Evaluation of the Carba-R NxG Assay in a Global Challenge Set of *Pseudomonas aeruginosa*

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ABSTRACT

Background: Carbapenem-resistant *P. aeruginosa* (CRPA) is a growing clinical challenge. Carbapenemase production is particularly problematic due to high transmissibility and limited treatment options. Carbapenemase prevalence and diversity are largely driven by geography and thus testing spectrum will dictate utility in certain regions. The purpose of this study was to evaluate the performance of the research-use-only# Xpert® Carba-R NxG (Carba-R NxG) and commercially available Xpert® Carba-R* (Carba-R) in a global collection of *P. aeruginosa*.

Methods: The challenge set included 123 clinical *P. aeruginosa* isolates from 11 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 per manufacturer instructions. Carba-R NxG testing was completed by Cepheid (Sunnyvale, CA) blinded to genotype. Test performances were tabulated for each assay by carbapenemase class.

Results: 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 (Carba-R NxG 100% vs. Carba-R 58% detection). All SPM and GES isolates, targets not present in the current 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. Table 1 provides the test performance of both assays. Overall, the Carba-R NxG successfully categorized 100% of isolates tested compared with 68% for its predecessor.

Conclusion: As the prevalence and diversity of carbapenemase-producing CRPA continues to expand, the Carba-R offers a rapid and sensitive assay to identify clinically relevant carbapenemase genotypes to inform infection prevention and therapeutic interventions. The Carba-R NxG will expand the current targets including SPM, GES, NMC/IMI, and IMP-subtypes, outside the previous testing spectrum, increasing the global utility of the test.

INTRODUCTION

- Carbapenem resistance in *P. aeruginosa* is typically mediated by efflux/porin loss and over-expression of cephalosporinases although carbapenemases are more common in certain geographic areas[1,2]
- Carbapenemase production is especially problematic as it is associated with nosocomial spread and limited treatment options.[3]
- Detection of carbapenemase-producing *P. aeruginosa* is imperative to guide infection control and targeted antimicrobial therapy.
- The Cepheid Xpert® Carba-R is a rapid, *in* vitro, diagnostic that utilizes real-time PCR.[4]
- The commercially available Carba-R assay targets VIM-, NDM-, OXA-48-, KPC- and some IMP-subtype carbapenemases.[4]
- The yet-to-market next-generation Carba-R (Carba-R NxG) expands the genotypic targets to include more IMP-subtypes, SPM, NMC/IMI, and GES carbapenemases (**Table 1**).

Table 1. Carba-R and Carba-R NxG genotypic carbapenemase targets.

Commercially Available Carba-R	RUO Carba-R NxG
KPC	KPC
OXA-48	OXA-48
VIM	VIM
NDM	NDM
IMP (certain subtypes)	IMP (expanded subtypes)
---	SPM
---	GES
---	NMC/IMI

[a]RUO = Research-use-only; not for diagnostic use, not reviewed by any regulatory body

OBJECTIVE

- To compare the test performance of the research-use-only Carba-R NxG compared with the commercially available Carba-R in a global challenge set of *P. aeruginosa* .

METHODS

Bacterial Isolates

- The challenge set comprised of 123 P. aeruginosa isolates (**Figure 1**).
- 52 Isolates were from the FDA-CDC Antimicrobial Resistance Bank while remaining were from the Center for Anti-Infective Research and Development Repository.
- All isolates were previously identified by whole-genome sequencing or PCR
- Carbapenemase positive strains (n =103/123) were tested.
  - Meropenem MICs ranged from 8 - >64 mg/L; imipenem MIC ranged from 8 - >64 mg/L.
  - VIM-, IMP-, NDM-, SPM-, KPC-, and GES-carbapenemases were represented.
  - Three isolates co-harbored 2 carbapenemases (VIM- and GES-, n=2; VIM- and NDM-, n=1).
- Non-carbapenemase producing isolates (n=20/123) with cephalosporinases and/or efflux/porin mutations were included as negative controls.
  - All isolates were meropenem or imipenem-resistant: Meropenem MIC range 1 - 64 mg/L; imipenem MIC range 0.25 - >64 mg/L.
- All isolates were stored at -80ºC in skim milk and transferred twice on Trypticase soy agar plates with 5% sheep's blood.

