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Evaluation of the Xpert Carba-R NxG Assay for Detection of Carbapenemase Genes in a Global Challenge Set of *Pseudomonas aeruginosa* Isolates

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ABSTRACT The growing prevalence and diversity of carbapenemase producers among carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates warrants an expansion of detection capabilities. The purpose of this study was to evaluate the performance of the commercially available Xpert Carba-R (Carba-R) and the research-use-only Xpert Carba-R NxG (Carba-R NxG) in a global collection of *P. aeruginosa*. The challenge set included 123 *P. aeruginosa* clinical isolates from 12 countries. Isolates were previously categorized via PCR or whole-genome sequencing. Carbapenemase classes tested include VIM, IMP, NDM, SPM, KPC, and GES. Non-carbapenemase (non-CP)-harboring isolates were also tested (negative control). Isolates were tested using the Carba-R NxG and the Carba-R tests per the manufacturer's instructions. Carba-R NxG testing was completed by Cepheid (Sunnyvale, CA), blinded to genotype. Both assays gave negative results for all non-CP isolates and positive results for all VIM, NDM, and KPC isolates. An improvement in IMP detection among isolates was observed (100% detection by Carba-R NxG versus 58% by Carba-R). All SPM and GES isolates, targets not present in commercially available Carba-R, were positive by Carba-R NxG. Two isolates harbored both VIM and GES, while a third isolate contained VIM and NDM. The Carba-R NxG identified both targets in all 3 isolates, while the Carba-R was negative for both GES-containing isolates. Overall, the Carba-R NxG successfully categorized 100% of isolates tested compared with 68% for its predecessor. The Carba-R NxG will expand the detection spectrum of the current Carba-R assay to include SPM, GES, and expanded IMP variants, increasing the global utility of the test.

KEYWORDS CarbaR NxG, carbapenemase, *Pseudomonas aeruginosa*, rapid diagnostics

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) continues to represent a public health concern (1, 2). Rapid identification of such organisms is vital to inform infection prevention measures; however, phenotypic and genotypic detection of these enzymes can be challenging (3–5). For instance, certain carbapenemase phenotypic tests (i.e., modified carbapenem inactivation method/EDTA-modified carbapenem inactivation method [mCIM/eCIM]) have the advantage of detecting enzyme production, if present, and can broadly group the detected carbapenemases as class B or non-class B, although it is not able to differentiate enzymes on a more specific level (i.e., VIM versus NDM or KPC versus GES) (4). Thus, enzyme subtypes are uncharacterized, limiting important epidemiological information and hampering a clinician's ability to utilize targeted antimicrobial therapy (4, 6). Additionally, recent reports have described the failure of the current eCIM and mCIM for identifying SPM/IMP- and GES-harboring *P. aeruginosa* isolates, respectively (7). Furthermore, the turnaround time of most pheno-

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typic tests is greater than 24 h, which may delay infection prevention interventions (4, 7, 8). Conversely, more rapid tests (i.e., the CarbaNP) cannot differentiate between metallo- and non-metallo-β-lactamases (4).

Of the numerous resistance mechanisms harbored by *P. aeruginosa* (9), carbapenemase production poses significant therapeutic challenges (10–12). Detection of serine carbapenemases (i.e., KPCs) can direct targeted therapy for carbapenemase-producing *P. aeruginosa* with ceftazidime-avibactam and imipenem-relebactam (13). Additionally, the detection of metallo-β-lactamases carries high resistance rates to our standard-of-care β-lactams for carbapenem-resistant *P. aeruginosa* (ceftolozane-tazobactam, imipenem-relebactam, and ceftazidime-avibactam), limiting treatment options, and would direct clinicians to alternative agents (i.e., cefiderocol) (14–17).

