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Rapid Detection of Carbapenemases in *Enterobacteriaceae*: Evaluation of the RESIST-3 O.K.N (OXA-48, KPC, NDM) Multiplexed Lateral Flow Assay

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Running title: Multiplexed CRE lateral flow cards

Text

The identification, treatment and control of carbapenem resistant *Enterobacteriaceae* (CRE) infections are a major challenge for healthcare institutions and diagnostic laboratories worldwide. Those producing plasmid mediated OXA-48, KPC, NDM or VIM-like carbapenemases (CPE) are most concerning, being frequently involved in nosocomial outbreaks that are difficult and very costly to manage (1). A simple and rapid test, able to provide sensitive and specific identification of CRE and also the carbapenemase present, is critical in any strategy aimed at addressing this problem.

Numerous phenotypic (MIC determination, disc diffusion, selective culture media, acidometric) and genotypic (PCR amplification, microarrays, DNA sequencing) methods have been used in the laboratory isolation, diagnosis and confirmation of CRE (2). Recently, two novel immunochromatographic lateral flow assays (Coris BioConcepts, Gembloux, Belgium) were developed for the specific detection of OXA-48 (OXA-48 K-SeT) and KPC-like carbapenemase producers (KPC K-SeT). These assays have been evaluated in multiple laboratories, using diverse sets of organisms (*Escherichia coli, Klebsiella, Enterobacter, Serratia, Providencia, Pseudomonas* spp) carrying multiple β-lactamase, OXA-48 (OXA-48, 162, 181, 204, 232, 242) and KPC (KPC-2/3/4) allellic variants. (3,4,5,6). Both have a reported sensitivity and specificity of 100 % when compared to molecular detection of carbapenemase genes as the gold standard (3,4,5,6). They have also been shown to be compatible with organisms recovered from most un-supplemented, selective, solid and liquid culture media currently in use in diagnostic laboratories and with bacteria taken directly from positive blood culture bottles (BacT/ALERT, bioMerieux, Macy L'Etoile, France) or culture positive urinary samples (3, 4). A modification of this system - RESIST-3

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O.K.N (Coris BioConcepts) designed for the simultaneous detection of OXA-48, KPC and NDM-like enzymes using a single disposable cartridge, has now been manufactured. Here we assessed the ability of the RESIST-3 O.K.N assay to detect OXA-48, KPC and NDM co-production in a collection of 112 non-replicate well defined CRE isolates received in our laboratories.

All isolates were carbapenem resistant *Enterobacteriaeceae* (ertapenem MIC > 1µg/ml) recovered from routine clinical samples submitted to Barts Health NHS Trust or the Antimicrobial Research Laboratory at Queen Mary University London between 1/2011 and 12/2016. Identification was performed using the Bruker MALDI-ToF mass spectrometry system (Bruker UK Ltd, Coventry, UK), with carbapenem MICs determined using MicroScan WalkAway Negative Combo 36 panels (Siemens Healthcare Diagnostics, Deerfield, IL) and confirmed with Etest gradient strips (bioMerieux). Multiplex PCRs were used to confirm the presence of class A (KPC, BKC, SME, VEB, PER, GES), B (IMP, VIM, NDM, SIM, SPM, DIM, GIM, KHM, FIM, AIM, DIM, TMB, FRI), and D (OXA-like) carbapenemase genes and all allelic variants identified were confirmed by Sanger sequencing of the entire coding regions (7, 8). The collection consisted of carbapenemase producing *K. pneumoniae* (n=57), *E. coli* (n=17), *Enterobacter spp* (n=14), *Providencia stuartii* (n=3) and single isolates of *Citrobacter koseri, Morganella morganii* and *Proteus mirabilis* (Table 1). Carbapenemases produced by the isolates were identified as KPC-2/4, NDM-1/5/7, VIM-1/4, IMP-1, OXA-48 and OXA-232. Nine isolates produced 2 carbapenemaes, either OXA-48 in combination with NDM-1 (n=6) or KPC-2 with VIM-1 (n=3).

Detection of OXA-48, KPC and NDM using the RESIST-3 O.K.N. cassettes was carried out according to the manufacturer's protocol. Bacteria were grown for 18 hrs at 37°C on Mueller-Hinton II agar plates (Oxoid) and a single colony emulsified in 5 drops of the lysis buffer. Cassettes were loaded with 3 drops of lysate and read within 5 minutes. Six carbapenem susceptible Gram-negative type strains (*K. pneumoniae* NCTC 9633, *E. coli* NCTC 12241, *E. cloacae* NCTC 13380, *E. aerogenes* NCTC 9375, *P. aeruginosa* ATCC 27852, *Acinetobacter baumannii* ATCC 19606) were used as negative controls. Eighteen additional *Enterobacteriacae* isolates [*K. pneumoniae* (8) / *oxytoca* (1), *E. coli* (4), *E. cloacae* (2) / *aerogenes* (2), *S. marcesens* (1)] with phenotypic resistance to ertapenem but without a known carbapenemase were also used as negative controls.

