primer pair IMP-f (5′-CTCGATCTATCCCCACGTATG-3′) and IMP-6r (5′-TCGTCTCCAACTTCACTGTGAGC-3′) generates a 350-bp *bla*$_{IMP-6}$ gene-specific fragment (underlined sequences indicate nucleotide alternations for allele-specific primers). The 3′ termini of the primers correspond to the specific nucleotide sequence at position 640 of IMP-1 (AGT, Ser) and IMP-6 (GGT, Gly), respectively. We performed multiplex ARMS PCR amplification using the Qiagen Multiplex PCR Master Mix (Qiagen, Venlo, The Netherlands) with the following conditions: denaturation for 15 minutes at 95°C and 32 cycles of 10 seconds at 95°C, 30 seconds at 50°C, and 20 seconds at 72°C. DNA fragments were analyzed by electrophoresis. We used IMP-1-producing *E. coli* (NR2406) and IMP-6-producing *E. coli* (NR2407) from Japanese clinical isolates as positive controls. The bands, 120- or 350-bp in size, were obtained

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Corresponding author: Ryuichi Nakano
Department of Microbiology and Infectious Diseases, Nara Medical University, 840 Shijo-chou, Kashihara, Nara 634-8521, Japan
Tel & Fax: +81-744-29-8839, E-mail: rnakano@naramed-u.ac.jp

*These authors contributed equally to this article.

within 90 minutes or less (Fig. 1).

We performed this method on 350 carbapenemase-producing clinical isolates collected from general hospitals across Japan from 2012 to 2016 and archived in our laboratory [4, 8].

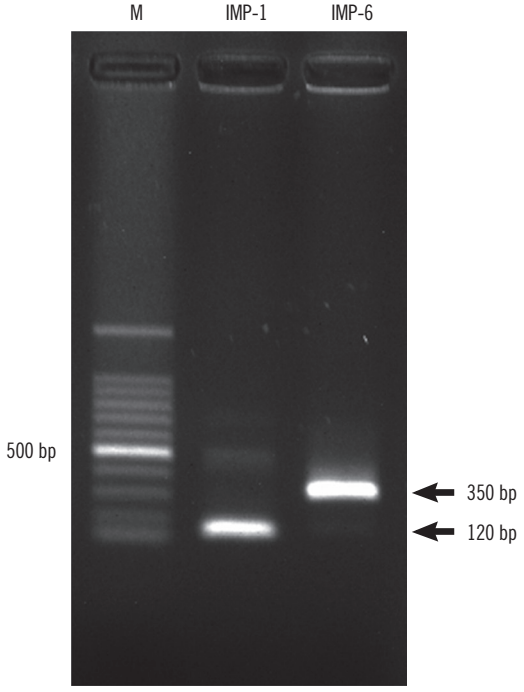

Fig. 1. Representative multiplex ARMS PCR patterns of IMP-1- and IMP-6-producing strains.
Abbreviations: ARMS, amplification refractory mutation system; Lane M, 100-bp DNA ladder marker.

We also determined the antibiotic susceptibility of the isolates by the agar dilution method in accordance with CLSI recommendations [9]. The multiplex ARMS PCR results and imipenem and meropenem minimum inhibitory concentration (MIC) ranges of the isolates are shown in Table 1. The IMP-1 and IMP-6 producers yielded PCR products of the expected size. We confirmed the accuracy of this method by DNA sequencing of the PCR products (120-bp and 350-bp bands) of three representative strains. Cross-reactivity for other types of carbapenemase producers was not detected, and false-positive or false-negative results were not observed (Table 1).

This multiplex ARMS PCR assay successfully discriminated between *bla*$_{IMP-1}$ and *bla*$_{IMP-6}$. Thus, this method could serve as a specific, rapid, and simple alternative for the detection of IMP-1 or IMP-6 producers and could facilitate clinical treatment, infection control, and large-scale IMP producer screening.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Table 1. Characteristics of carbapenemase-producing strains used in this study and the multiplex ARMS PCR results

Species	Strains (N)	β-lactamase	MIC range (μg/mL)		Specific band* (N)
			IPM	MEPM	
Klebsiella pneumoniae	31	IMP-1	0.125–64	0.125–64	120 bp (31)
Enterobacter cloacae	18	IMP-1	0.125–4	≤0.06–4	120 bp (18)
Klebsiella oxytoca	9	IMP-1	0.125–1	0.125–2	120 bp (9)
Escherichia coli	5	IMP-1	0.125–1	0.5–2	120 bp (5)
Citrobacter freundii	2	IMP-1	0.25–1	0.125–0.5	120 bp (2)
Escherichia coli	170	IMP-6	≤0.06–1	0.125–32	350 bp (170)
Klebsiella pneumoniae	87	IMP-6	≤0.06–0.5	0.25–16	350 bp (87)
Enterobacter cloacae	1	IMP-6	0.125	0.25	350 bp (1)
Klebsiella pneumoniae	12	KPC	2–32	2–64	None
Escherichia coli	12	NDM	1–16	0.5–32	None
Klebsiella pneumoniae	2	OXA-48 like	0.5–1	0.5	None
Citrobacter freundii	1	VIM	1	1	None

*120-bp band, IMP-1-specific band; 350-bp band, IMP-6-specific band.
Abbreviations: MIC, minimum inhibitory concentration; IPM, imipenem; MEPM, meropenem; ARMS, amplification refractory mutation system.

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