



Rapid Identification of OXA-48-like, KPC, NDM, and VIM Carbapenemase-Producing Enterobacteriaceae From Culture: Evaluation of the RESIST-4 O.K.N.V. Multiplex Lateral Flow Assay

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There is an urgent need for accurate and rapid diagnostic assays capable of identifying carbapenemase-producing Enterobacteriaceae (CPE). We assessed the performance of the RESIST-4 O.K.N.V. (OKNV) assay (Coris BioConcept, Gembloux, Belgium) for the identification of oxacillinase (OXA)-48-like-, *Klebsiella pneumoniae* carbapenemase (KPC)-, New Delhi metallo- β -lactamase (NDM)-, and Verona integron-encoded metallo- β -lactamase (VIM)-producing Enterobacteriaceae grown on sheep blood agar (SBA) and the CHROMagar KPC medium. Sixty-five carbapenem-resistant Enterobacteriaceae (CRE) isolates with characterized carbapenemase content were used to evaluate the OKNV assay. The assay correctly identified all 30 isolates that produced one of the four targeted carbapenemase families. Additionally, it correctly identified 15 isolates that co-produced KPC and NDM, VIM and NDM or OXA-48-like and NDM, but failed to identify an NDM-1 and OXA-232 co-producing *Klebsiella pneumoniae* isolate. All 16 non-carbapenemase-producing CRE and four CPE isolates exhibited negative results, and no cross-reaction was observed. Overall, the sensitivity and specificity of the assay were 97.8% and 100%, respectively. The OKNV assay is an accurate and rapid assay for identifying OXA-48-like, KPC, NDM, and VIM carbapenemases produced by Enterobacteriaceae isolates cultured on both SBA and the CHROMagar KPC media in the clinical microbiology laboratory.

Key Words: Carbapenemase-producing Enterobacteriaceae, OXA-48-like, KPC, NDM, VIM, Evaluation, Performance, RESIST-4 O.K.N.V. assay

Received: August 27, 2019
Revision received: October 14, 2019
Accepted: November 12, 2019

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The global dissemination of carbapenemase-producing Enterobacteriaceae (CPE) is a major public health concern. Accurate and rapid detection of CPE is essential for patient management and for implementation of appropriate infection control measures [1, 2]. Globally, *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo- β -lactamase (NDM), Verona integron-encoded metallo- β -lactamase (VIM), imipenem-resistant *Pseudomonas* (IMP), and oxacillinase (OXA)-48-like are the most

prevalent carbapenemases in Enterobacteriaceae. Thus, it is essential not only to identify carbapenemase production but also to characterize the enzyme. As the identification of CPE in clinical microbiological laboratories based solely on the resistance phenotype is difficult [3], a number of rapid colorimetric assays have been developed for the identification of carbapenemase activity in cultured bacteria. However, a major limitation of this approach is the absence of reliable assays for the identifica-

tion of OXA-48-like producers [4, 5].

Recently, lateral flow immunochromatographic assays based on monoclonal antibodies generated by immunization of mice have been developed for rapid and easy identification of OXA-48-like, KPC, and NDM carbapenemases. This technology exhibited excellent accuracy (100% sensitivity and 100% specificity) in the identification OXA-48-like, KPC, and NDM producers directly from bacterial colonies within 15 minutes [6, 7]. We evaluated the new RESIST-4 O.K.N.V. (OKNV) multiplex lateral flow assay developed by Coris BioConcept (Gembloux, Gembloux, Belgium), which identifies specific antibodies against OXA-48, KPC, NDM, and VIM carbapenemases directly from different culture media such as sheep blood agar (SBA) and a chromogenic medium. Different types of chromogenic media, such as CHROMagar KPC, chromID CARBA SMART (BioMérieux, Marcy-l'Étoile, France), and Brilliance CRE (ThermoFisher Scientific, Illkirch, France), are often used in rectal surveillance culture for identifying carbapenem-resistant Enterobacteriaceae (CRE) and/or CPE [8, 9].

Globally, the OKNV assay shows 97–100% sensitivity and 100% specificity, with unequivocal results [10–12]. Therefore, additional confirmatory assays were not required. Unlike other studies, the number of carbapenemase co-producing isolates included was relatively high, and this is the first study to include isolates grown on a chromogenic medium.