There was complete agreement between the carbapenemases detected by PCR and the results obtained with RESIST-3 O.K.N (Table 1). The lateral flow device was able to correctly identify and differentiate OXA, KPC and NDM production amongst all CRE species and enzyme variants tested, including those carrying >1 carbapenemase. No cross reactions were observed for strains with carbapenem resistance due to IMP-1 or VIM-1/4 (Table 1), with any of the susceptible type strains and, with the 18 CRE that had no OXA, KPC or NDM carbapenemase detectable by PCR (Figure 1).

We found the RESIST-3 O.K.N assay to be 100 % sensitive and specific in the detection and differentiation of OXA-48, KPC and NDM-like carbapenemases amongst CRE recently referred to our laboratory. Although the assay does not currently extend to the detection of VIM or IMP-like metallo-β-lactamases, the ability

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to detect 3 of the 5 most prevalent and transmissible carbapenemases found worldwide is a significant advantage. The ease of use, speed and low cost (< $15) of the cassettes make it attractive as a diagnostic tool for use in the management of CRE outbreaks. It's role either as a primary screening, confirmatory or rapid point-of care test should be assessed further, ideally prospectively in the setting of a polyclonal nosocomial CRE outbreak.

Acknowledgements

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118 **Table 1:** Detection of Carbapenemase Production using the RESIST-3 O.K.N assay

		RESIST-3 O.K.N Result		
Isolate	Carbapenemase Carried	OXA-48-like (O)	KPC-like (K)	NDM-like (N)
Klebsiella pneumoniae	OXA-48 (23)	23	0	0
	OXA-48 + NDM-1 (4)	4	0	4
	OXA-232 (6)	6	0	0
	KPC-2 (8)	0	8	0
	KPC-2 + VIM-1 (1)	0	1	0
	NDM-1 (14)	0	0	14
	VIM-4 (1)	0	0	0
Escherichia coli	OXA-48 (1)	1	0	0
	KPC-2 (3)	0	3	0
	KPC-2 + VIM-1 (1)	0	1	0
	NDM-1 (6)	0	0	6
	NDM-5 (3)	0	0	3
	NDM-7 (2)	0	0	2
	VIM-1 (1)	0	0	0
Enterobacter cloacae	OXA-48 + NDM-1 (2)	2	0	2
	NDM-1 (2)	0	0	2
	KPC-4 (2)	0	2	0
	IMP-1 (1)	0	0	0
	VIM-1 (4)	0	0	0
Enterobacter aerogenes	KPC-2 (2)	0	2	0
	NDM-1 (1)	0	0	1
Citrobacter koseri	KPC-2 + VIM-1 (1)	0	1	0
Providencia stuartii	NDM-1 (1)	0	0	1
	VIM-1 (2)	0	0	0
Morganella morganii	VIM-1 (1)	0	0	0
Proteus mirabilis	VIM-1 (1)	0	0	0

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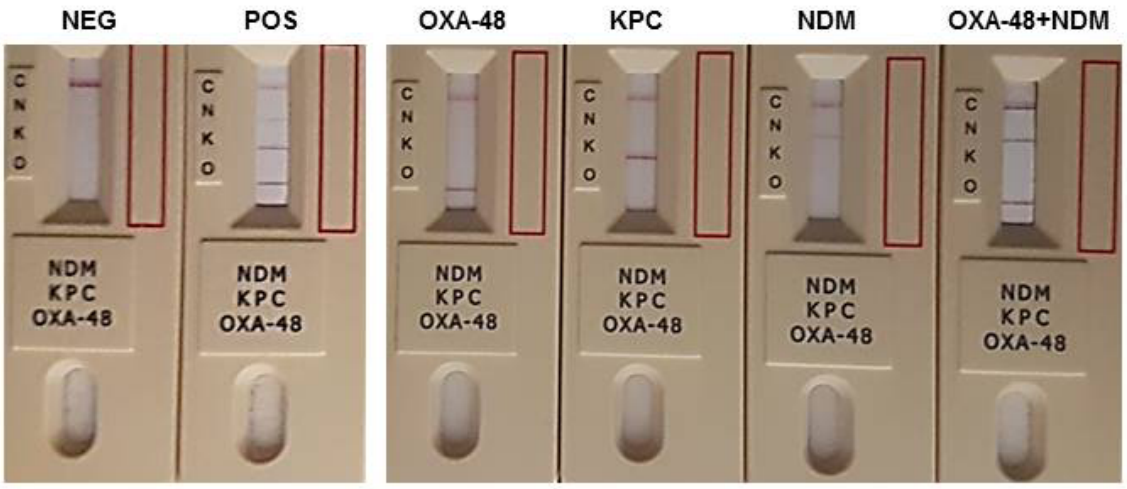
124 **Figure 1:** Detection of OXA-48, KPC and NDM-like carbapenemase in isolates producing single and dual enzymes using the
125 RESIST-3 O.K.N assay



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