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Multicenter Evaluation of the Xpert Carba-R Assay for Detection and Identification of Carbapenemase Genes in Sputum Specimens

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ABSTRACT Rapid diagnosis of infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) is crucial for proper treatment and infection control. The Xpert Carba-R assay is a qualitative multiplex real-time PCR method that qualitatively detects and differentiates five common carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP}) directly from rectal swabs or purified colonies within approximately 1 h. We performed a multicenter evaluation of the investigational use of the Carba-R assay for detection and differentiation of carbapenemase genes from sputum specimens in patients with a clinical diagnosis of pneumonia. The intra- and interassay coefficients of variation values for the Carba-R assay were 0.2% to 2.0% and 1.4% to 2.3%, respectively. A total of 301 sputum specimens were collected and tested. Compared to bacterial culture followed by PCR identification of resistance genes from colonies, the Carba-R assay reduced turnaround time from 56 to 84 h to less than 2 h. Carbapenemase genes were detected by the Carba-R assay in *Klebsiella pneumoniae* ($n = 236$), *Escherichia coli* ($n = 22$), *Enterobacter cloacae* ($n = 23$), *Klebsiella oxytoca* ($n = 8$), *Serratia marcescens* ($n = 6$), *Citrobacter freundii* ($n = 4$), and *Klebsiella aerogenes* ($n = 2$). The Carba-R assay detected 112 bla_{KPC} (33.5%), 70 bla_{NDM} (21.0%), 8 bla_{IMP} (2.4%), and 2 bla_{VIM} (0.6%) genes, with positive percent agreement, negative percent agreement, and concordance rates of 92.9%, 86.7%, and 88.3%, respectively, for the dominant bla_{KPC} and 85.0%, 87.8%, and 87.4%, respectively, for the bla_{NDM} genes. Neither method detected the bla_{OXA-48} carbapenemase gene. The convenient, rapid, and simple characteristics of the Xpert Carba-R assay make it a potential tool for CRE detection and identification directly in sputum specimens.

KEYWORDS Carba-R assay, sputum, bla_{KPC}, bla_{NDM}, carbapenem-resistant *Enterobacteriaceae*

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During the past several decades, the increase of multidrug-resistance (MDR) in Gram-negative bacteria (GNB) has become a major threat throughout the world. Carbapenem antimicrobial agents are currently considered the last line of defense to treat severe Gram-negative bacterial infections. Since the early 2000s, carbapenem-resistant *Enterobacterales* (CRE) isolates have emerged worldwide; these isolates are mainly due to acquired carbapenemases. CRE isolates now represent a significant threat in hospitals as well as an emerging public-health problem (1).

The most common mechanism of resistance to carbapenems in CRE strains is production of a carbapenemase. Carbapenemases are found in three classes of β-lactamases, class A or D serine β-lactamases and class B metallo-β-lactamases (MBLs). The most common carbapenemase genes are the *Klebsiella pneumoniae* carbapenemase gene (bla_{KPC}, class A), the New Delhi metallo-β-lactamase gene (bla_{NDM}, class B), the Verona integron-encoded metallo-β-lactamase gene (bla_{VIM}, class B), the imipenemase metallo-β-lactamase gene (bla_{IMP}, class B), and the oxacillinase-48 gene (bla_{OXA-48}, class D). Because most of these carbapenemase genes are plasmid mediated, carbapenem resistance can spread quickly throughout different regions. Moreover, CRE strains frequently carry other resistance genes, which result in resistance to other classes of antimicrobial agents, such as fluoroquinolones and aminoglycosides. Consequently, these MDR CRE strains are very difficult to treat (2).

A rapid diagnostic method to detect CRE is required both to appropriately treat patients and to control the spread of CRE (3). Until recently, methods for detecting CRE resistance genes directly from clinical specimens have been limited. Previously, testing resistance genes of CRE strains required isolation of these strains from clinical specimens followed by PCR assay methods done on the purified colony as well as antibiotic susceptibility testing. With this previous approach, a diagnostic time of 54 to 86 h was typical and thus was not suitable for guiding antimicrobial therapy of these patients (4).