In total, 65 CRE clinical isolates were included in the study (49 CPE and 16 non-CP-CRE). The CRE isolates were obtained from six Hallym University Medical Centers (two hospitals in Seoul, two hospitals in Gyeonggi, and one hospital in Gangwon) in Korea between 2012 and 2018, and all isolates were sent to one institution (Kangnam Sacred Heart Hospital, Seoul) and frozen before study. The study protocol was approved by the Institutional Review Board of each institution, which waived the need for informed consent. All isolates were tested for carbapenemase by PCR and DNA sequencing according to previously described methods [13, 14]. The CPE isolates included nine KPC, eight NDM-1, seven OXA-48-like, five VIM, two IMP, and two Guiana extended-spectrum β -lactamase (GES)-5, carbapenemase variants. In addition, 16 Enterobacteriaceae isolates co-producing carbapenemases (OXA-48-like and NDM [N=9], KPC and NDM [N=5], and VIM and NDM [N=2]) were included. Sixteen non-CP-CRE isolates were also included (Table 1).

The OKNV assay is a multiplex lateral flow immunochromatographic assay for the detection of OXA-48-like, KPC, NDM, and VIM carbapenemases in two lateral flow cassettes (one for OXA-48-like and KPC and the other one for NDM and VIM). The as-

sayed isolates were grown on SBA (SPL Life Sciences, Gyeonggi-do, Korea) and CHROMagar KPC medium (CHROMagar, Paris, France) for 16–24 hours at 37°C, and the assays were performed according to the manufacturer's instructions. An example of the identification results for OXA-48, KPC, NDM, and VIM using the OKNV assay is shown in Fig. 1. The sensitivity and specificity of the assay were calculated for all isolates. Sensitivity was calculated from the number of true-positive isolates, whereas specificity was calculated from the number of true-negative isolates.

All isolates that grew on both SBA and CHROMagar KPC media showed the same results in the OKNV assay. The OKNV assay could identify all isolates that produced one of the four targeted carbapenemase families, including eight OXA-48-like (OXA-48 [N=1], OXA-181 [N=3], and OXA-232 [N=4]), nine KPCs (KPC-2 [N=7] and KPC-4 [N=2]), eight NDMs (all NDM-1), and five VIMs (VIM-1 [N=2], and VIM-2 [N=3]). Additionally, it correctly identified 15 isolates that co-produced KPC and NDM (N=5), VIM and NDM (N=2), or OXA-48-like and NDM (N=8), but failed to identify one *K. pneumoniae* isolate co-producing NDM-1 and OXA-232 (negative for NDM and positive for OXA-48). One NDM-1-producing *Serratia marcescens* isolate, one NDM-1 and OXA-232 co-producing *Enterobacter cloacae* isolate, and one NDM-1 and OXA-232 co-producing *K. pneumoniae* isolate showed a weak positive NDM band, and two NDM-1 and VIM-2 co-producing *E. cloacae* isolates showed a weak positive VIM band. All 16 non-CP-CRE and four CPE (two GES-5, one IMP-1, and one IMP-4) were negative for carbapenemase production, and no cross-reaction was observed (Table 1). Based on the overall results, the sensitivity and specificity of the OKNV assay for identifying isolates grown on both SBA and CHROMagar KPC were 97.8% (95% CI, 88.2%–99.9%) and 100% (95% CI, 83.2%–100%), respectively.

The OKNV assay performed well, when using colonies grown on both SBA and CHROMagar KPC. It is possible that the negative result obtained for the NDM-1 and OXA-232 co-producing *K. pneumoniae* isolate was due to plasmid loss during serial passage [15]. However, subsequent PCR and sequencing analysis of colonies grown on the same culture medium demonstrated that the isolate was NDM-1- and OXA-232-positive. Thus, we confirmed that the NDM-negative result obtained using the OKNV assay was a real false-negative result.

