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Evaluation of the K-SeT R.E.S.I.S.T. immunochromatographic assay for the rapid detection of KPC and OXA-48-like carbapenemases

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Sir,

The worldwide emergence of carbapenemases is a major health issue leaving clinicians with few treatment options. Implementation of infection prevention and control (IPC) procedures relies on early detection of carbapenemase producers and distinction from other carbapenem resistance mechanisms.

Various carbapenem hydrolysis methods allow detection of carbapenemase activity but do not differentiate between the carbapenemase families.¹ Inhibitor-based tests can help with identification of KPC and metallo- β -lactamases (MBLs), but only high-level temocillin resistance has been proposed as a marker for OXA-48-like carbapenemases.^{1–3} Recently, the use of discs impregnated with imipenem and EDTA with or without phenyl boronic acid has been proposed for detecting OXA-48-like carbapenemases.⁴ Molecular detection methods represent the gold standard, but they require expertise and laboratory facilities that may be unaffordable in some settings. Both biochemical and molecular methods have their limitations; e.g., both in-house and commercial assays may not reliably detect OXA-48-like variants.^{5,6}

Recently, Glupczynski *et al.*⁷ reported the development of an immunochromatographic lateral-flow assay that detects specific epitopes of KPC and OXA-48-like carbapenemases directly from bacterial colonies in 15 min. We evaluated the performance of the K-SeT R.E.S.I.S.T. assays (Coris BioConcept, Gembloux, Belgium) to detect KPC and OXA-48-like carbapenemases.

Bacteria ($n=101$) were submitted from UK laboratories to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit and included isolates with defined carbapenemases ($n=92$) or where carbapenem resistance was mediated by combinations of ESBL/AmpC plus porin loss ($n=9$) (Table 1). Isolates represented the diversity of carbapenemase alleles seen in the UK, based, for most isolates, on WGS results (AMRHAI, unpublished data).

MICs were determined by BSAC agar dilution using AMRHAI's standard Gram-negative antibiotic panel, which includes imipenem (tested with or without EDTA to detect likely MBLs), ertapenem and

meropenem. Carbapenemase genes were detected using in-house PCRs. WGS of isolates was performed on a HiSeq sequencer (Illumina, Little Chesterford, UK), and data were analysed using an in-house bioinformatics pipeline.⁸

Isolates were plated on nutrient or MacConkey agars, and the detection of KPC and OXA-48-like carbapenemases by the K-SeT R.E.S.I.S.T. assays was performed in parallel according to the manufacturer's instructions. Briefly, a single colony was suspended in 10 drops of lysis buffer; three drops of the suspension were then dispensed onto the test strip. The results were scored visually within 10 min by comparison with a control band.

The KPC K-SeT assay identified all isolates previously identified as KPC-positive by PCR, including isolates producing KPC-2, KPC-3, KPC-4 and KPC-9 (Table 1). The assay also detected KPC-2 when it was coproduced with NDM-1. Similarly, all 36 isolates producing OXA-48-like carbapenemases were detected by the OXA-48 K-SeT assay. The assay detected 'classic' OXA-48 and also OXA-181, OXA-204, OXA-232, OXA-244, OXA-245, OXA-436 and OXA-484 variants (Table 1). It also detected OXA-181 and OXA-232 when they were coproduced with either NDM-1 or NDM-5. The two K-SeT R.E.S.I.S.T. assays both gave positive results for three isolates in which KPC-2 and OXA-48 were coproduced. The tests performed equally well on isolates grown on MacConkey or nutrient agars.

Although both tests are marketed for the detection of carbapenemases in Enterobacteriaceae, the OXA-48 K-SeT assay detected OXA-181 in a *Pseudomonas aeruginosa* isolate and OXA-48 in a *Shewanella* sp. isolate.

