

Performance of Xpert Carba-R Assay for Identification of Carbapenemase Gene in the Clinical Microbiology Laboratory

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Objectives: The Xpert Carba-R Assay is a diagnostic test designed for the rapid detection and differentiation of the *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{IMP-1} genes. We verified the performance of Xpert Carba-R Assay for identification of carbapenemase gene in the clinical microbiology laboratory.

Methods: The analytical limit of detection was determined with two suspensions of carbapenemase-producing *Enterobacteriaceae* (CPE) isolates (KPC and NDM). A total of 52 specimens were evaluated: 21 bacterial isolates from clinical specimens, 21 rectal swabs, and 10 contrived stool specimens.

Results: In bacterial isolates, concordant results between the Xpert Carba-R Assay and PCR were found in 20 of 21; 8 KPC, 8 NDM, 1 IMP, and 2 multiple carbapenemase genes (KPC/NDM, NDM/OXA) were detected both by Xpert Carba-R Assay and PCR. In one GES-positive isolate, Xpert Carba-R Assay showed a negative result as expected. One VIM-positive isolate tested negative by Xpert Carba-R Assay. Complete concordance was seen in rectal swab specimens: 4 specimens with KPC and 17 specimens with negative results both by Xpert Carba-R Assay and surveillance culture. Among the 10 contrived stool specimens, Xpert Carba-R Assay detected carbapenemase genes in 9 specimens as expected according to the CPE strains spiked into the contrived stool; 2 KPC, 4 NDM, 1 IMP, and 2 multiple carbapenemase genes (NDM/KPC, NDM/OXA). One VIM-positive specimen tested negative by Xpert Carba-R Assay.

Conclusion: In conclusion, the Xpert Carba-R Assay can be used to identify carbapenemase gene in bacterial isolates cultured from clinical specimens and detect CPE carrier using rectal swab in clinical laboratories. (Ewha Med J 2020;43(3):39-42)

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Key Words

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Introduction

Rapid detection of carbapenemase-producing *Enterobacteriaceae* (CPE) is important for directing appropriate treatment and preventing nosocomial spread of these bacteria [1]. The Xpert Carba-R Assay (Cepheid, Sunnyvale, CA, USA) is a diagnostic test designed for the rapid detection and differentiation of the *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{IMP-1} genes. It is a

fully automated and integrated system for sample preparation, DNA extraction, amplification and qualitative detection of target genes using multiplex real-time PCR technique. Initially, it was approved for the use on rectal swab specimens, but later also for pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa* [2,3]. We verified the performance of the Xpert Carba-R Assay for identification of carbapenemase gene with bacterial isolates from clinical speci-

mens, rectal swabs, and contrived stool specimens to implement it in the clinical microbiology laboratory.

Methods

To determine the analytical limit of detection of the assay, 2 suspensions of *Klebsiella pneumoniae* isolates from clinical specimens were obtained: one with KPC-2 and the other with NDM-1 production. Suspensions were prepared to equal the density of a 0.5 McFarland standard (1.5×10^8 CFU/mL). Serial 10-fold dilutions to 1.5×10^2 CFU/mL were prepared in phosphate buffered saline.

A total of 52 specimens were evaluated to assess the reproducibility of the assay: 21 bacterial isolates from clinical specimens, 21 rectal swabs, and 10 contrived stool specimens (Table 1). Bacterial isolates from clinical specimens were retrieved from CPE previously proven by culture, PCR and sequencing. Carbapenemase production was confirmed by PCR and sequencing of carbapenemase genes including *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, *bla*_{IMP-1}, and *bla*_{GES}. Rectal swabs were compared to surveillance culture results. Surveillance culture were processed according to the Centers for Disease Control and Prevention laboratory protocol (overnight selective enrichment in 5 mL tryptic soy broth with a 10- μ g meropenem disk, followed by plating onto MacConkey agar) [4]. Ten contrived stool speci-

mens were prepared. Carbapenemase-negative stool specimens were obtained and spiked with CPE strains, which were selected from the bacterial isolates included in this study. Suspensions were prepared to equal the density of a 0.5 McFarland standard (1.5×10^8 CFU/mL) and diluted to 1:100. One milliliter of the diluted bacterial suspensions was added to the stool specimens and mixed well.

The Xpert Carba-R Assay was run on the GeneXpert platform as per the manufacturer's instructions, and the results were compared to previously performed culture and/or sequencing results.

This study was considered exempt from institutional review board oversight (EUMC 2018-06-029).

Results

The Xpert Carba-R Assay detected KPC and NDM to a final concentration of 1.5×10^4 CFU/mL, with threshold cycle 33 for KPC, and 32 for NDM. Gene amplification of KPC and NDM were occurred at the detection limit concentration of 1.5×10^3 CFU/mL, but were reported negative because the threshold cycle value were outside the effective range.

