



HAL
open science

Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases

Katie L Hopkins, Danièle Meunier, Thierry Naas, Hervé Volland, Neil Woodford

► To cite this version:

Katie L Hopkins, Danièle Meunier, Thierry Naas, Hervé Volland, Neil Woodford. Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. *Journal of Antimicrobial Chemotherapy*, 2018, 73 (12), pp.3523-3526. 10.1093/jac/dky342 . hal-04455800

HAL Id: hal-04455800

<https://hal.science/hal-04455800>

Submitted on 13 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

J Antimicrob Chemother 2018; **73**: 3523–3526
doi:10.1093/jac/dky342
Advance Access publication 5 September 2018

Evaluation of the NG-Test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases

Katie L. Hopkins^{1*}, Danièle Meunier¹,
Thierry Naas^{2–5}, Hervé Volland⁶ and Neil Woodford¹

¹*Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, National Infection Service, Public Health England, London NW9 5EQ, UK;* ²*Bacteriology-Hygiene Unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France;* ³*EA7361 'Structure, dynamic, function and expression of broad spectrum β -lactamases', Université Paris-Sud, Université Paris-Saclay, LabEx Lermite, Faculty of Medicine, Le Kremlin-Bicêtre, France;* ⁴*Associated French National Reference Center for Antibiotic Resistance: Carbapenemase-Producing Enterobacteriaceae, Le Kremlin-Bicêtre, France;* ⁵*Evolution and Ecology of Resistance to Antibiotics Unit, Institut Pasteur—APHP—Université Paris-Sud, Paris, France;* ⁶*SPI, CEA, Université Paris-Saclay, F-91191 Gif-sur-Yvette, France*

*Corresponding author. Tel: +44-(0)208-327-7061;
E-mail: katie.hopkins@phe.gov.uk

Sir,
No single method currently commercialized for detecting acquired carbapenemases offers comprehensive coverage, but local testing is needed to support rapid detection and prompt action. Within the UK and globally most carbapenemase producers harbour one or more of the 'big 5' families: KPC, OXA-48-like, NDM, VIM and IMP.^{1,2} Diagnostic laboratories should therefore consider tests that reliably detect at least four or, preferably, all five of these families. Here, we evaluated the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, Guipry, France),³ for detecting the 'big 5' carbapenemases in isolates referred to the UK's national reference laboratory.

The NG-Test CARBA 5 assay was evaluated using 197 previously characterized bacterial isolates including 177 confirmed carbapenemase producers and 20 carbapenem-resistant but carbapenemase-negative isolates (Table 1). Isolates represented the diversity of carbapenemase genes (but not the prevalence), and host organisms thereof, identified in the UK. One colony of overnight growth harvested from Columbia blood agar plates was tested according to the manufacturer's instructions. Time until appearance of one or more red lines in the test region of the

cassette was recorded by comparison to a line in the control region, but with the final reading performed at 15 min as per the manufacturer's instructions. Any discrepancies between the results obtained with the NG-Test CARBA 5 and those obtained with in-house PCRs and/or WGS were investigated by retesting using the NG-Test CARBA 5 and in-house PCR. Where discrepancies still remained, the carbapenem inactivation method (CIM) was used to screen for carbapenemase activity.⁴

The NG-Test CARBA 5 identified all KPC ($n = 30$), OXA-48-like ($n = 31$), NDM ($n = 31$) and VIM ($n = 29$) producers with no false-positives. One NDM producer initially gave an additional false-positive signal for VIM. On initial testing, IMP carbapenemases were detected in 12/17 isolates producing IMP-1 ($n = 5$), IMP-4 ($n = 2$), IMP-6 ($n = 1$), IMP-7 ($n = 1$), IMP-8 ($n = 1$) and IMP-29 ($n = 2$). Repeat testing by in-house PCR and NG-Test CARBA 5 confirmed 1/5 'failed' isolates was a false-negative. The four remaining isolates encoding variants most closely related to IMP-13 ($n = 2$) and IMP-14 ($n = 2$) were again negative by NG-Test CARBA 5 and carbapenemase activity was confirmed by CIM; therefore, these isolates were also considered false-negatives.

Among the 25 isolates producing two carbapenemases, discrepancies were observed between the NG-Test CARBA 5 and in-house PCR for two isolates; both were thereafter considered as true-negatives owing to gene loss. One isolate initially gave an additional false-positive band for KPC. All 14 isolates producing carbapenemases belonging to families other than the 'big 5' and the 20 carbapenem-resistant isolates without carbapenemases were correctly identified as negative with no false-positives.

When calculating analytical sensitivity and specificity, we considered each NG-Test CARBA 5 assay to be five individual tests, meaning a total of 985 (197×5) tests were performed. After the first round of testing, i.e. including the false-positives but excluding the true-negatives due to gene loss, the overall sensitivity and specificity of the NG-Test CARBA 5 were 97.31% (95% CI 93.84%–99.12%) and 99.75% (95% CI 99.12%–99.97%), respectively. Although both sensitivity and specificity increased following repeat testing, the first NG-Test CARBA 5 assay results would be recorded in a frontline laboratory, with no reason to repeat.