In this study, the overall sensitivities were 100% for both the KPC and OXA-48 K-SeT assays. Recently, Wareham *et al.*⁹ also reported a sensitivity of 100% when using the OXA-48 K-SeT assay on Enterobacteriaceae that produced OXA-48-like enzymes. The overall specificity of both assays was 100%, with no false-positive results observed for isolates that produced only MBLs, GES, IMI or SME class A carbapenemases or that were carbapenem resistant but carbapenemase negative.

The specificity of the antibodies against the epitopes of OXA-48-like carbapenemases was further investigated by testing three OXA-23-positive *Acinetobacter baumannii* and one OXA-198-positive *P. aeruginosa* isolate. No cross-reactivity was observed between the OXA-48 K-SeT assay and these carbapenemases or with other oxacillinases detected by WGS in 45 of the isolates, namely: OXA-1 ($n=33$), OXA-9 ($n=17$), OXA-33 ($n=1$), OXA-50 ($n=2$), OXA-56 ($n=1$) and OXA-129 ($n=2$).

In the UK, the major carbapenemase families are KPC, OXA-48-like, NDM, VIM and IMP (AMRHAI, unpublished data). The K-SeT R.E.S.I.S.T. assays do not detect all of these families, and each currently detects a single enzyme family, necessitating multiple tests per isolate. At present, they are unsuitable for general testing of colonies for carbapenemase production in the UK. In this context, the clinical value of negative tests would be increased if similar assays for detecting MBLs become available, but the cost and performance of multiple immunochromatographic tests would then need to be considered alongside those of molecular assays. Nevertheless, the ability to detect carbapenemase producers cheaply (£5/test) and rapidly in a local hospital setting would significantly decrease the time taken to implement IPC measures.¹⁰ The speed and ease of use of the current K-SeT R.E.S.I.S.T. assays make them attractive tools for outbreak

Table 1. Isolates tested with defined carbapenem resistance mechanism(s)

Species	Carbapenemase status, including allele where known (number of isolates)				
	KPC (n=30)	OXA-48- like (n=36)	KPC or OXA-48-like + other carbapenemase (n=11)	other carbapenemase (n=15)	no carbapenemase (n=9)
<i>Klebsiella pneumoniae</i>	19 KPC (2) ^a KPC-2 (14) KPC-3 (2) KPC-9 (1)	17 OXA-48 (5) OXA-181 (5) OXA-232 (2) OXA-245 (1) OXA-436 (1) OXA-484 (3)	7 KPC-2 + NDM-1 (1) KPC-2 + OXA-48 (2) NDM-1 + OXA-181 (1) NDM-1 + OXA-232 (3)	3 NDM-1 (1) VIM-4 (1) GES-5 (1)	3
<i>Klebsiella oxytoca</i>		1 OXA-48		1 GES-5	
<i>Escherichia coli</i>	4 KPC-2 (3) KPC-3 (1)	7 OXA-48 (2) OXA-181 (3) OXA-232 (1) OXA-244 (1)	3 KPC-2 + OXA-48 (1) NDM-5 + OXA-181 (2)	2 NDM-7 (1) VIM-1 (1)	2
<i>Enterobacter cloacae</i> complex	2 KPC-4 (2)	5 OXA-48 (3) OXA-204 (1) OXA-244 (1)	1 NDM-1 + OXA-181	3 IMI-4 (1) IMP-1 (1) GES-5 (1)	3
<i>Enterobacter aerogenes</i>	1 KPC-2	1 OXA-48			1
<i>Citrobacter freundii</i>	3 KPC-2 (1) KPC-3 (1) KPC-4 (1)	2 OXA-48			
<i>Serratia marcescens</i>	1 KPC ^a	1 OXA-48		1 SME-2	
<i>Acinetobacter baumannii</i>				3 OXA-23	
<i>Pseudomonas aeruginosa</i>		1 OXA-181		2 SPM-1 OXA-198	
<i>Shewanella</i> sp.		1 OXA-48			

^aThe *bla*_{KPC} alleles for three tested isolates were not defined by sequencing.

investigations associated with either KPC or OXA-48-like enzymes, or in countries where these carbapenemases dominate.

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