The Xpert Carba-R assay (Cepheid, Sunnyvale, CA) is a molecular diagnostic device specifically designed for the qualitative detection of the carbapenemase genes, including bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48}, using real-time PCR with results available within less than an hour (5). The Carba-R assay has been cleared by the U.S. Food and Drug Administration for detection of carbapenemase genes directly from rectal and peri-rectal swabs and pure colonies. Published studies have shown that the Carba-R assay is able to detect carbapenemase genes from pure bacterial colonies (6, 7) as well as rectal swabs (8) with high sensitivities and specificities. Studies using the Carba-R assay for detecting carbapenemase genes from respiratory specimens rarely have been reported and are not included in the intended use of the assay (3, 9). Notably, unlike North America and Europe, where CRE were more frequently isolated from urinary samples, approximately two-thirds of CRE in China were isolated from lower respiratory tract infection specimens (10). It is thus essential to evaluate whether the Carba-R assay is able to directly detect CRE from sputum specimens in order to shorten the diagnostic turnaround time. In this study, we investigated the prevalence of carbapenemase genes in sputum specimens from patients admitted to six hospitals in different geographic areas in China by using the Carba-R assay in a study to detect resistance genes and compared these results with conventional methods (11).

MATERIALS AND METHODS

Specimen collection and preparation. From March 2017 to February 2018, sputum specimens were collected from patients with a clinical diagnosis of pneumonia at six tertiary care Chinese hospitals. Gram stains were performed to assess the quality of the sputum prior to specimen processing. The specimens were collected and aliquoted into two portions. One aliquot was used for bacterial culture per routine microbiology practice. The other aliquot was stored in a −80°C freezer for Carba-R testing later. Information including patient's gender, age, and accession number, as well as the bacterial species recovered and antimicrobial susceptibility profile, was acquired from each hospital.

Bacterial culture and isolate identification. Sputum specimens were cultured on Columbia agar with 5% sheep blood and China blue lactose rosolic acid agar (Oxoide, Hampshire, England) for pathogen recovery (10). After 24 h of incubation at 35°C in 5% CO_2, suspicious pathogen colonies (blue) were selected from the agar for identification using a Vitek 2 GN card and antimicrobial susceptibility testing using Vitek 2 AST-GN04 (bioMérieux, Marcy l'Etoile, France). Two ATCC prototype strains, *Klebsiella pneumoniae* BAA-1705 and BAA-1706, were included in each run as controls (12). CRE were identified

according to the Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints (13). All participating laboratories performed testing according to the same protocol to minimize the bias in sample processing and strain collection.

PCR characterization. Nucleic acids were extracted from purified bacterial colonies and tested by PCR with primers specific to five carbapenemase target genes as previously described (3, 14). The appropriately sized PCR products were determined by DNA sequence analysis for confirmation (7, 14).

Xpert Carba-R assay. All clinical sputum specimens were tested directly with the Carba-R assay. The sputum was preprocessed as previously described (3) before entering the Xpert cartridge. In brief, 300 μl of the specimen was mixed with 600 μl of 4% NaOH for 30 to 60 min. After the specimen was fully liquefied, 900 μl of sample reagent was added, and 1.7 ml of the mixture was transferred into the cartridge for testing. Quality control for the Xpert assay includes an internal probe check control (PCC) and a sample processing control (SPC). The GeneXpert instrument system (Cepheid, Sunnyvale, CA) is an automated real-time fluorescent quantitative PCR (qPCR) instrument designed for detecting the five carbapenemase genes bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP}. The Carba-R assay was performed on all validation specimens according to the manufacturer's package insert, and results were interpreted directly from the report generated by the GeneXpert instrument.

