Evaluation of a new multiplex immunochromatographic assay OKN K-SeT for the rapid detection of OXA-48, KPC and NDM carbapenemases from cultured bacteria

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Introduction

•Accurate and timely detection of CPE is essential for patient management and for rapid implementation of appropriate infection control measures.[1]

•Recently, lateral flow assays based on monoclonal antibodies generated by immunization in mice have been developed for easy and rapid detection of OXA-48-like and KPC carbapenemases.

•This technology was first shown by our group[2] and subsequently confirmed by several other investigators in different countries[3-6] as a powerful means (100% sensitivity and 100% specificity) to identify OXA-48 and KPC producers within 15 minutes directly from bacterial colonies but as single confirmatory test.

• The present study aimed to evaluate the performance of a new triplex assay (OKN *K*-SeT) which incorporates specific antibodies against OXA-48, NDM and KPC and aims to detect these three carbapenemases in a single test from culture colonies.

Methods

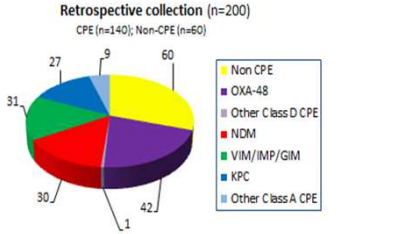
•**Retrospective analysis :** 200 collection isolates of Enterobacteriaceae with characterized β-lactamases collected at the French associated NRC (period 2008-2014) including 60 non CPE (mostly ESBLs or AmpC+/- impermeability) and 140 CPE producers (see Figure 1a and Table 1). All isolates were verified before testing for the presence of carbapenemase(s) by an updated version of the Carba NP test; PCR/sequencing of β-lactam genes was used as reference gold standard.

•**Prospective analysis (2 months in 2016):** 183 non-duplicate clinical isolate with decreased susceptibility or resistance to carbapenems (meropenem and/or ertapenem) referred to the Belgian NRC as putative CPE (Fig. 1b, Table 2).

- All isolates were verified for the presence of carbapenemase by BYG Carba test (BYG Carba v2.0).
- Two in house multiplex PCR assays (ISO15189 certified) targeting the major carbapenemases were performed on all isolates. Amplicons were sequenced using external sequencing services for allele identification (Macrogen Inc., South Korea).
- The OKN *K*-SeT assay (Coris BioConcept, Gembloux, Belgium) was used according to the recommendations of the manufacturer. All strains tested were grown on trypticase soy agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) for 16-24 h at 37°C.

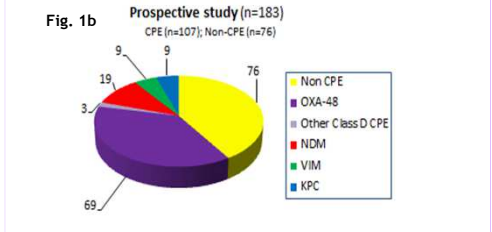
Results I

Fig. 1a



OXA-48: OXA-48 (n=22); OXA-162 (n=1); OXA-181 (n=10); OXA-204 (n=5); OXA-232 (n=2); OXA-244 (n=2)
KPC: KPC-2 (n=25); KPC-3 (n=5)
NDM: NDM-1 (n=24); NDM-4 (n=2); NDM-5 (n=1); NDM-6 (n=1), NDM-7 (n=1); NDM-9 (n=1)

Fig. 1b



OXA-48: OXA-48 (n=68); OXA-181 (n=1)
KPC: KPC-3 (n=9) (including one isolate harboring KPC-3+VIM-1)
NDM: NDM-1 (n=18); NDM-5 (n=1) (including one isolate harboring NDM-1+OXA-181

Table 1. Description of the retrospective collection panel

200 collection strains	27 strains tested positive for KPC with the Trio K-SeT	27 strains carrying a KPC carbapenemase	Escherichia coli, Klebsiella ozaenae, Enterobacter cloacae, Citrobacter freundii, Serratia marcescens (positive for KPC-2 or KPC-3).
	30 strains tested positive for NDM with the Trio K-SeT	30 strains carrying a NDM carbapenemase	E. coli, K. pneumoniae, E. cloacae, Morganella morganii, Providencia spp., Salmonella spp. (positive for NDM-1, NDM-4,-5,-6,-7 or -9)
	43 strains tested positive for OXA-48 with the Trio K-SeT	43 strains carrying a OXA-48 carbapenemase	E. coli, E. cloacae, Enterobacter aerogenes, C. freundii, Citrobacter koseri, K. pneumoniae (positive for OXA-48, -162, -181, -204, 232, -244)
	100 strains tested negative with theTrio K-SeT	40 strains carrying a carbapenemase other than KPC, NDM or OXA-48 like	IMI-1/-2, Sme-1/-2, NmcA, GES-5, FRI-1, GIM-1, IMP-1,IMP-8, IMP-10, IMP-11 , VIM-1, VIM-2,-VIM-4, VIM-19
		60 strains not carrying a carbapenemase	

Prospective study part:

