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Rapid Detection and Typing of Carbapenemase Genes from Carbapenem-Resistant Enterobacteriaceae Isolates Collected in a Japanese Hospital Using the Xpert Carba-R Assay

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Carbapenem-resistant Enterobacteriaceae (CRE) are a growing threat to infectious disease treatment and control (1). Rapid detection of CRE, particularly carbapenemase-producing CRE (CP-CRE), and typing of carbapenemases are important for preventing and controlling outbreaks of CRE (2). GeneXpert[®] systems have recently been developed for the automated detection of target genes. Integrated with the Xpert[®] Carba-R assay, GeneXpert[®] systems can detect 5 types of carbapenemase genes (*bla*_{IMP-1}, *bla*_{KPC}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{NDM}) from rectal swabs or isolates in 2 easy steps that can be completed within 1 h (3). However, few studies to verify the accuracy of the Xpert[®] Carba-R assay have been conducted in North America and Europe (3–5), and no such studies have been conducted in Japan, where the epidemiology of CRE is far different from that in Western countries (6). First, IMP-type carbapenemases are most prevalent in Japan. Although the manufacturer reported high sensitivity of the Xpert[®] Carba-R assay for *bla*_{IMP-1} detection (3), few other studies have confirmed this result. However, several studies have evaluated the sensitivity of the Xpert[®] Carba-R assay for the detection of other types of carbapenemases. Second, *Enterobacter* species are the most common CRE in Japan (7), while *Klebsiella* species are dominant in Western countries (1). Although the manufacturer evaluated the sensitivity of the Xpert[®] Carba-R assay using *Enterobacter* strains, the number of tested strains was small (3). The most important challenge for Japanese clinicians is the ability to accurately identify CP-CRE among a large number of carbapenem-resistant *Enterobacter* isolates. Therefore, the clinical utility of the Xpert[®] Carba-R assay in Japan remains unclear. Thus, the aim of this study was to evaluate the clinical utility of the Xpert[®] Carba-R assay using CRE isolated in a university hospital in Japan.

This study included all CRE isolates collected in Keio

University Hospital between January 2014 and December 2014. Duplicate isolates collected from the same patient were excluded. We used the following criteria to define CRE, in accordance with the Infectious Diseases Control Law in Japan: minimum inhibitory concentration (MIC) of meropenem was no less than 2 µg/mL, or MIC of imipenem was no less than 2 µg/mL and that of cefmetazole was no less than 64 µg/mL. During the study period, 43 CRE isolates met the inclusion criteria, including 29 *Enterobacter* isolates, 10 *Klebsiella pneumoniae* isolates, 2 *Escherichia coli* isolates, 1 *Citrobacter freundii* complex isolate, and 1 *Serratia marcescens* isolate.

CRE isolates stored at –80°C were thawed and cultured on sheep blood agar plates (Nissui, Tokyo, Japan) for 20 h to obtain colonies. Each isolate was evaluated for the presence of carbapenemase genes using the Xpert[®] Carba-R assay cartridge, which was loaded into the GeneXpert[®] VI system according to the manufacturer's instructions (3). We also evaluated the isolates for the presence of carbapenemase genes using a conventional PCR assay. DNA was extracted from each CRE isolate by the boiling method (100°C, 10 min), adjusted to a concentration of 50 ng/µL, and used as template. Amplification was performed using previously described primer pairs for *bla*_{IMP-1} and *bla*_{VIM-2} (8), *bla*_{NDM} (9), *bla*_{KPC} (10), and *bla*_{OXA-48} (11). One microliter of each primer (10 µM) was added to 10 µL of PCR master mix (Ampdirect[®], Shimadzu, Kyoto, Japan), 0.1 µL of BioTaq[™] HS DNA polymerase (Shimadzu), 6.9 µL of H₂O, and 1 µL of DNA template. The thermocycling conditions were as follows: 1 cycle at 95°C for 10 min, followed by 30 cycles at 94°C for 15 s, 55°C for 30 s, and 72°C for 60 s, and a final cycle at 72°C for 7 min. The PCR products were electrophoresed using the MCE[®]-202 MultiNA Microchip Electrophoresis System (Shimadzu).

We calculated the sensitivity and specificity of the Xpert[®] Carba-R assay for the detection of *bla*_{IMP-1}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{NDM}, and *bla*_{VIM-2}. The PCR results were used as the reference for the Xpert[®] Carba-R assay. The study protocol was approved by the Ethics Committee of Keio University School of Medicine.

The results of the Xpert[®] Carba-R assay and the PCR assay are shown in Table 1. No isolates harbored *bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48}, or *bla*_{VIM-2}. PCR analysis identified 14 isolates as positive for *bla*_{IMP-1}. The

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Table 1. The number of carbapenemase-positive strains of each Enterobacteriaceae species

Identification	Number (CRE)	<i>bla</i> _{IMP-1} -positive strain		
		Xpert™	Carba-R	PCR
<i>Enterobacter cloacae</i>	21	5		5
<i>Klebsiella pneumoniae</i>	10	7		7
<i>Enterobacter aerogenes</i>	7	0		0
<i>Escherichia coli</i>	2	0		0
<i>Enterobacter</i> spp. ¹⁾	1	0		0
<i>Citrobacter freundii</i>	1	1		1
<i>Serratia marcescens</i>	1	0		1
Total	43	13		14

¹⁾; *Enterobacter* strains other than *E. cloacae* and *E. aerogenes*. No isolates harbored *bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48}, or *bla*_{VIM-2}.