Ethics statement. The six hospitals waived the need for written informed consent from patients, as included patients were not subject to extra procedures or questions. Samples were collected as part of standard care. This was purely a laboratory-based intervention without involvement of patients.

Data analysis and statistics analysis. A positive result reported by the Carba-R assay means that at least one carbapenemase gene was detected in this specimen. A negative result means that there were no bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, or bla_{IMP} carbapenemase genes detected by the Carba-R assay. Test turnaround time (TAT), defined as hours between specimen processing and result reported, was calculated. For each carbapenemase gene, the first positive specimen with enough leftover volumes was chosen to determine the assay reproducibility by the coefficient of variation (CV) between three triplicate repeats within each run (intra-assay) and between three runs by different operators on different days (interassay). The positive percent agreement (PPA), negative percent agreement (NPA), and concordance rate of the Carba-R assay were calculated in comparison with culture followed by PCR assay using GraphPad Prism 8 statistical software analysis.

RESULTS

A total of 301 sputum specimens were included in this study. All specimens were collected from patients with a clinical diagnosis of pneumonia from six hospitals in China in the cities of Chengdu, Kunming, Soochow, Beijing, Yinchuan, and Guangzhou (Fig. 1). Among them, 221 (73.4%) patients were males and 80 (26.6%) were females, and the ages ranged from 3 months to 102 years.

The reproducibility of the Carba-R assay for detecting carbapenemase genes was assessed first. Three sputum specimens, which were positive for bla_{KPC}, bla_{NDM}, and bla_{IMP}, respectively, were run in triplicate by three different operators. The intra-assay coefficient of variation (CV) values of the Carba-R assay ranged from 0.2 to 1.8% for bla_{KPC}, 0.7 to 2.0% for bla_{NDM}, and 0.9 to 1.3% for bla_{IMP} (Table 1). The interassay CV values were 1.4%, 1.4%, and 2.3% for bla_{KPC}, bla_{NDM}, and bla_{IMP}, respectively (Table 1).

The test turnaround time of the Carba-R assay was less than 2 h for detecting carbapenemase genes, which included specimen liquefication, testing, and result analysis. In contrast, the total turnaround time for culture was 56 to 84 h, followed by conventional culture (48 to 72 h), followed by PCR (8 to 12 h). The culture followed by PCR assay detected 122 positive results from 301 specimens, bla_{KPC} ($n = 79$), bla_{NDM} ($n = 32$), bla_{IMP} ($n = 2$), bla_{VIM} ($n = 1$), $bla_{NDM} + bla_{KPC}$ ($n = 6$), and $bla_{IMP} + bla_{NDM}$ ($n = 2$). The Carba-R assay detected 162 positive results, bla_{KPC} ($n = 86$), bla_{NDM} ($n = 45$), bla_{IMP} ($n = 2$), bla_{VIM} ($n = 1$), $bla_{NDM} + bla_{KPC}$ ($n = 22$), $bla_{IMP} + bla_{KPC}$ ($n = 3$), $bla_{IMP} + bla_{NDM}$ ($n = 1$), $bla_{IMP} + bla_{NDM} + bla_{VIM}$ ($n = 1$), and $bla_{IMP} + bla_{NDM} + bla_{KPC}$ ($n = 1$). bla_{OXA48} was not detected by culture PCR or the Carba-R assay.

The distribution of carbapenemase genes detected by the Carba-R assay varied among the six locations, with bla_{KPC} and bla_{NDM} predominating across China (Fig. 1). The concordance rate of the two methods for detecting carbapenemase genes ranged from 87.4% to 99.1% with bla_{KPC} (88.3%), bla_{NDM} (87.4%), bla_{IMP} (98.2%), and bla_{VIM} (99.1%) (Table 2). PPA values ranged from 0% to 92.9%, with high values occurring for bla_{KPC} and bla_{NDM} and low values for the rare bla_{IMP} and bla_{VIM} genes (Table 2). The NPA value of the Carba-R assay ranged from 86.7% to 99.4%. Relatively low NPA values were noted for bla_{KPC} and bla_{NDM} (Table 2). It was noticed that the low values for bla_{IMP} and bla_{VIM} represented a single missed strain each, with the Carba-R assay detecting significantly more bla_{IMP}- and bla_{VIM}-positive specimens.

