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Xpert CARBA-R Assay for the Detection of Carbapenemase-Producing Organisms in Intensive Care Unit Patients of a Korean Tertiary Care Hospital

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Carbapenemase-producing organisms (CPO) are rapidly disseminating worldwide, and their presence in tertiary care hospitals poses a significant threat to the management of nosocomial infections. There is a need to control CPO, especially in intensive care unit (ICU) patients, because these organisms are resistant to most β-lactam antibiotics and are easily transmitted. At present, the identification of CPO is time-consuming; hence, this study focused on the use of the Xpert CARBA-R assay (Cepheid, USA) to determine intestinal colonization rates of CPO in patients admitted to the ICU of a tertiary care hospital in Korea. Forty clinical stool samples were collected and inoculated both in a CARBA-R cartridge and in conventional culture plates. The CARBA-R assay required only ~one hour to screen CPO, while the time required for conventional culture was over three days. We also found that the prevalences of intestinal colonization by carbapenem-resistant organisms and *Enterobacteriaceae* were 17.5% (7 out of 40) and 7.5% (3 out of 40), respectively. Among the colonizing strains, three that contained carbapenemase, including *Klebsiella pneumonia* carbapenemase (KPC), and imipenem (IMP) and Verona integron-mediated metallo-β-lactamase (VIM) were found. With its convenience, the Xpert CARBA-R assay can be included in CPO surveillance strategies.

Key Words: Carbapenemase-producing organisms, Carbapenem-resistant organisms, Intensive care unit, Colonization, Xpert CARBA-R

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A rapid increase in the prevalence of carbapenem-resistant organisms (CRO) has been reported in Korea, particularly in tertiary care hospitals [1, 2]. Among the different resistance mechanisms in CRO, the most important is the production of the carbapenemase enzyme. The reason for concern about these carbapenemase-producing organisms (CPO), which are a subset of CRO, is that these enzymes can induce high levels of resistance to most β-lactam antibiotics, including carbapenems, which are the last line of defense in the treatment of gram-negative *Enterobacteriaceae* infections. In addition, CPO can spread and have been related to outbreaks of carbapenem-resistant bacteria in both developed and developing countries [3-8]. In Korea, six multidrug-resistant organisms, including CPO, have been implicated as the main agents of nosocomial infections according to the Korean government. It has been mandated for hospitals to report infections by these organisms to the Korean Center for Disease Control and Prevention since 2012 (http://www.cdc.go.kr/CDC/).

The risk factors for infection with multidrug-resistant organisms include a previous invasive procedure, diabetes mellitus, solid tumors, tracheostomy, urinary catheter insertions, and receipt of antipseudomonal penicillin [9]. Because the above factors are common in intensive care unit (ICU) patients, the detection of CRO or CPO colonization, which easily leads to true infec-

tions or to the horizontal transfer of carbapenem resistance determinants to other species [10], is very important for infection prevention. According to the study reported in 2012, the prevalence of fecal carriage of carbapenem-resistant *Enterobacteriaceae* (CRE) was 0.3%; however, to date, there have been no carbapenemase-producing *Enterobacteriaceae* (CPE) reported in the ICUs of Korean tertiary care hospitals [11]. Furthermore, a Chinese study in 2012 reported the prevalences of CRE and CPE, including *Klebsiella pneumonia* carbapenemase (KPC)-2, imipenem (IMP)-4, and New Delhi metallo-β-lactamase (NDM)-1 producers, to be 6.6% and 2.6%, respectively [12]. However, these previous studies utilized conventional methods, which were time-consuming and laborious. Recently, the Xpert CARBA-R assay (Cepheid, Sunnyvale, CA, USA) has been introduced for the detection of CPO from clinical samples. This assay is based on a multiplex real-time PCR technique and can detect bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC}, and bla_{OXA-48}-like alleles. However, reports on its clinical application have been scarce [13]. Hence, we applied the CARBA-R assay to determine the colonization rate of CPO in ICU patients in a tertiary care hospital in Korea and compared its results with those of conventional culture methods.

From July to August 2013, a total of 102 clinical samples were collected from ICU patients. Of these, the samples from patients in neonatal ICUs or patients with less than five-day stay in ICU were excluded. Additionally, duplicate samples from the same patients were excluded. In total, 40 clinical samples, including 23 stool samples and 17 rectal swabs, from 40 patients were evaluated. One of the rectal swabs was inoculated into the sample reagent and loaded into the cartridge of the CARBA-R assay according to the manufacturer's instructions, while another swab was inoculated in MacConkey broth containing 1 μg/mL meropenem (MEM) and incubated for 24 hr. The organisms from the broth containing MEM were then sub-cultured on MacConkey agar plates, each containing an MEM disk. Colonies growing near the MEM disks were sub-cultured on MacConkey agar containing 2 μg/mL MEM to ascertain resistance to carbapenem. Species were identified by using Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry (Bruker Daltonics, Bremen, Germany). For carbapenemase screening, a modified Hodge test using an IMP disk and a double disk synergy (DDS) test using aminophenyl boronic acid (APBA) and dipicolinic acid (DPA) vs. MEM were performed [14].