The NG-Test CARBA 5 assay was easy to perform and set-up took ~5 min per isolate with relatively little hands-on time. Although the final result was read at 15 min, positive results started to appear within 2–6 min.

All KPC, OXA-48-like, NDM and VIM gene variants identified in the UK to date could be detected with the NG-Test CARBA 5 assay and this study adds carbapenemase variants KPC-4/-23, OXA-245/-436/-484, NDM-2/-3 and IMP-4/-6/-7/-10/-29 to the range previously detected.³ However, IMP-13- and IMP-14-like carbapenemases were not detected. Problems with detecting these IMP variants were also observed during an evaluation of the Cepheid Xpert® Carba-R assay.⁵ Sequence diversity within the IMP family and the even distribution of mutations throughout the sequence⁶ make it difficult to design primer/probe sets that can detect all variants. However, IMP carbapenemases are reported to have two well-conserved amino acid regions, H2 (amino acids 124–130) and S6

Table 1. Details of clinical isolates with previously characterized mechanisms conferring resistance to carbapenems

Species	Carbapenemase status, including allele where known (number of isolates)									
	KPC (n = 30)	OXA-48-like (n = 31)	NDM (n = 31)	VIM (n = 29)	IMP (n = 17)	combination (n = 25)	other carbapenemase (n = 14)	no carbapenemase (n = 20)		
<i>Klebsiella pneumoniae</i>	8	10	5	7	3	12	1	4		
	KPC-2 (5)	OXA-48 (2)	NDM-1 (1)	VIM-1 (2)	IMP-1 (2)	KPC-2+OXA-48 (1)	GES-5 (1)			
	KPC-3 (2)	OXA-181 (3)	NDM-5 (2)	VIM-4 (3)	IMP-6 (1)	NDM-1+OXA-48 (2)				
	KPC-9 (1)	OXA-232 (1)	NDM-7 (2)	VIM-19(2)		NDM-1+OXA-181 (3)				
		OXA-245 (1)				NDM-1+OXA-232 (1)				
		OXA-436 (1)				NDM-4+OXA-48 (1)				
		OXA-484 (2)				NDM-5+OXA-48 (1)				
						NDM-5+OXA-181 (2)				
						IMP-1+VIM-4 (1)				
<i>Klebsiella oxytoca</i>	2	2	2	5	1					
	KPC-2+GES-7 (1)	OXA-181 (2)	NDM-1 (2)	VIM-1 (2)	IMP-1 (1)					
	KPC-2 (1)			VIM-4 (3)						
<i>Klebsiella variicola</i>	1									
	KPC-2 (1)									
<i>Raoultella ornithinolytica</i>			1							
			NDM-1 (1)							
<i>Escherichia coli</i>	4	9	10	4	1	7		1		
	KPC-2 (3)	OXA-48 (3)	NDM-1 (1)	VIM-1 (2)	IMP-14 (1)	KPC-2+OXA-48 (1)				
	KPC-3 (1)	OXA-181 (1)	NDM-3 (1)	VIM-4 (2)		NDM-4+OXA-181 (2)				
		OXA-232 (3)	NDM-4 (3)			NDM-5+OXA-181 (2)				
		OXA-244 (2)	NDM-5 (2)			NDM-7+OXA-181 (1)				
			NDM-7 (3)			NDM-5+OXA-232 (1)				
<i>Enterobacter cloacae</i> complex	8	3	2	5	3	4	6	4		
	KPC-2 (5)	OXA-181 (1)	NDM-1 (2)	VIM-1 (2)	IMP-1 (2)	NDM-1+OXA-48 (2)	IMI-4 (1)			
	KPC-4 (3)	OXA-204 (1)		VIM-4 (3)	IMP-4 (1)	NDM-1+OXA-181 (1)	IMP-8 (1)			
		OXA-244 (1)				NDM-1+VIM-1 (1)	NMC-A (1)			
							FRI-1 (1)			
							FRI-2 (1)			
							OXA-427 (1)			
<i>Klebsiella aerogenes</i>	1	2	1					1		
	KPC-2 (1)	OXA-48 (2)	NDM-7 (1)							
<i>Citrobacter freundii</i>	4	2	2	4	1					
	KPC-2 (2)	OXA-48 (1)	NDM-1 (2)	VIM-1 (2)	IMP-10 (1)					
	KPC-3 (1)	OXA-232 (1)		VIM-4 (2)						
	KPC-4 (1)									