In Korea, KPC, NDM, and OXA-48-like genes are expressed by 71%, 14%, and 9% of CPE, respectively, while VIM and IMP genes are very rare (<2% of CPE) [16]. As the OKNV assay does not identify certain carbapenemases (mostly those belonging to the IMP family), the use of other diagnostic methods, such as

Table 1. Results of the OKNV assay for carbapenem-resistant Enterobacteriaceae isolates

Organism	Carbapenemase content	N of isolates	Results of the OKNV assay from SBA/CHROMagar KPC			
			OXA-48	KPC	NDM	VIM
CPE (N=49)						
<i>Klebsiella pneumoniae</i>	GES-5*	2	N/N	N/N	N/N	N/N
<i>Enterobacter cloacae</i>	KPC-2	2	N/N	P/P	N/N	N/N
<i>Escherichia coli</i>	KPC-2	2	N/N	P/P	N/N	N/N
<i>Klebsiella aerogenes</i>	KPC-2	1	N/N	P/P	N/N	N/N
<i>Klebsiella oxytoca</i>	KPC-2	1	N/N	P/P	N/N	N/N
<i>K. pneumoniae</i>	KPC-2	1	N/N	P/P	N/N	N/N
<i>K. pneumoniae</i>	KPC-4	2	N/N	P/P	N/N	N/N
<i>Citrobacter freundii</i>	NDM-1	2	N/N	N/N	P/P	N/N
<i>E. cloacae</i>	NDM-1	1	N/N	N/N	P/P	N/N
<i>E. coli</i>	NDM-1	1	N/N	N/N	P/P	N/N
<i>K. aerogenes</i>	NDM-1	1	N/N	N/N	P/P	N/N
<i>K. pneumoniae</i>	NDM-1	1	N/N	N/N	P/P	N/N
<i>Providencia rettgeri</i>	NDM-1	1	N/N	N/N	P/P	N/N
<i>Serratia marcescens</i>	NDM-1	1	N/N	N/N	wP/wP	N/N
<i>Enterobacter asburiae</i>	VIM-2	1	N/N	N/N	N/N	P/P
<i>E. cloacae</i>	VIM-2	1	N/N	N/N	N/N	P/P
<i>Enterobacter kobei</i>	VIM-2	1	N/N	N/N	N/N	P/P
<i>K. pneumoniae</i>	VIM-1	2	N/N	N/N	N/N	P/P
<i>E. asburiae</i>	IMP-1*	1	N/N	N/N	N/N	N/N
<i>K. aerogenes</i>	IMP-4*	1	N/N	N/N	N/N	N/N
<i>K. pneumoniae</i>	OXA-48	1	P/P	N/N	N/N	N/N
<i>E. coli</i>	OXA-181	1	P/P	N/N	N/N	N/N
<i>K. pneumoniae</i>	OXA-181	1	P/P	N/N	N/N	N/N
<i>C. freundii</i>	OXA-232	1	P/P	N/N	N/N	N/N
<i>E. cloacae</i>	OXA-232	1	P/P	N/N	N/N	N/N
<i>E. coli</i>	OXA-232	1	P/P	N/N	N/N	N/N
<i>K. pneumoniae</i>	OXA-232	1	P/P	N/N	N/N	N/N
<i>C. freundii</i>	KPC-2+NDM-1	1	N/N	P/P	P/P	N/N
<i>Raoultella ornithinolytica</i>	KPC-2+NDM-1	1	N/N	P/P	P/P	N/N
<i>Raoultella planticola</i>	KPC-2+NDM-1	1	N/N	P/P	P/P	N/N
<i>R. planticola</i>	KPC-4+NDM-1	1	N/N	P/P	P/P	N/N
<i>K. pneumoniae</i>	KPC-4+NDM-5	1	N/N	P/P	P/P	N/N
<i>E. cloacae</i>	NDM-1+VIM-2	2	N/N	N/N	P/P	wP/wP
<i>K. pneumoniae</i>	NDM-1+OXA-181	1	P/P	N/N	P/P	N/N
<i>E. cloacae</i>	NDM-1+OXA-232	1	P/P	N/N	P/P	N/N
<i>E. cloacae</i>	NDM-1+OXA-232	1	P/P	N/N	wP/wP	N/N
<i>E. coli</i>	NDM-1+OXA-232	1	P/P	N/N	P/P	N/N
<i>K. pneumoniae</i>	NDM-1+OXA-232	2	P/P	N/N	P/P	N/N
<i>K. pneumoniae</i>	NDM-1+OXA-232	1	P/P	N/N	wP/wP	N/N
<i>K. pneumoniae</i>	NDM-1+OXA-232	1	P/P	N/N	N/N†	N/N
<i>K. pneumoniae</i>	NDM-5+OXA-181	1	P/P	N/N	P/P	N/N

(Continued to the next page)