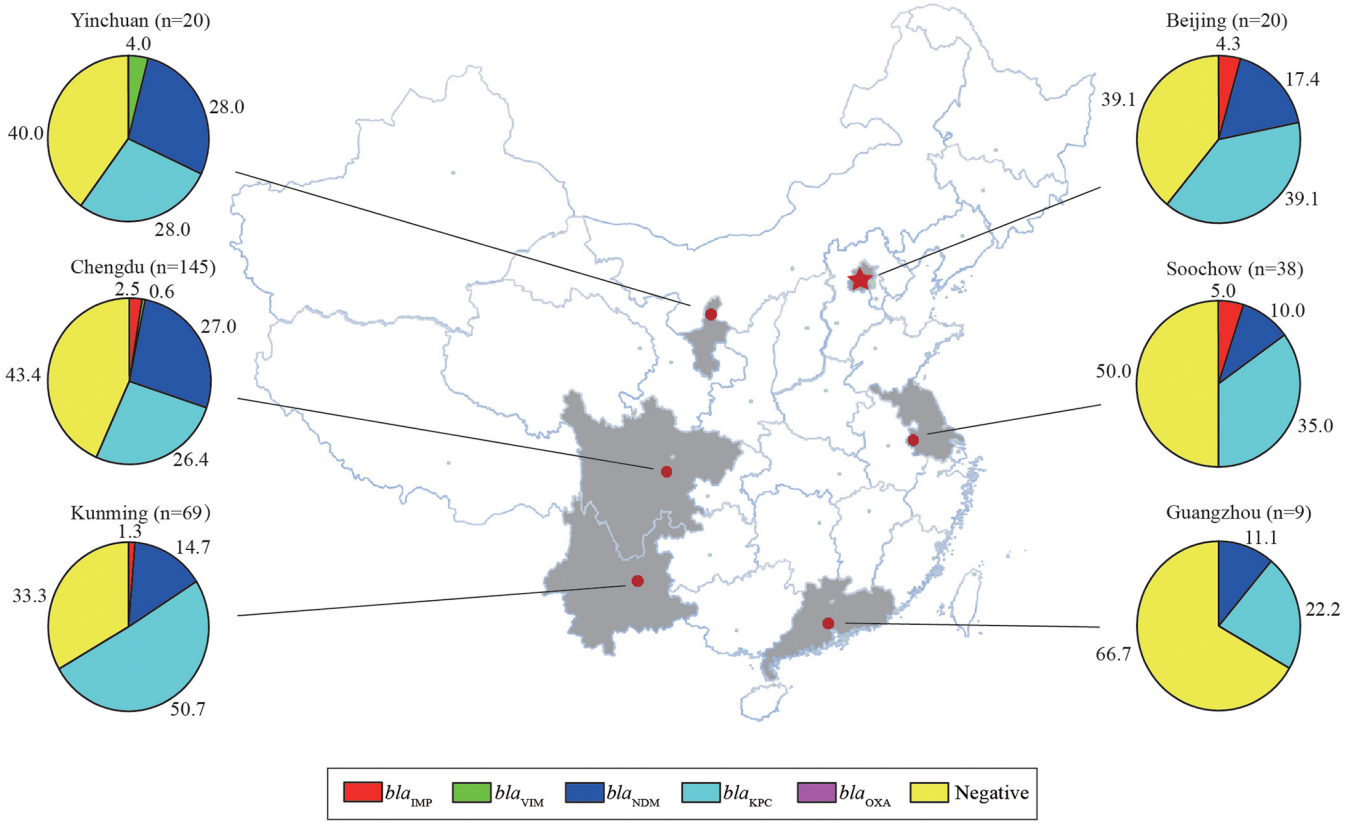

FIG 1 Distribution of five carbapenemase genes in six locations in China detected by the Xpert Carba-R assay.