<i>Citrobacter sedlakii</i>	1					
	NDM-1 (1)					
<i>Serratia marcescens</i>	2	OXA-48 (2)				2
	1	NDM-1 (1)				SME-2 (1) SME-4 (1)
<i>Proteus mirabilis</i>	1					
	1	NDM-1 (1)				
<i>Providencia rettgeri</i>	1					
	2	NDM-1 (1)				
<i>Providencia stuartii</i>	2		2	1		
	1	NDM-1 (2)	VIM-1 (1) VIM-4 (1)		IMP-1+NDM-1 (1)	
<i>Pantoea species</i>	1					
	KPC-2 (1)					
<i>Acinetobacter baumannii</i>	1					2
	NDM-1 (1)					OXA-23 (2)
<i>Acinetobacter species</i>				2		
<i>Pseudomonas aeruginosa</i>	1	1	1	1	1	3
	KPC (1)	OXA-181 (1)	VIM-2 (1)	IMP-29 (2) 6	IMP+NDM (1)	SPM-1 (1) OXA-198 (1) DIM-1 (1)
				IMP-4 (1) IMP-7 (1) IMP-8 (1) IMP-13 (2) IMP-14 (1)		
<i>Pseudomonas putida</i>			1			
			VIM-2 (1)			
<i>Morganella morganii</i>	1					
	NDM-1+GES-1 (1)					

(amino acids 134–140), and these have been targeted in an immunochromatographic test reported to detect all IMP variants.^{7,8}

This study highlights that coverage *within* families may vary between assays designed to detect the main carbapenemase families. Although IMP producers only account for 379/13682 (2.8%) carbapenemase-producing organisms confirmed by AMRHAI since 2000, they may be becoming more widespread, with 29 laboratories referring 167 isolates in 2017 compared with 20 laboratories referring 75 isolates in 2016 (K. L. Hopkins and N. Woodford, unpublished data). Laboratories should maintain a high level of suspicion regarding any isolate with a meropenem MIC above the EUCAST screening cut-off for carbapenemase-producing Enterobacteriaceae (MIC >0.12 mg/L) but negative in an assay marketed as covering the ‘big 5’ carbapenemases and refer any such isolates to a national reference laboratory for further investigation.

Acknowledgements

We wish to thank NG Biotech for providing the NG-Test CARBA 5 cassettes and AMRHAI staff for their role in characterization of these isolates.

Funding

This study was carried out as part of our routine work.

Transparency declarations

PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Accelerate Diagnostics, Achaogen Inc., Allegra Therapeutics, Amplex, AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, ECDC, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp., Meiji Seika Pharma Co., Ltd, Mobidiag, Momentum Biosciences Ltd, Neem Biotech, NIHR, Nordic Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Shionogi & Co., Ltd, Trius Therapeutics, VenatoRx Pharmaceuticals, Wockhardt Ltd and WHO. All other authors: none to declare.

References

- 1 PHE. *English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR), Report 2017*. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/656611/ESPAUR_report_2017.pdf.
- 2 Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017; **215** Suppl 1: S28–36.
- 3 Boutal H, Vogel A, Bernabeu S *et al*. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like

carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother* 2018; **73**: 909–15.

4 van der Zwaluw K, de HA, Pluister GN *et al*. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. *PLoS One* 2015; **10**: e0123690.

5 Findlay J, Hopkins KL, Meunier D *et al*. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. *J Antimicrob Chemother* 2015; **70**: 1338–42.

6 Widmann M, Pleiss J, Oelschlaeger P. Systematic analysis of metallo- β -lactamases using an automated database. *Antimicrob Agents Chemother* 2012; **56**: 3481–91.

7 Kitao T, Miyoshi-Akiyama T, Tanaka M *et al*. Development of an immunochromatographic assay for diagnosing the production of IMP-type metallo- β -lactamases that mediate carbapenem resistance in *Pseudomonas*. *J Microbiol Methods* 2011; **87**: 330–7.

8 Notake S, Matsuda M, Tamai K *et al*. Detection of IMP metallo- β -lactamase in carbapenem-nonsusceptible Enterobacteriaceae and non-glucose-fermenting Gram-negative rods by immunochromatography assay. *J Clin Microbiol* 2013; **51**: 1762–8.

J Antimicrob Chemother 2018; **73**: 3526–3529

doi:10.1093/jac/dky338

Advance Access publication 29 August 2018

Influence of sublethal concentrations of vancomycin and quinupristin/dalfopristin on the persistence of viable but non-culturable *Staphylococcus aureus* growing in biofilms

Sonia Pasquaroli¹, Barbara Citterio², Gianmarco Mangiaterra¹, Francesca Biavasco¹ and Carla Vignaroli^{1*}

¹Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy; ²Department of Biomolecular Science, Biotechnology Section University of Urbino ‘Carlo Bo’, Urbino, Italy

*Corresponding author. Tel: +39-071-2204638; E-mail: c.vignaroli@univpm.it

Sir,
Staphylococcus aureus is frequently associated with biofilm-related infections in both nosocomial and community settings.¹ Infections