The Xpert Carba-R assay (Cepheid, Sunnyvale, CA) is FDA approved for the genotypic detection of enzymatic resistance mechanisms in *P. aeruginosa*, *Acinetobacter baumannii*, and *Enterobacterales*. The Carba-R and other approved platforms (i.e., GenePOC) capture the "big five" carbapenemase classes clinically encountered (i.e., bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$) (5, 18–20). However, the growing diversity of enzyme subtypes exceeds the capabilities of current molecular tests. Indeed, the rising incidence of non-big five carbapenemases in certain regions of the world, such as SPM in Brazil or GES in Canada and Mexico, warrants the expansion of genotypic targets to improve detection (21–23).

The Xpert Carba-R NxG (Carba-R NxG) offers expanded carbapenemase targets relevant to the global epidemiology of *P. aeruginosa*, including GES, SPM, and certain IMP subtypes, that were outside the scope of the commercially available Xpert Carba-R assay (Carba-R). The purpose of this study was to compare the test performance of the commercially available Carba-R to the research-use-only Carba-R NxG (not for use in diagnostic procedures and not reviewed by any regulatory body) assay against a challenge set of *P. aeruginosa* isolates from across the globe.

MATERIALS AND METHODS

Bacterial isolates. The challenge set was comprised of 123 clinical *P. aeruginosa* isolates. Fifty-two isolates were obtained from the FDA-CDC Antimicrobial Resistance Bank, while all others were from the Center for Anti-Infective Research and Development repository. Isolates were geographically diverse, including strains isolated from Brazil, Czech Republic, Germany, India, Japan, Kenya, Kuwait, Nigeria, the Philippines, South Korea, Thailand, and the United States. All isolates were previously categorized via PCR or whole-genome sequencing (WGS), and these results were used as the reference genotypes. A total of 103 CRPA isolates (meropenem MIC range, 8 to >64 mg/liter; imipenem MIC range, 8 to >64 mg/liter) harboring the following carbapenemase classes were evaluated: VIM, IMP, NDM, SPM, KPC, and GES. Three isolates coharbored two carbapenemase classes (VIM and GES, $n = 2$; NDM and VIM, $n = 1$). Isolates were included in the analysis if they contain carbapenemase types that are within the expanded scope of the Carba-R NxG (i.e., GES, SPM, and IMP subtypes) and enzymes that should be captured by both assays (i.e., KPC, NDM, and VIM) to evaluate the expanded targets of the Carba-R NxG relative to the commercially available Carba-R. Twenty non-carbapenemase-producing (non-CP) isolates, harboring various cephalosporinase and/or efflux/porin mutations, were tested as negative controls. All non-CP isolates were resistant to at least meropenem or imipenem (meropenem MIC range, 1 to 64 mg/liter; imipenem MIC range, 0.25 to >64 mg/liter).

All isolates were stored at −80°C in skim milk (Becton, Dickinson, Sparks, MD). Prior to genotypic testing, isolates were subcultured twice on Trypticase soy agar with 5% sheep blood (Becton, Dickinson, Sparks, MD). Subcultures were incubated for 18 to 20 h at 37°C prior to testing. Isolates were sent between study sites on Trypticase soy agar slants (Becton, Dickinson, Sparks, MD, USA) and subcultured for testing and storage within 14 days of slant preparation.

Genotypic carbapenemase testing. All isolates underwent two genotypic carbapenemases tests: (i) the commercially available Xpert Carba-R assay (Cepheid, Sunnyvale, CA) and (ii) the research-use-only Carba-R NxG (Cepheid, Sunnyvale, CA) (not for use in diagnostic procedures, not reviewed by any regulatory body). The Carba-R NxG tests for the genes encoding the following enzymes: KPC, NDM, VIM, IMP, OXA-48-like, OXA-58, GES, SPM, and IMI/NMC. Conversely, the commercially available Carba-R contains targets for KPC, NDM, VIM, OXA-48, and certain IMP subtypes. Testing using the current commercially available assay was conducted at the Center for Anti-Infective Research and Development as previously described (18, 20). Quality control for Carba-R was conducted weekly during the testing period using a molecular standard positive for all five genotypic targets (Xpert Carba-R QC panel M219; lots D03JAN19B and E03JAN19B; Maine Molecular Quality Control, Saco, ME). All testing with the Carba-R NxG was conducted by Cepheid. During the preanalytic, testing, and assay evaluation periods, all employees of Cepheid were blinded to the reference genotypic profile of the organisms. Quality control procedures were conducted according to the manufacturer's guidelines for the test system.