The ability of the Carba-R assay to detect carbapenemase genes from specimens containing different CRE species was evaluated. Each species carried different carbapenemase genotypes. Compared with culture followed by PCR, the Carba-R assay detected more genotypes from specimens with most of the bacterial species (see Table S1 in the supplemental material). Our data indicated that bla_{KPC} and bla_{NDM} are the two predominant genes detected in all bacterial species. The Carba-R assay demonstrated a high PPA for these genes in each species, with bla_{NDM} ranging from 76.2% to 100.0% and bla_{KPC} from 95.0% to 100.0% (except for *Escherichia coli*). However, the Carba-R assay had a relatively low NPA rate for bla_{KPC}, with 80.0% in *Citrobacter freundii*, 66.7%

TABLE 1 Intra- and interassay variations of the Xpert Carba-R assay

Carbapenemase gene	Operator	C_T 1	C_T 2	C_T 3	Mean C_T[a]	SD C_T	CV (%)[b]
Intra-assay							
bla_{KPC}	1	24.7	24.4	25.0	24.7	0.3	1.2
	2	24.6	24.5	24.6	24.6	0.1	0.2
	3	24.6	25.5	24.9	25.0	0.5	1.8
bla_{NDM}	1	22.0	21.6	22.0	21.9	0.2	1.1
	2	21.6	21.5	21.8	21.6	0.2	0.7
	3	22.2	21.5	22.3	22.0	0.4	2.0
bla_{IMP}	1	35.4	34.8	35.2	35.1	0.3	0.9
	2	36.7	37.4	36.5	36.9	0.5	1.3
	3	36.1	36.6	36.0	36.2	0.3	0.9
Interassay							
bla_{KPC}					24.8	0.3	1.4
bla_{NDM}					21.8	0.3	1.4
bla_{IMP}					36.1	0.8	2.3

[a]C_T, threshold cycle.
[b]CV, coefficient of variation.

TABLE 2 PPA, NPA, and concordance rate of the Xpert Carba-R assay ($n = 334$)[a]

Carbapenemase gene	No. of specimens by result				Test performance (95% CI) (%)		
	P + X+	P − X+	P + X−	P − X−	PPA (range)	NPA (range)	Concordance (range)
bla_{NDM}	34	36	6	258	85.0 (70.9–92.9)	87.8 (83.5–91.0)	87.4 (83.4–90.6)
bla_{KPC}	79	33	6	216	92.9 (85.4–96.7)	86.7 (82.0–90.4)	88.3 (84.4–91.3)
bla_{IMP}	3	5	1	325	75.0 (30.1–98.7)	98.5 (96.5–99.4)	98.2 (96.1–99.2)
bla_{VIM}	0	2	1	331	0.0 (0.0–94.9)	99.4 (97.8–99.9)	99.1 (97.4–99.8)

[a]P, culture followed by PCR; X, Xpert Carba-R assay; +, detected; −, not detected; CI, confidence interval; PPA, positive percent agreement; NPA, negative percent agreement.

in *Klebsiella aerogenes*, 84.9% in *K. pneumoniae*, and 83.3% in *Serratia marcescens*. In comparison to bla_{KPC} and bla_{NDM}, bla_{IMP} and bla_{VIM} were relatively rare genes which were only detected in *Enterobacter cloacae*, *K. pneumoniae*, and *Klebsiella oxytoca*. For the rarely detected bla_{VIM} and bla_{IMP} genes, the Carba-R assay performed poorly, detecting none of bla_{VIM} genes in *E. cloacae* and only 66.7% of bla_{IMP} in *K. pneumoniae*. In all three species, the Carba-R assay showed a high NPA rate for bla_{IMP} and bla_{VIM} genes, with bla_{IMP} ranging from 98.1% to 100.0% and bla_{VIM} from 99.2% to 100.0% (Table S1).