Genotypic Carbapenemase Detection Testing

- All isolates underwent gene detection on: i) the commercially available Xpert® Carba-R assay (Cepheid, Sunnyvale, CA); ii) the research-use-only Carba-R NxG (Cepheid, Sunnyvale, CA, USA).
- Testing with the current commercially available assay was conducted at the Center for Anti-Infective Research and Development per the package insert.[4]
- Quality control using the Xpert® Carba-R QC Panel (Maine Molecular) was conducted weekly.
- Discordant results from the Carba-R NxG were repeated in triplicate, interspersed in a collection of other carbapenemase positive isolates to maintain blinding.
- Whole genome-sequencing (WGS) was used to reconfirm the presence of the targeted carbapenemase genes.

Data Analysis

- Sensitivity and specificity were calculated individually for each assay by carbapenemase class compared with the reference genotype.
- 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.

* IVD. In vitro diagnostic device

RESULTS

Table 2. Test performance of the Carba-R NxG and Carba-R against a challenge set of *P. aeruginosa*

Target	True Positive	False Positive	True Negative	False Negative	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	---	---[a]	---[a]
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	---[a]	---[a]
KPC	8	0	115	0	100% (63-100)	100% (97-100)
GES	0	0	109	14	---[a]	---[a]
Non-CP	---	0	20	---	---[a]	---[a]

[a]Not calculated; Non-CP = non-carbapenemase producing

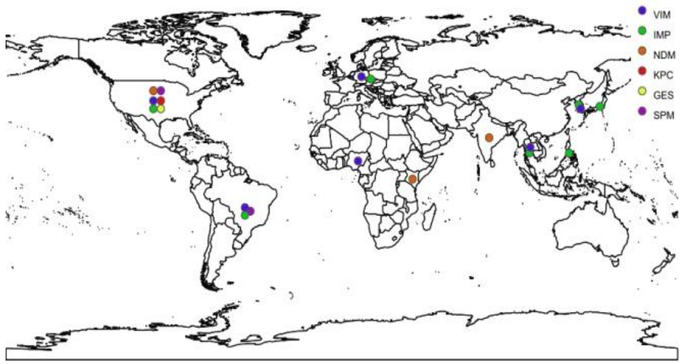
- 2 of the 103 carbapenemase positive strains evaluated (harboring IMP-6 and IMP-7) required re-testing on the Carba-R NxG due to discordant results attributed to a pre-analytical error.
- Retesting resolved this discordance with the Carba-R NxG accurately identifying both genotypic targets.

Table 3. Number of isolates and carbapenemase subtypes tested.

Carbapenemase Class	Number of Isolates Tested	Subtypes Represented
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**

Subtypes in **bold and colored** were expanded coverage provided by the RUO **Carba-R NxG**

Figure 1. Geographic distribution of carbapenemase-producing *P. aeruginosa* in the challenge set.



DISCUSSION/CONCLUSIONS

- The Cepheid Carba-R NxG successfully identified all 123 *P. aeruginosa* isolates included in the challenge set (**Table 2**).
- The Carba-R NxG offers an expanded testing spectrum including GES-, SPM-, and expanded coverage of IMP-subtypes previously not targeted by the commercially available Carba-R (**Table 3**).
- Introduction of the Carba-R NxG to the clinic will expand the utility of the assay both in the United States (i.e., GES-) and abroad (i.e., IMP-, GES-, SPM-) to identify carbapenemase-producing *P. aeruginosa*.

ACKNOWLEDGMENTS

- The authors thank Courtney Bouchard, Janice Cunningham, Nicole DeRosa, Lauren McLellan, Alissa Padgett, Debora Santini, Jennifer Tabor-Rennie, Rebecca Stewart, Charlie Cote, Max Lasko, and Christina Sutherland from the Center for Anti-Infective Research and Development for their assistance with the conduct of this study.
- The Cepheid Carba-R and Cepheid Carba-R NxG were provided by and conducted by Cepheid, Sunnyvale, Ca., respectively.
- All other funds and resources were provided by the Center for Anti-Infective Research and Development, Hartford Hospital.

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