Three out of 40 samples (7.5%) tested positive by the CARBA-R assay, i.e., they showed one bla_{VIM}-, one bla_{IMP}-, and one bla_{KPC}-positive signal, indicating that the prevalence of CPO was substantial in these ICU patients (Table 1). By conventional culture, 16 out of 40 samples showed bacterial growth close to the MEM disk on MacConkey agar. Of these, seven isolates (three *Klebsiella pneumoniae*, three *Pseudomonas aeruginosa*, and one *Pseudomonas monteilii*) were resistant to MEM with a minimum inhibitory concentration (MIC) over 2 μg/mL. Among the seven MEM-resistant strains, two *K. pneumoniae* and three *P. aeruginosa* were isolated from samples that tested negative by Xpert CARBA-R assay. For these five strains, the modified Hodge test was negative. In addition, enhanced inhibition zones were observed around APBA disks but not around DPA disks for four strains, and one strain showed no inhibition zone around either the APBA and DPA disks. This indicated that over-expression of

Table 1. Summary of the results showing positivity in CARBA-R assay or conventional culture assay

Case No.	Duration (day)	Specimen	CARBA-R assay	Species of CRO	MHT	Double disk potentiation test	Possible mechanism
5	225	Stool	Negative	*Klebsiella pneumoniae*	Negative	Negative	AmpC β-lactamase with porin loss
7	10	Stool	Negative	*Pseudomonas aeruginosa*	Negative	APBA+	AmpC β-lactamase with porin loss
9	5	Stool	VIM+	*Pseudomonas monteilii*	Negative	DPA+	Class B carbapenemase
16	11	Rectal swab	IMP+	NT*	NT*	NT*	-
23	29	Rectal swab	Negative	*Klebsiella pneumoniae*	Negative	APBA+	AmpC β-lactamase with porin loss
25	44	Rectal swab	Negative	*Pseudomonas aeruginosa*	Negative	APBA+	AmpC β-lactamase with porin loss
26	20	Rectal swab	Negative	*Pseudomonas aeruginosa*	Negative	APBA+	AmpC β-lactamase with porin loss
33	25	Stool	KPC+	*Klebsiella pneumoniae*	Positive	APBA+	Class A carbapenemase

*Not tested owing to the lack of bacterial growth after overnight incubation in an enrichment (MacConkey) broth with 1 μg/mL MEM or no MEM-resistant colony around the MEM disk on MacConkey agar.
Abbreviations: APBA, aminophenylboronic acid; CCU, coronary care unit; CRO, carbapenem-resistant organism; DPA, dipicolinic acid; ERP, ertapenem; ICU, intensive care unit, IMP, imipenem; MEM, meropenem; MHT, modified Hodge test; NCU, neurosurgical care unit; NT, not tested; PCCU, pediatric critical care unit; VIM, Verona integron-mediated metallo-β-lactamase; KPC, *Klebsiella pneumonia* carbapenemase.

Amp-C β-lactamase along with porin loss might be the causes of carbapenem resistance in these strains.

One *P. monteilii* isolate from a *bla*$_{VIM}$-positive sample was negative for the modified Hodge test using an IPM disk but positive for the DDS test, indicating the limitations of the modified Hodge test in carbapenemase screening owing to its relatively low sensitivity [15-17]. Additionally, no bacterial growth was observed from the sample showing a positive signal for *bla*$_{IMP}$-, indicating that the CARBA-R assay is sensitive enough to detect CPO even in a rectal swab sample with a very low bacterial concentration. Alternatively, this might indicate a limitation of this study: we followed the MEM resistance cut-off value for *Enterobacteriaceae* in the CLSI guidelines [18]; however, this cut-off can cause low levels of CPO resistance to be missed. In addition, a recent report showed that the CARBA-R assay does not perform well in the detection of OXA-48-producing *Escherichia coli* [19]. Therefore, further studies are required to determine the prevalence of CPO in specific settings and to determine the accuracy of the CARBA-R assay with organisms that exhibit a low level of resistance to carbapenems.

Regarding the turn-around time, while over three days were required for the conventional culture, the CARBA-R assay required only about one hour, including 48 min running time.

The prevalences of intestinal colonization by CRO and CRE were 17.5% and 7.5%, respectively, in the ICUs of a tertiary care hospital in Korea, which is higher than those previously reported in similar settings [11, 12]. However, owing to the limited number of samples, further study will be needed to determine the true prevalence of CPO and CPE in the guts of ICU patients. The Xpert CARBA-R assay was found to be an easy-to-use assay, considering the labor and processing time required for conventional culture. The Xpert CARBA-R assay t should be adopted for surveillance and the determination of CPO colonization rates in clinical settings.

Authors' Disclosures of Potential Conflicts of Interest

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

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