- 100% sensitivity and specificity for the detection of OXA-48 like, KPC & NDM
- 100% positive predictive value and 100 % negative predictive value for the detection of these three carbapenemase families
- Rapid detection of 97 carbapenemases out of 109 (89%) in 107 CPE isolates
- Additional confirmatory tests would have been required only for the detection of 12 CPE isolates (Table 2)
- The multiplex OKN assay allows rapid, direct identification from culture colonies of 90% of the CPE in countries such as Belgium and France

Table 2. Results of the OKN *K*-SeT assay in the prospective study phase

β-lactamase content	Species	N° of isolates	RESIST-3 O.K.N assay[2]	BYG Carba*
Carbapenemases producers				
OXA-48	*K. pneumoniae*	42	OXA-48	P
	E. cloacae	12	OXA-48	P
	E. cloacae	1	OXA-48	N
	E. coli	7	OXA-48	P
	K. oxytoca	3	OXA-48	P
	C. freundii	2	OXA-48	P
	Kluyvera georgiana	1	OXA-48	P
OXA-48 + NDM-1	*E. coli*	1	OXA-48+ NDM	P
NDM-1	*K. pneumoniae*	11	NDM	P
	E. cloacae	3	NDM	P
	E. coli	2	NDM	P
	E. aerogenes	1	NDM	P
NDM-5	*E. coli*	1	NDM	P
KPC-3	*K. pneumoniae*	7	KPC	P
	E. coli	1	KPC	P
KPC-3 + VIM-1	*C. freundii*	1	KPC	P
VIM-1	*E. cloacae*	4	N	P
	E. coli	1	N	P
	C. freundii	1	N	N
	S. marcescens	1	N	P
VIM-4	*K. oxytoca*	1	N	P
OXA-23	*P. mirabilis*	2	N	P
OXA-58	*P. mirabilis*	1	N	P
Non carbapenemase producers				
ESBL + impermeability	*K. pneumoniae*	19	N	N
	E. cloacae	11	N	N
	E. coli	8	N	N
	C. freundii	1	N	N
	K. oxytoca	1	N	N
Cephalosporinase + impermeability	*E. cloacae*	13	N	N
	E. aerogenes	3	N	N
	C. freundii	1	N	N
	H. alvei	1	N	N
	S. marcescens	1	N	N
DHA-1 + impermeability	*K. pneumoniae*	1	N	N
Cephalosporinase + ESBL + impermeability	*E. cloacae*	4	N	N
	E. aerogenes	1	N	N
	K. pneumoniae	1	N	N
	E. coli	1	N	N
	C. freundii	1	N	N
Other	*E. coli*	3	N	N
	K. pneumoniae	1	N	N
	K. oxytoca	3	N	N
	E. cloacae	1	N	N

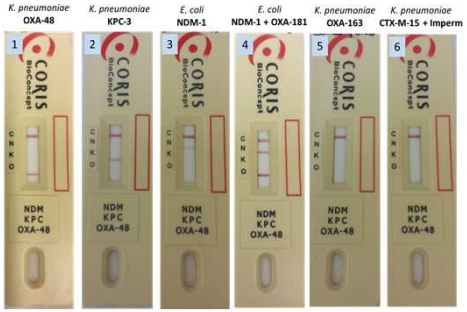
*P refers to a positive result and N a negative result. For the OKN K-SeT Assay, the detected carbapenamases are indicated by their family name (OXA-48, NDM, KPC)

Results II

Retrospective study part:

- 100% sensitivity and specificity for the detection of OXA-48 like, KPC and NDM carbapenemases
- Correct detection of all targeted carbapenemases independently of the species or of their association with other types of β-lactamases
- Accurate detection of strains harbouring a combination of two carbapenemases (seven isolates positive for OXA-181 + NDM-1)
- Absence of detection of OXA-48 like variants without carbapenem hydrolytic activity (e.g. OXA-163, OXA-405)
- No false positive results with the non targeted carbapenemases

Figure 2. Multiplex lateral flow assay for the detection of NDM, KPC and OXA-48 like carbapenemase in a single test



For negative result a single line appears at the position of the control line (C). Examples of positive (1., 2., 3., 4.) and of negative results (5., 6.) are shown here

Conclusions

- Globally, the performance of the OKN *K*-SeT assay reached **100% sensitivity** and **100% specificity** for the detection of OXA-48 like, KPC and NDM carbapenemases
- The OKN *K*-SeT assay did correctly detect isolates that contained a **combination of different carbapenemases.**
- In the prospective study, the OKN assay allowed the detection of 97 of the 109 carbapenemases (89%), and yielded a **positive and a negative predictive value of 100%** for the three targeted carbapenemases.
- The assay is **efficient and very easy to implement in the workflow** of a clinical microbiology laboratory for the confirmation of OXA-48, NDM and KPC carbapenemases.
- The OKN *K*-SeT assay test represents a **powerful diagnostic tool** as it enables the **rapid detection** (<15 minutes) **from culture colonies** of three among the most important carbapenemase families without the need of more costly and less frequently available molecular assays.

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