European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:331–335
https://doi.org/10.1007/s10096-018-3432-2

ORIGINAL ARTICLE

Rapid detection of OXA-48-like, KPC, NDM, and VIM carbapenemases in Enterobacterales by a new multiplex immunochromatographic test

Christopher Greissl[1] · Ahmad Saleh[1,2] · Axel Hamprecht[1,2]

Received: 21 September 2018 / Accepted: 11 November 2018 / Published online: 17 November 2018

Abstract

The rapid detection of carbapenemase-producing Gram-negative bacteria is indispensable to optimize treatment and avoid the further spread of these organisms. While phenotypic tests are time-consuming and PCR is expensive and not available in many routine laboratories, immunochromatographic tests (ICT) can provide rapid results at moderate cost. The aim of this study was to determine the performance of the new ICT RESIST-4 O.K.N.V. K-SeT (Coris BioConcept, Gembloux, Belgium) which can detect the four most prevalent carbapenemases: OXA-48-like, KPC, NDM, and VIM. Additionally, we analyzed the impact of different culture conditions on the sensitivity. The new ICT was challenged with 169 carbapenem-resistant isolates. Of these, 125 were carbapenemase producers: 43 OXA-48-like, 15 KPC, 29 NDM, and 43 VIM. The ICT correctly detected 129 of the 130 carbapenemases resulting in a sensitivity of 99.2% and specificity of 100% when tested from Mueller-Hinton agar (MHA). The sensitivity of the assay increased to 100% when performed from zinc-supplemented MHA and sheep blood agar (SBA) or when the inoculum was harvested from the inhibition zone of an ertapenem disk. All carbapenemase-negative carbapenem-resistant bacteria tested negative and no cross-reaction was observed. The new ICT is an excellent test for rapid diagnostic of carbapenemase-producing Gram-negatives in the routine laboratory. It is easy to handle and provides rapid results with a high sensitivity. For best results, we recommend to obtain the inoculum from a medium with sufficient zinc or from the inhibition zone of an ertapenem disk.

Keywords Carbapenemase · Immunochromatographic test · Enterobacterales · Enterobacteriaceae · New Delhi metallo-beta-lactamase · Verona integron-encoded metallo-beta-lactamase · Mueller-Hinton agar

Introduction

The increase of carbapenemase-producing Enterobacterales (CPE) is of great concern as the treatment options in infections are severely compromised, resulting in higher mortality, longer hospital stay, and higher costs. Rapid detection of CPE is of great importance, both for the initiation/optimization of antibiotic treatment and for infection control purposes. The most prevalent carbapenemases in Enterobacterales worldwide are the OXA-48-like, KPC, and metallo-β-lactamases VIM, NDM, and IMP [1].

The phenotypic detection of CPE can be challenging due to a high variation of minimal inhibitory concentrations (MICs) for the antibiotics meropenem, imipenem, and ertapenem. Some CPE isolates show low MICs for these carbapenems and can therefore be easily missed [2]. In addition, many confirmatory tests are either time-consuming (e.g., phenotypic tests) or expensive and not available in many institutions, especially on weekends (e.g., PCR). The newly introduced immunochromatographic tests (ICT) detect carbapenemase-specific epitopes using monoclonal antibodies and are a rapid alternative, taking only around 15 min. Previous studies of various lateral flow tests detecting different spectra of carbapenemases showed high sensitivity and specificity for OXA-48-like, KPC, and NDM carbapenemases [3–5].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-018-3432-2) contains supplementary material, which is available to authorized users.

✉ Axel Hamprecht
axel.hamprecht@uk-koeln.de

[1] Institute for Medical Microbiology, Immunology and Hygiene, University Hospital of Cologne, Goldenfelsstrasse 19-21, 50935 Cologne, Germany

[2] DZIF (German Centre for Infection Research), partner site Bonn-Cologne, Cologne, Germany

The RESIST-4 O.K.N.V. K-SeT (Coris BioConcept, Gembloux, Belgium) is a new immunochromatographic assay which also detects VIM carbapenemases in addition to OXA-48-like, KPC, and NDM. These four are the most prevalent carbapenemases in Germany and most other countries [6].