Overall, concordant results were shown in 50 out of 52 samples (96.2%; Kappa value, 0.92; 95% confidence interval [CI], 0.81 to 1.00). In bacterial isolates, concordant results between the Xpert Carba-R Assay and PCR were found in 20 of 21. Nineteen CPE isolates were detected both by the Xpert Carba-R Assay and PCR: 8 KPC-2, 7 NDM-1, 1 NDM-3, 1 IMP-1, 2 CPE with multiple carbapenemase genes (KPC-2/NDM-1, NDM-5/OXA-181). In one GES-producing isolate, the Xpert Carba-R Assay showed a negative result as expected. One VIM-2-producing isolate tested negative by the Xpert Carba-R Assay. Complete concordance was seen in rectal swab specimens (21 out of 21): 4 specimens with KPC-1, and 17 specimens with negative results both by the Xpert Carba-R Assay and surveillance culture. Among the 10 contrived stool specimens, the Xpert Carba-R Assay detected carbapenemase genes in 9 specimens as expected according to the CPE strains spiked into the contrived stool: 2 KPC-2, 3 NDM-1, 1 NDM-3, 1 IMP-1, and 2 multiple carbapenemases specimens (KPC-2/NDM-1, NDM-5/OXA-181). One VIM-2-positive contrived stool specimen tested negative by the Xpert Carba-R Assay. In conclusion, there were two

Table 1. Detection rates of carbapenemase genes by Xpert Carba-R Assay

Carbapenemase type	% of isolates		
	Bacterial isolates	Contrived stool specimens	Rectal swab specimens
KPC-2	100 (8/8)	100 (2/2)	100 (4/4)
NDM			
NDM-1	100 (7/7)	100 (3/3)	-
NDM-3	100 (1/1)	100 (1/1)	-
VIM-2	0 (0/1)	0 (0/1)	-
IMP-1	100 (1/1)	100 (1/1)	-
GES	0 (0/1)	-	-
Multiple types			
KPC-2/NDM-1	100 (1/1)	100 (1/1)	-
NDM-5/OXA-181	100 (1/1)	100 (1/1)	-
Negative	-	-	100 (17/17)
Total	90 (19/21)	90 (9/10)	100 (21/21)

discrepant results due to the one VIM-2-producing isolate, one from the bacterial isolate test and the other from the contrived stool specimen test (Table 1).

Discussion

In this study, we verified the performance of the Xpert Carba-R Assay to introduce into routine use in the clinical microbiology laboratory and it showed complete concordance with comparable methods for major carbapenemases except VIM. The assay showed good performance for all bacterial isolates, rectal swab and contrived stool specimen, and we decided to use the assay for carbapenemase identification in the bacterial isolates cultured from clinical specimens and CPE carrier detection using rectal swab.

The limit of detection of this study was higher than that for the KPC/NDM-producing standard strain provided by the manufacturer [2] and also higher compared with data published in other studies [5]. The limit of detection may be increased according to the concentration of the resistant gene in the isolate. Clinical isolates were used in this study and the burden of gene in bacteria is difficult to measure.

Several studies have evaluated the performance of the assay for the bacterial isolates and/or rectal swabs [3,6-9]. Despite the limitations and variations of these studies, the overall Xpert Carba-R Assay showed excellent performance: 96.6% to 100% of sensitivity and 95.3% to 99.13% of specificity.

OXA-181, which is one of the OXA-48 variants, was detected as OXA-48 positive. Previously, the Xpert Carba-R Assay failed to detect the new emerging OXA-48 variants, such as OXA-181 and OXA-232 carbapenemases [10-13]. However, this poor performance was improved in a novel version of the assay, which accurately detected the OXA-48 variants [3-6]. OXA-181 is one of the most prevalent carbapenemases in South Africa and is increasingly recognized in Asia and Europe [14-16].

Our study included one VIM-2-producing *Serratia marcescens*. The Xpert Carba-R Assay was performed using bacterial isolate and contrived stool specimen with the isolate, and both results were negative. Repeated PCR confirmed the presence of VIM gene in two cases, therefore the results of the Xpert Carba-R Assay were falsely negative. Even though previous studies demonstrated excellent performance of the Xpert Carba-

R Assay [5-9], the performance for VIM was relatively lower than other targets; sensitivity was 93.5% (95% CI, 78.6% to 99.2%) [6], positive percentage of agreement was 60% (95% CI, 31.3% to 83.2%) [8]. Due to the low prevalence of VIM-producing strains, only one VIM-positive CPE was included in our study. Further study with more specimens would be necessary.

The Xpert Carba-R Assay has several limitations. Most of all, it is not for bacterial identification. Detection of these gene sequences does not indicate the presence of viable organisms. Therefore, when the assay is performed on rectal swab specimens from patients, concomitant cultures are necessary to recover organisms for epidemiological typing, antimicrobial susceptibility testing, and for further confirmatory bacterial identification.

Due to the absence or low prevalence of IMP-1, VIM, and OXA carbapenemases in isolates found in clinical specimens in our laboratory, only one isolate of each carbapenemase was included in this study. And we intentionally included more positive rectal swab specimens than negative samples, consequently the prevalence of carbapenemases (4/21, 19.0%) was higher than that of the routine surveillance culture in our hospital. The performance of assays depends largely on the local prevalence; in low prevalence setting, false-positive results could be higher.

In conclusion, the Xpert Carba-R Assay can be used to identify carbapenemase gene in bacterial isolates cultured from clinical specimens and detect CPE carrier using rectal swab in clinical laboratories. It can allow the earlier implementation of appropriate treatment and infection control as it is less labor-intensive and yields reliable results quickly.

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