TABLE 1 Carbapenemase profile of *P. aeruginosa*

Carbapenemase genotype[a]	No. of *P. aeruginosa* isolates	Carbapenemase subtype(s) tested
VIM	31	1, 2, 4, 5, 11
IMP	26	1, 10, 6, 7, 18, 48, 62
NDM	13	1
SPM	14	1
KPC	8	2, 5
GES	14	1, 5, 19, 20, 26
Non-CP	20	NA[b]

[a]Three isolates coharbored two carbapenemases. VIM, Verona integron-encoded metallo-β-lactamase; IMP, imipenemase; NDM, New Delhi metallo-β-lactamase; SPM, Sao Paulo metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; GES, Guiana extended spectrum; non-CP, non-carbapenemase producing.
[b]NA, not applicable.

Discordant results from the Carba-R NxG were repeated in triplicate, interspersed in a collection of other carbapenemase-positive isolates previously tested to maintain blinding. WGS was used to reconfirm the presence of the targeted carbapenemase genes. WGS was performed as follows. The Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, CA) was used for nucleic acid extraction. DNA concentrations were quantified using the NanoPhotometer system (Implen, Munich, Germany). The Nextera XT (Illumina, San Diego, CA) kit was used to generate sequencing libraries, which were quantified using a Qubit 4 fluorometer and double-stranded DNA high-sensitivity assay kit (Invitrogen, Carlsbad, CA). Finally, specimens were sequenced with an Illumina MiSeq (Illumina, San Diego, CA) sequencer. All testing was in compliance with the manufacturer's instructions. The Center for Genomic Epidemiology (CGE) ResFinder online tools (https://cge.cbs.dtu.dk/services/ResFinder/) were utilized to analyze genomes (24).

Data analysis. Sensitivity and specificity were calculated separately for each assay by carbapenemase class. True-positive results were isolates that tested positive for the same carbapenemase class as the reference genotype. False-positive results were isolates that tested positive for a carbapenemase that was not identified by the previous genotype profile. Isolates that were positive for a carbapenemase genotype but tested negative on Carba-R or Carba-R NxG were considered false negatives. True-negative results were isolates that did not test positive on Carba-R for carbapenemase, in concordance with previous genotypic testing. The sensitivity and specificity values with their respective 95% confidence intervals (CI) were calculated using SPSS, version 23 (IBM, Armonk, NY, USA). Successful categorization of isolates was also recorded and defined as positive identification of all carbapenemases in isolates harboring such genes or appropriately negative assay results in isolates without known carbapenemases.

RESULTS

Genotypic carbapenemase testing. Table 1 describes the carbapenemase class distribution and the enzyme subtypes represented in the 123 *P. aeruginosa* isolates evaluated in the current study.

Both the Carba-R and Carba-R NxG assays resulted in carbapenemase-negative tests for all non-CP isolates. Similarly, both assays appropriately identified all VIM-, NDM-, and KPC-harboring *P. aeruginosa* isolates. The calculated test performance for each assay by carbapenemase class is presented in Table 2.

Carba-R detected 58% (15/26) of IMP-harboring *P. aeruginosa* isolates; undetected genotypes included IMP-7 ($n = 5$), IMP-48 ($n = 4$), IMP-18 ($n = 1$), and IMP-62 ($n = 1$), none of which would be predicted to be detected with Carba-R based on *in silico* analysis. Initial testing of these isolates on the Carba-R NxG revealed two negatives (one IMP-6 and one IMP-7); however, due to concerns regarding initial differences between the phenotype and expected genotype of these two isolates and potential for preanalytical errors, retesting was warranted. Both isolates underwent WGS to reconfirm the presence of the enzyme and subtype. Subsequent blinded retesting in triplicate was positive for all three replicates of each isolate on the Carba-R NxG. Unlike the Carba-R, the Carba-R NxG detected the IMP gene in all 26 isolates. All isolates that harbored SPM and GES, genotypic targets not present in the current Carba-R, were positively detected by Carba-R NxG and negative by Carba-R.

