Evaluation of Xpert® Carba-R and NG-Test® CARBA-5 for detection of carbapenemases from clinical isolates

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INTRODUCTION

The global spread of carbapenemase-producing organisms (CPOs) is a critical medical and public health issue.

This evaluation was designed to assess the performance of the q-PCR based Xpert® Carba-R assay (Cepheid) and the NG-Test® CARBA-5 immunoassay (NG Biotech) in detecting *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$ carbapenemases in a panel of bacterial species received at the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) under the Victorian guideline on carbapenemase producing organisms.

Xpert® Carba-R

- Automated real-time PCR
- Detects genes associated with carbapenem non-susceptibility: KPC, OXA-48-like, IMP, VIM and NDM
- 60 minute time to result
- Requires GeneXpert instrument
- Sample types include bacterial cultures and rectal swabs

Image source: Xpert® Carba-R (cepheid.com)

NG-Test® CARBA-5

- Immunochromatographic assay
- Detects carbapenemase enzymes KPC, OXA-48-like, IMP, VIM and NDM
- 20 minute time to result
- Does not require specialist equipment
- Sample types include bacterial cultures only

Image source: https://www.ngbiotech.com/antibiotic-resistance

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METHODS

- A panel of 67 carbapenemase-producing bacterial isolates representative of those seen in Victoria over the last 10 years containing one or more carbapenemase gene variants (previously characterised by whole genome sequencing) were tested on the Xpert® Carba-R and the NG-test® CARBA-5.
- Additional bacterial species and carbapenemase gene variants to those validated by the commercial assays were selected for this study (see table below).
- Samples were tested in pools of up to 4 bacterial isolates of different species and genotypes, and were retested individually where discrepant results were observed.
- Additional testing was performed on the Xpert® Carba-R to verify the limit of detection (LoD).

Bacterial species and genotypes tested

Species or genus	Variants tested
Acinetobacter baumanii	NDM-1, OXA-94
Citrobacter sp.	KPC-2, VIM-1
Escherichia coli	IMP-26, VIM-1, NDM-1, NDM-19, NDM-5, KPC-2, OXA-181, OXA-484
Enterobacter sp.	IMP-4, VIM-1, NDM-7, KPC2, KPC-4, OXA-181
Klebsiella sp.	IMP-14, VIM-5, VIM-19, NDM-1, NDM-5, NDM-4, KPC-2, KPC-3, KPC-33, KPC-23, OXA-232, OXA-181
Proteus sp.	IMP-4, VIM-1, NDM-1, OXA-181
Providencia sp.	OXA-48, IMP-26
Pseudomonas aeruginosa	IMP-7, IMP-10, IMP-34, IMP-62, IMP-1, VIM-2, VIM-4, NDM-1
Serratia marcescens	IMP-4, NDM-1, NDM-7

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RESULTS

NG-test® CARBA-5 Performance Characteristics

	Target	Number of pools	True positives	False positives	True negatives	False negatives	Positive predictive value	Negative Predictive value	Accuracy	Sensitivity	Specificity
✓	IMP	22	15	0	7	0	1.0	1.0	100%	100%	100%
✓	VIM	23	13	0	10	0	1.0	1.0	100%	100%	100%
✓	NDM	22	19	0	3	0	1.0	1.0	100%	100%	100%
✓	KPC	23	12	0	11	0	1.0	1.0	100%	100%	100%
✓	OXA-48-like	21	10	0	11	0	1.0	1.0	100%	100%	100%

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- 100% accuracy, sensitivity and specificity for all targets.

Xpert® Carba-R Performance Characteristics

	Target	Number of pools	True positives	False positives	True negatives	False negatives	Positive predictive value	Negative predictive value	Accuracy	Sensitivity	Specificity
→	IMP	22	11	0	9	2	1.0	0.8	90.9%	84.6%	100%
✓	VIM	23	13	0	10	0	1.0	1.0	100%	100%	100%
✓	NDM	22	19	0	3	0	1.0	1.0	100%	100%	100%
→	KPC	23	12	1	10	0	0.9	1.0	95.6%	100%	90.9%
✓	OXA-48-like	21	10	0	11	0	1.0	1.0	100%	100%	100%

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- 100% accuracy, sensitivity and specificity for OXA-48-like, NDM and VIM gene targets.
- Reduced sensitivity (84.6%) and accuracy (90.9%) for the detection of **IMP.**
- Reduced specificity (90.9%) and accuracy (95.6%) for the detection of **KPC.**
- Two false-negative IMP results were obtained; one ***P. aeruginosa*** with an **IMP-62** genotype and one ***S. marcescens*** with an **IMP-4** genotype.
- A KPC positive result was detected for a ***K. pneumoniae*** with a **KPC-33** genotype. As KPC-33 induces an ESBL phenotype with susceptibility to carbapenems, this result was recorded as a false positive.

