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

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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 sequence[6] make it difficult to design primer/probe sets that can detect all variants. However, IMP carbapenemases are reported to have two

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

	Carbapenemase status, including allele where known (number of isolates)							
Species	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 ($n = 20$)
Klebsiella pneumoniae	8	10	5	7	3	12	1	4
	KPC-2 (5) KPC-3 (2) KPC-9 (1)	OXA-48 (2) OXA-181 (3) OXA-232 (1) OXA-245 (1) OXA-436 (1) OXA-484 (2)	NDM-1 (1) NDM-5 (2) NDM-7 (2)	VIM-1 (2) VIM-4 (3) VIM-19(2)	IMP-1 (2) IMP-6 (1)	KPC-2+OXA-48 (1) NDM-1+OXA-48 (2) NDM-1+OXA-181 (3) NDM-1+OXA-232 (1) NDM-4+OXA-48 (1) NDM-5+OXA-48 (1) NDM-5+OXA-181 (2) IMP-1+VIM-4 (1)	GES-5 (1)	
Klebsiella oxytoca	2	2	2	5	1			
	KPC-2+GES-7 (1) KPC-2 (1)	OXA-181 (2)	NDM-1 (2)	VIM-1 (2) VIM-4 (3)	IMP-1 (1)			
Klebsiella variicola	1							
	KPC-2 (1)							
Raoultella ornithinolytica			1					
			NDM-1 (1)					
Escherichia coli	4	9	10	4	1	7		1
	KPC-2 (3) KPC-3 (1)	OXA-48 (3) OXA-181 (1) OXA-232 (3) OXA-244 (2)	NDM-1 (1) NDM-3 (1) NDM-4 (3) NDM-5 (2) NDM-7 (3)	VIM-1 (2) VIM-4 (2)	IMP-14 (1)	KPC-2+OXA-48 (1) NDM-4+OXA-181 (2) NDM-5+OXA-181 (2) NDM-7+OXA-181 (1) NDM-5+OXA-232 (1)		
Enterobacter cloacae complex	8	3	2	5	3	4	6	4
	KPC-2 (5) KPC-4 (3)	OXA-181 (1) OXA-204 (1) OXA-244 (1)	NDM-1 (2)	VIM-1 (2) VIM-4 (3)	IMP-1 (2) IMP-4 (1)	NDM-1+OXA-48 (2) NDM-1+OXA-181 (1) NDM-1+VIM-1 (1)	IMI-4 (1) IMP-8 (1) NMC-A (1) FRI-1 (1) FRI-2 (1) OXA-427 (1)	
Klebsiella aerogenes	1	2	1					1
	KPC-2 (1)	OXA-48 (2)	NDM-7 (1)					
Citrobacter freundii	4	2	2	4	1			
	KPC-2 (2) KPC-3 (1)	OXA-48 (1) OXA-232 (1)	NDM-1 (2)	VIM-1 (2) VIM-4 (2)	IMP-10 (1)			

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

well-conserved amino acid regions, H2 (amino acids 124–130) and S6 (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 379/13 682 (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.

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