Three isolates harbored two carbapenemases. The Carba-R NxG detected the genes encoding both enzymes in all 3 isolates (genotypes were the following: VIM and GES, $n = 2$; NDM and VIM, $n = 1$), while the Carba-R assay does not contain the primers and probes to detect the gene encoding the GES enzyme present in two of the three isolates.

TABLE 2 Performance characteristics of the Carba-R NxG and commercially available Carba-R assays in a genotypically diverse cohort of *P. aeruginosa* ($n = 123$)

Test and target[a]	True positive (*n*)	False positive (*n*)	True negative (*n*)	False negative (*n*)	Test performance [% (CI)]	
					Sensitivity	Specificity
Carba-R NxG						
VIM	31	0	92	0	100 (89–100)	100 (95–100)
IMP	26	0	97	0	100 (87–100)	100 (96–100)
NDM	13	0	110	0	100 (75–100)	100 (97–100)
SPM	14	0	109	0	100 (77–100)	100 (97–100)
KPC	8	0	115	0	100 (63–100)	100 (97–100)
GES	14	0	110	0	100 (75–100)	100 (97–100)
Non-CP		0	20		—[b]	—
Commercially available Carba-R						
VIM	31	0	92	0	100% (89–100)	100% (95–100)
IMP	15	0	97	11	58% (37–77)	100% (96–100)
NDM	13	0	110	0	100% (75–100)	100% (97–100)
SPM	0	0	109	14	—	—
KPC	8	0	115	0	100% (63–100)	100% (97–100)
GES	0	0	109	14	—	—
Non-CP		0	20		—	—

[a]Carba-R NxG, research-use-only Carba-R NxG; Carba-R, commercially available Carba-R; VIM, Verona integron-encoded metallo-β-lactamase; IMP, imipenemase; NDM, New Delhi metallo-β-lactamase; SPM, Sao Paulo metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; GES, Guiana extended spectrum; Non-CP, non-carbapenemase producing.
[b]—, not calculated.

Evaluating the challenge set as a whole, the Carba-R NxG successfully categorized 100% of isolates included compared to 68% for the current commercially available cartridge.

DISCUSSION

Using a challenge set of *P. aeruginosa* isolates, the commercially available Carba-R assay demonstrated reliable test performance for targets within the big five family of carbapenemase genes, including most, but not all, IMP variants, as identified by the manufacturer (20). Importantly, the evaluation of the Carba-R NxG assay also demonstrated excellent testing performance for carbapenemases within the tested big five members as well as regionally relevant carbapenemases, including SPM, GES, and IMP subtypes not present in the current Carba-R assay (18).

Continuous refinement and expansion of genotypic detection assays is important to screen for clinically relevant carbapenemases in concordance with increasingly prevalent carbapenemase-producing pathogens (3). While other metallo-β-lactamases have been identified in both *P. aeruginosa* and other Gram-negative organisms, SPMs are only found in *P. aeruginosa* and are endemic to South America, particularly Brazil (21, 25). Indeed, a Brazilian teaching hospital analyzed 43 ceftazidime-resistant *P. aeruginosa* isolates, finding 63% of isolates harbored SPMs after a targeted extended-spectrum beta-lactamase (ESBL) and metallo-β-lactamases screen on extracted DNA (26). This finding highlights the potential role of the automated Carba-R NxG as a simplified method for routine SPM detection and expanded access outside individual teaching institutions. Additionally, the current CLSI-endorsed eCIM procedure failed to differentiate 100% ($n = 14$) of tested SPM-harboring isolates from serine carbapenemase-producing strains in a recent analysis, highlighting the challenges of phenotypic detection (7).