Table 1. Continued

Organism	Carbapenemase content	N of isolates	Results of the OKNV assay from SBA/CHROMagar KPC			
			OXA-48	KPC	NDM	VIM
Non-CP-CRE (N=16)						
<i>C. freundii</i>	None	2	N/N	N/N	N/N	N/N
<i>E. cloacae</i>	None	3	N/N	N/N	N/N	N/N
<i>E. coli</i>	None	3	N/N	N/N	N/N	N/N
<i>K. aerogenes</i>	None	2	N/N	N/N	N/N	N/N
<i>K. pneumoniae</i>	None	3	N/N	N/N	N/N	N/N
<i>S. marcescens</i>	None	3	N/N	N/N	N/N	N/N

*The OKNV assay can only detect OXA-48, KPC, NDM, and VIM type carbapenemases; †Boldface represents false-negative results. Abbreviations: OKN, RESIST-4 O.K.N.V; CP-CRE, carbapenemase-producing carbapenem-resistant Enterobacteriaceae; SBA, sheep blood agar; P, positive; wP, weakly positive; N, negative; CPE, carbapenemase-producing Enterobacteriaceae; OXA, oxacillinase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo- β -lactamase; VIM, Verona integron-encoded metallo- β -lactamase.

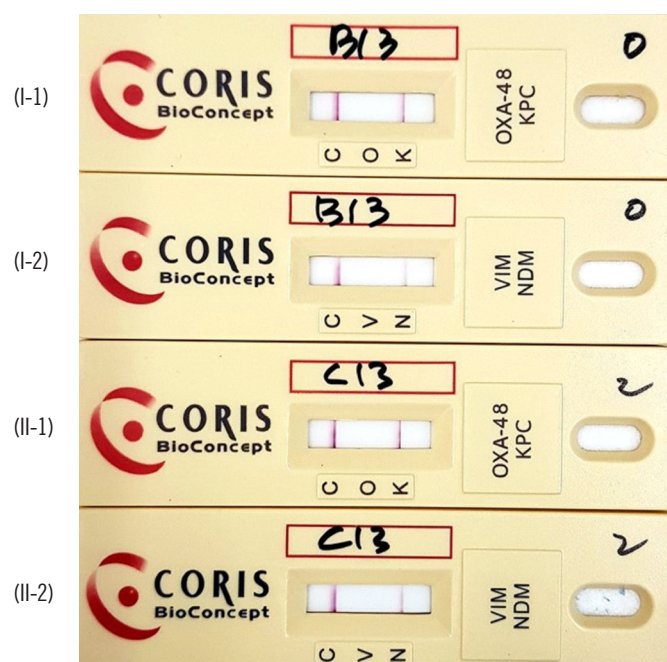


Fig. 1. Example of results for a KPC-2 and NDM-1 co-producing *Citrobacter freundii* isolate grown on (I) sheep blood agar and (II) CHROMagar KPC (1, cassette for (OXA)-48-like (O) and KPC (K); 2, cassette for VIM (V) and NDM (N)). In the case of a negative result, only a single line appears at the position of the control band (C). Abbreviations: OXA, oxacillinase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo- β -lactamase; VIM, Verona integron-encoded metallo- β -lactamase.

PCR, is still required. Furthermore, another lateral flow assay, including IMP-type monoclonal antibodies, is currently under development [17]. Although a few NDM-positive or VIM-positive isolates showed weak positive results, the OKNV assay was easy to use and provided easy-to-read results after only 15 min of in-

cubation.

A limitation of this study is that a relatively small number of isolates were assayed; however, the sensitivity and specificity of the OKNV assay were similar to those reported in previous studies [10-12].

Overall, the speed and ease-of-use of the OKNV assay represent significant technical advances; the assay also shows excellent performance for detecting the OXA-48-like, KPC, NDM, and VIM-type carbapenemases from Enterobacteriaceae isolates cultured on both SBA and CHROMagar KPC medium.

ACKNOWLEDGEMENTS

We thank BioPark Diagnostics for support with the assay reagents.

AUTHOR CONTRIBUTIONS

WS wrote the manuscript and supervised this study. MJP, SJ, DHS, JSK, HSK, HSK, and NL collected the isolates and analyzed the data, JSH performed the assays, and SHJ analyzed the data.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this paper were reported.

RESEARCH FUNDING

None declared.

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