In this study, we evaluated the new ICT with carbapenemase-producing and carbapenemase-negative isolates using different culture conditions. The aim was to determine the sensitivity and specificity for OXA-48-like, KPC, NDM, and especially the new VIM monoclonal antibody and to analyze the impact of different culture media and antibiotic disks on the sensitivity.

Materials and methods

Clinical isolates

A total number of 169 isolates were included in this study; of these, 125 produced 130 carbapenemases. Forty-four carbapenemase-negative isolates with different β-lactamases served as controls.

The isolates were all clinical isolates from Germany, mostly from the University of Cologne or from other studies [5, 7, 8]. Isolates of 11 different species were analyzed, with most strains belonging to the species *Klebsiella* spp. ($n = 60$), *E. coli* ($n = 49$), and *Enterobacter* spp. ($n = 39$) (Table 1).

The study included 17 different carbapenemases, 6 VIM variants, 3 NDM variants, 6 variants of the OXA-48-like carbapenemase, and 2 KPC variants (Table 1). All carbapenemases were molecularly characterized as previously described [7–11]. Briefly, isolates were analyzed for the presence of the carbapenemase genes $bla_{\text{OXA-48-like}}$, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{GIM}, and bla_{KPC} by PCR and subsequent DNA Sanger sequencing. MICs of ertapenem, meropenem and imipenem were determined using MIC test strips (Liofilchem, Roseto degli Abruzzi, Italy). For phenotypic identification of metallo-β-lactamases, KPC, or AmpC, a combination disk test was used with meropenem and one of the following β-lactamase inhibitors: EDTA, cloxacillin, or boronic acid (Liofilchem). Results were interpreted according to the manufacturer's recommendations.

RESIST-4 O.K.N.V. K-SeT ICT

Bacteria were harvested from MHA after overnight incubation (37 °C) using a 1-μL inoculating loop, which has previously been shown to be the optimal amount for an earlier version of the ICT, RESIST-3 O.K.N. K-SeT [5]. The RESIST-4 O.K.N.V. K-SeT (Coris BioConcept, Gembloux, Belgium) uses the same monoclonal antibodies for OXA-48-like, KPC, and NDM as the O.K.N., but additionally detects VIM carbapenemases.

It has been shown that the zinc content of culture media and antibiotic pressure increased the sensitivity of the RESIST-3 O.K.N. assay for NDM. Zinc is bound by metallo-β-lactamases at the enzyme's active site and leads to an increase of its activity. We therefore also investigated the impact of different culture conditions for the detection of the two metallo-β-lactamases NDM and VIM [5]. For these two carbapenemases, four different conditions were used: (a) from colonies grown on sheep blood agar (SBA, Oxoid, Wesel, Germany), (b) from MHA without antibiotics (Oxoid), (c) from MHA but harvested at the inhibition zone of a 10-μg ertapenem disk, and (d) from MHA supplemented with 50 mg/L of zinc sulfate ($ZnSO_4$).

The RESIST-4 O.K.N.V. ICT was performed according to the manufacturer's instructions. Briefly, colonies were harvested from the plates, resuspended in LY-A buffer solution and then transferred to the lateral flow test.

One test contains two lateral flow cassettes—one to identify KPC and OXA-48, the other for NDM and VIM. A positive result is displayed by a red band next to the letters of each carbapenemase (Fig. 1). Both the qualitative result and the time to positivity were documented. In case of doubtful result, e.g., faint bands or long time to positivity, the test was repeated for verification.

Statistical analysis

The Wilcoxon matched pairs signed rank test was employed to compare nonparametric data and the two-sided Fisher's exact test was used for analysis of frequency data. P values of < 0.05 were considered statistically significant.

Results

When tested from MHA, 129/130 (99.2%) carbapenemases from 125 clinical isolates were correctly identified. A single NDM-1 from a *Proteus mirabilis* isolate was not detected by the ICT. Of the 125 carbapenemase producers, five isolates expressed two different carbapenemases and all of these were correctly detected. All 44 carbapenemase-negative isolates gave a negative result and no cross-reaction was observed (Table 2).