DISCUSSION

Currently, sputum specimens are the major specimen type submitted by clinicians for diagnosis of pneumonia in China. In this study, we evaluated the performance of the Xpert Carba-R assay for the detection of carbapenemase genes from sputum, a specimen type that has not been explored previously. The intra- and interassay CV values determined by the Carba-R assay ranged from 0.1 to 2.3%, demonstrating its excellent repeatability for detecting carbapenemase genes in sputum specimens. The assay had a high PPA for detecting the predominant bla_{KPC} and bla_{NDM} genes and a high NPA for detecting rare genes, including bla_{IMP} and bla_{VIM}. The convenient, rapid, and simple characteristics of the Carba-R assay make it a potential tool for CRE detection and identification directly in lower respiratory tract specimens.

CRE strains are among the major causes of pneumonia in hospitalized patients, and the choice of antimicrobial therapy for these types of pneumonia is difficult (15). Identifying CRE isolates from sputum specimens has been an important step for managing patients with the clinical diagnosis of pneumonia (16, 17). However, the methods for detection of CRE have required starting with a pure colony from a culture (18). Therefore, the development of a rapid and convenient method for detection and identification of CRE genes directly from sputum would improve the therapy of pneumonia. We found that the Carba-R assay showed a high PPA of 92.9% but a relatively low NPA of 86.7% in sputum specimens for the detection of bla_{KPC}. One reason for the low NPA rate produced by the Carba-R assay for detecting bla_{KPC} is that the Carba-R assay detects carbapenemase genes from specimens where no carbapenem-resistant organisms were detected by culture. The negative cultures could be due to testing patients who had already received antibiotics, resistant bacterial species that were fastidious and did not grow on the selective medium, or bacteria that contained resistance genes that were not expressed or only expressed at low levels. Importantly, the Carba-R assay can detect carbapenemase genes from other or non-Enterobacterales organisms (19). It has been reported that bla_{KPC} genes that have been recovered from species other than *Enterobacterales*, including *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Acinetobacter baumannii* (20–23). Additionally, molecular tests detect all nucleic acids even if the bacteria are dead.

We noticed that the Carba-R assay resulted in a poor PPA in detecting bla_{IMP} and bla_{VIM} genes in comparison with culture followed by PCR assay, but this was only a single specimen for each resistance gene. bla_{IMP} and bla_{VIM} isolates are rare in China, and only limited positive samples were detected in this study. In addition, mutations in primer or probe binding regions may also affect detection of bla_{IMP}- or bla_{VIM}-containing variants (24). False-negative results were observed using the Carba-R assay

when specimens contained a low concentration of the microorganisms harboring bla_{IMP} or bla_{VIM} genes (25). Limitations for bla_{IMP-7}, bla_{IMP-13}, and bla_{IMP-14} are listed in the package insert of the product. Larger numbers of sputum specimens with positive bla_{IMP} and bla_{VIM} genes are needed to further validate the performance of the Carba-R assay for detection and identification of rare carbapenemase genes.

Our study indicated that the Xpert Carba-R assay is a convenient and rapid method with high PPA and NPA rates compared with culture followed by PCR assay for detecting CRE genes from deep expectorated sputum, which remains a widely used specimen type in clinical practice in China. Our results suggest the possibility that the Carba-R assay can be used to screen for CRE genes directly from deep expectorated sputum specimens associated with high bla_{KPC} and bla_{NDM} prevalence.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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Y.-W.T. is an employee of Cepheid, the commercial manufacturer of the Xpert Carba-R test. The rest of the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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