Xpert® Carba-R assay identified 13 of these isolates as positive and 1 as negative for *bla*_{IMP-1}. Thus, the sensitivity and specificity of the Xpert® Carba-R assay for detecting the 5 carbapenemase genes were 92.9% and 100%, respectively, in reference to the conventional PCR assay.

The only carbapenemase gene detected in this study was *bla*_{IMP-1}. This result is consistent with the findings of a previous epidemiological study of carbapenemases in Japan (7). In Japan, IMP-type carbapenemases are the most prevalent, whereas OXA-48, NDM, and KPC carbapenemases are extremely rare.

Our results indicate that the Xpert® Carba-R assay has high sensitivity and specificity for the detection of carbapenemase genes in CRE. To the best of our knowledge, only 4 previous studies have tested the accuracy of carbapenemase gene detection using the Xpert® system (3–5,12), and strains positive for *bla*_{IMP-1} were included in only 2 of those studies. In a multisite prospective study performed by Cepheid Inc. (3), 27 isolates positive for *bla*_{IMP-1} were collected from 3 reference laboratories in the United States and Europe. The sensitivity and specificity of the Xpert® Carba-R assay for *bla*_{IMP-1} detection were 96.3% and 100%, respectively. In the other study (4), performed in England, the sensitivity of the assay for *bla*_{IMP} detection was very low (71%). The sensitivity in the present study was much higher than that in the latter study.

In the present study, an *S. marcescens* isolate was identified as positive for *bla*_{IMP-1} by the conventional PCR assay but not by the Xpert® Carba-R assay. Although the exact cause of this discrepancy is unknown, there are several possibilities. The first possibility is manipulation error; however, the isolate was tested twice using the Xpert® Carba-R assay and 3 times using conventional PCR, so this possibility is unlikely. The second possibility is that the primers for

*bla*_{IMP-1} used in the Xpert® Carba-R assay and those used for conventional PCR in this study amplify variant IMP-1 carbapenemase genes (8). The final possibility is suboptimal performance of the Xpert® Carba-R assay. Since the complete process of carbapenemase gene detection has not been disclosed by the manufacturer, the efficacy of DNA extraction and amplification is not clear. Despite the discrepancy, we conclude that the Xpert® Carba-R assay may be useful in regions where carbapenem-resistant *Enterobacter* strains producing IMP-type carbapenemases are most prevalent, such as in Japan.

Conflict of interest The authors wish to thank Cepheid Inc. for supplying the proprietary machinery and kits.

REFERENCES

- Centers for Disease Control and Prevention (CDC). Vital signs: carbapenem-resistant *Enterobacteriaceae*. MMWR Morb Mortal Wkly Rep. 2013;62:165-70.
- Hrabak J, Chudackova E, Papagiannitsis CC. Detection of carbapenemases in *Enterobacteriaceae*: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect. 2014;20: 839-53.
- Cepheid Xpert™ Carba-R user manual Rev B June 2014. Available at <http://www.cephheid.com> Accessed November 14, 2015.
- Findlay J, Hopkins KL, Meunier D, et al. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. J Antimicrob Chemother. 2015;70:1338-42.
- Anandan S, Damodaran S, Gopi R, et al. Rapid screening for carbapenem resistant organisms: current results and future approaches. J Clin Diagn Res. 2015;9:DM01-3.
- Yano H, Ogawa M, Endo S, et al. High frequency of IMP-6 among clinical isolates of metallo-β-lactamase-producing *Escherichia coli* in Japan. Antimicrob Agents Chemother. 2012;56: 4554-5.
- Shibata N, Doi Y, Yamane K, et al. PCR typing of genetic determinants for metallo-β-lactamases and integrases carried by gram-negative bacteria Isolated in Japan, with focus on the class 3 integron. J Clin Microbiol. 2003;41:5407-13.
- Wachino J, Yoshida H, Yamane K, et al. SMB-1, a novel subclass B3 metallo-beta-lactamase, associated with ISCR1 and a class 1 integron, from a carbapenem-resistant *Serratia marcescens* clinical isolate. Antimicrob Agents Chemother. 2011;55:5143-9.
- Arakawa N. The Research of Emerging Drug Resistant Organisms. Annual Report of Health Labour Sciences Research Grant. Available at <http://mhlw-grants.niph.go.jp/niph/search/NIDD02.do?resrchNum=201028026A>, 20128026A0001.pdf, 20128026A0002.pdf. Accessed December 20, 2016. Japanese.
- Poirer L, Heritier C, Tolun V, et al. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48:15-22.
- National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Carbapenem-resistant *Enterobacteriaceae* Infection, Japan. Infect Agents Surveillance Rep. 2014;35:281-2'.
- Tenover FC, Canton R, Kop J, et al. Detection of colonization by carbapenemase-producing gram-negative bacilli in patients by use of the Xpert MDRO assay. J Clin Microbiol. 2013;51:3780-7.