Xpert® Carba-R LoD results

	Target	Gene variant tested	Manufacturer's reported LoD (CFU/ml)	LoD this study (cfu/ml)	LoD within manufacturer's specifications?
→	IMP	IMP-4	24-127	1.5×10^7	NO
✓	VIM	VIM-1	61-180	50	YES
✓	NDM	NDM-1	11-50	50	YES
✓	KPC	KPC-2	75-100	50	YES
✓	OXA-48	OXA-181	21-45	50	YES

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- LoD of approximately 50cfu/ml for VIM, NDM, OXA-48-like and KPC genes was consistent with the manufacturers IFU.
- The manufacturers LoD was unable to be achieved for the detection of IMP-4. We calculated LoD for IMP to be ~1.5×10^7cfu/ml.

Overall Performance Characteristics

Test	Number of isolates	True positives	False positives	True negatives	False negatives	Positive predictive value	Negative Predictive value	Accuracy	Sensitivity	Specificity
Xpert® Carba-R	67	53	1	11	2	0.98	0.8	95.5%	96.4%	91.7%
NG-Test® CARBA-5	67	56	0	11	0	1.0	1.0	100%	100%	100%

Note: IMP-7 and IMP-14 recorded as true positives for NG-Test® CARBA-5 and true negatives for Xpert® Carba-R due to known limitations of the assay.

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- Xpert® Carba R:
  Accuracy = 95.5%, Sensitivity = 96.4%, Specificity = 91.7%.
  This assay did not meet the manufacturers specifications for performance (Sn=100%, Sp=97.1%).
- NG-test® CARBA-5:
  Accuracy = 100%, Sensitivity = 100%, Specificity = 100%.
  This assay met the manufacturers specifications for performance (Sn=100%, Sp=100%).

Xpert® Carba-R TEST LIMITATIONS

Inconsistent detection of IMP variants:

- A total of 7 isolates with IMP-4 genotypes were tested from *E. coli, Enterobacter* sp., *Proteus* sp., and *S. marcescens.* It was observed that *S. marcescens* isolates harbouring **IMP-4** genes (n=2) exhibited late amplification of the target (C_t = 39.1, C_t = 39.3) which resulted in inconsistent interpretation of the sample as positive or negative by the instrument.
- The LoD achieved for IMP genes was ~1.5×10^7 cfu/ml which was higher than claimed in the manufacturers IFU (25-150 cfu/ml). This is likely due to the IMP variant tested (IMP-4) and the assays reduced sensitivity to this target.
- A false negative result was recorded for a *P. aeruginosa* with an **IMP-62** genotype. This variant was not predicted to be detected by *in silico* analysis[1], and is not commonly seen in Victoria.
- The test is unable to detect IMP-7, IMP-13 (not tested in our study) and IMP-14 due to variation at the primer sites[2]. This was confirmed in our testing with negative results for these gene targets recorded as true negatives for this evaluation.

Reduced specificity for KPC:

- A false positive KPC result was recorded for a *K. pneumoniae* with a **KPC-33** genotype.
- KPC-33 is a *bla*$_{KPC-2}$ gene variant belonging to the KPC family. Phenotypically, isolates with this genotype show carbapenem susceptibility and resistance to ceftazidime-avibactam, and are therefore classified as extended-spectrum beta-lactamase producing *Enterobacterales* (ESBLs) rather than CPOs. For our evaluation, detection of this target was recorded as a false positive result.
- The NG-test® CARBA-5 did not detect the KPC-33 subvariant. This result was recorded as a true negative.

DISCUSSION

- The NG-test® CARBA-5 performed with high accuracy for a range of subvariants. The test is user friendly, rapid and cost efficient, and does not require specialist equipment or storage conditions, making it very useful in low resource settings.
- The Xpert® Carba-R is a helpful diagnostic tool for clinical settings as it has a much more sensitive limit of detection and may also detect carbapenemase genes from rectal swabs, however the assay did not meet the manufacturers specifications for performance of IMP and KPC targets.
- IMP-4 is a common carbapenemase gene-allele in Victoria, and was detected in 22.3% (n=230/1030) confirmed CPO cases notified to the Victorian state-wide surveillance program between Jan 2020 – Apr 2024. The reduced sensitivity of the Xpert® Carba-R seen for this target in our evaluation raises some concern for its utility in Australian clinical diagnostic settings if used in isolation.
- Our results highlight a potential opportunity for enhancement of the IMP primers in order for the Xpert® Carba-R to encompass a greater range of IMP variants.
- It is important to be cognisant of the different targets and test types for both assays (nucleic acid versus enzyme), and the impact this has on the interpretation of detection of 'Carbapenemases'. KPC-33 is an exemplar of this.

CONCLUSION

Xpert® Carba-R and NG-test® CARBA-5 are rapid and cost-efficient tests to detect carbapenemases across numerous bacterial species, and provide a useful resource in the identification/confirmation of CPOs. The limitations observed in this evaluation are important to be aware of when relying solely on these assays for diagnostic purposes.

REFERENCES

1. Gill CM et.al., 2020, "Evaluation of the Xpert Carba-R NxG Assay for Detection of Carbapenemase Genes in a Global Challenge Set of Pseudomonas aeruginosa Isolates.", J Clin Microbiol. e01098-20.
2. Xpert Carba-RP Only CE IVD ENGLISH Package Insert 301-9242, Rev. C. June 2020

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