GES-harboring *P. aeruginosa* isolates represent another detection challenge. Previous reports describe difficulty in identifying GES-harboring organisms using the standard mCIM, making detection problematic (7, 27). With endemicity of GES-harboring isolates in both Mexico and Canada as well as recent case reports of GES-harboring *P. aeruginosa* isolates in Texas, it is likely that GES-harboring isolates are an underrepresented carbapenemase class in the United States given limited testing (22, 23, 28). In the United Kingdom and Japan, healthcare-associated outbreaks with GES-harboring

Gram-negative organisms have been published, highlighting the potential for patient-to-patient spread (29–31). GES subtypes are widely heterogeneous, with certain subtypes more characteristic of carbapenemases, while others lack carbapenem hydrolytic activity (i.e., more like ESBLs). Of note, single point mutations can dictate the carbapenem hydrolytic activity, making differentiation challenging (32). All GES-harboring strains tested in this study were meropenem and imipenem resistant. On-label utilization of the Carba-R NxG will necessitate carbapenem nonsusceptibility, although it is possible such strains harbor ESBL subtype GES enzymes. Clinically, the relevance of identifying an ESBL versus carbapenemase GES enzyme remains to be defined for *P. aeruginosa*. Although ceftolozane-tazobactam is a reliable empirical option for carbapenem-resistant *P. aeruginosa* due to preserved activity despite alterations in porin and efflux mutations and consistently high susceptibility (14, 33, 34), resistance has been reported due to ESBL and carbapenemase enzymes, including GES-harboring strains (35, 36). Thus, early detection of GES enzymes can direct clinicians to alternative agents. The treatment of choice for this challenging enzymology has yet to be defined; however, *in vitro* and *in vivo* data have suggested imipenem-relebactam or ceftazidime-avibactam is a therapeutic option (35, 37). Reliable identification may be instrumental in defining the epidemiology and treatment outcomes of GES-harboring organisms.

The Carba-R NxG successfully identified 24/26 IMP-harboring isolates on initial testing. Subsequent WGS reconfirmed the target gene for each aberrant result, and blinded triplicate retesting using the Carba-R NxG found positive results for both isolates in all replicates. Since WGS and blinded retesting successfully identified the isolates, the initial negative results are suggestive of a preanalytical error as opposed to the IMP variant being outside the spectrum of the Carba-R NxG. The Carba-R NxG, which incorporates additional primers and probes to broaden the detection of IMP subtypes, was able to identify all IMP variants tested in the present study, including 11 isolates not captured by the commercially available Carba-R. This finding highlights the improved testing spectrum of the Carba-R NxG for detecting these challenging IMP variants that are found in portions of Southeast Asia but have been increasingly detected globally (21).

A strength of the present study was the number and diversity of carbapenemase classes represented in these clinical *P. aeruginosa* isolates. Nonetheless, while the Carba-R NxG provides genotypic targets to bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC}, bla_{SPM}, bla_{GES}, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}58}$, and $bla_{NMC/IMI}$, none of the evaluated isolates in this study harbored OXA-48, OXA-58, or NMC/IMI, warranting further assessments despite their low prevalence in *P. aeruginosa* (21, 38, 39). Future studies evaluating these expanded targets that are increasingly common in *Enterobacterales*, including SME, GES, and NMC/IMI, warrant further investigation. In addition, the positive predictive value and negative predictive value of the assay will vary depending on local carbapenemase epidemiology.

In summary, the Carba-R NxG assay provided rapid detection of carbapenemase producers among CRPA isolates and demonstrated an expanded spectrum of genotypic targets that includes bla_{SPM}, bla_{GES}, and bla_{IMP} variants previously outside the testing targets. Such enrichments to the carbapenemase detection spectrum can aid in the identification of carbapenemase-producing organisms across the globe as the prevalence and diversity of these enzymes continues to expand.

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