The overall sensitivity when performed from MHA was 99.2%, and the specificity was 100%. For OXA-48-like and KPC, sensitivity was 100%. In addition to MHA, isolates producing the metallo-β-lactamases NDM and VIM were tested from zinc-supplemented MHA, from SBA and from MHA with an ertapenem disk.

For NDM, the sensitivity was 96.6% if tested from MHA (28/29). When harvested from SBA, zinc-supplemented MHA or adjacent to the inhibition zone of an ertapenem disk, sensitivity increased to 100% ($p > 0.05$, Table 2). Time to

Table 1 Isolates included in this study; other Enterobacterales: *Klebsiella oxytoca* ($n = 1$), *Escherichia hermannii* ($n = 1$), *Raoultella ornithinolytica* ($n = 1$), *Serratia marcescens* ($n = 1$)

Carbapenemase production	*K. pneumoniae*	*E. coli*	*Enterobacter* spp.	*Citrobacter* spp.	*P. mirabilis*	Other Enterobacterales	All species
Carbapenemase-positive	49	29	28	14	1	4	125
OXA-48-like	19	17	3	1			40
OXA-48	12	8	3				23
OXA-181		5					5
OXA-232	3	2					5
OXA-162	2	1		1			4
OXA-244	1	1					2
OXA-204	1						1
KPC	12			1			13
KPC-2	11			1			12
KPC-3	1						1
NDM	12	6	5		1	2	26
NDM-1	12	4	5		1	2	24
NDM-5		1					1
NDM-7		1					1
VIM	3	4	20	12		2	41
VIM-1	3	4	12	9		1	29
VIM-2			1	1			2
VIM-4			2	2		1	5
VIM-26			1				1
VIM-27			1				1
VIM-39			3				3
VIM-1 + KPC-2	2						2
NDM-1 + OXA-232	1						1
NDM-1 + OXA-48		1					1
NDM-5 + OXA-181		1					1
Carbapenemase-negative	11	20	11	1	1		44
Total	60	49	39	15	2	4	169

positivity was used to quantify the effect of different culture conditions on the detection. For NDM-producing isolates, the mean time to positivity was 64 ± 59 s when taken from MHA, 61 ± 57 s from the ertapenem inhibition zone, and 69 ± 81 s from SBA (n.s.). Colonies harvested from zinc-containing MHA yielded positive ICT results after an average time of 40 ± 35 s ($p = 0.07$, online resource).

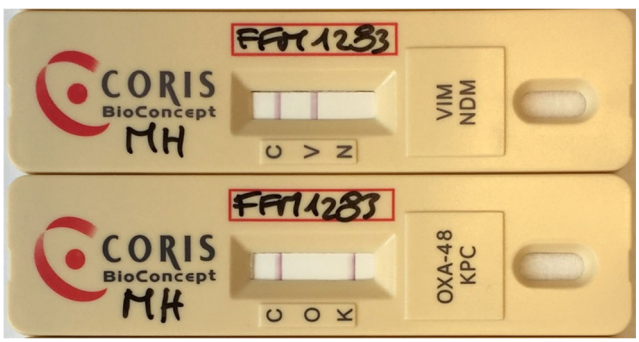

Fig. 1 Example of a positive test for KPC and VIM carbapenemase; left, cassette for OXA-48-like and KPC; right, cassette for NDM and VIM. C, control band; O, OXA-48-like; K, KPC; V, VIM; N, NDM carbapenemases

For the new monoclonal VIM antibody which was integrated in the ICT, 100% sensitivity was recorded for all testing conditions. The mean time to positivity was 99 ± 96 s when taken from MHA, 82 ± 68 s from the ertapenem inhibition zone, and 93 ± 131 s from SBA. As for NDM, time to positivity decreased for VIM-producing isolates when harvested from zinc-supplemented MHA (68 ± 57 s), $p = 0.07$ (Supplement Fig. 2B). In the subgroup of MBL-producing *E. coli* ($n = 13$), the time to positivity was shorter when harvested from zinc-containing MHA (51 ± 20 s) when compared to colonies harvested from MHA (124 ± 90 s, $p = 0.0086$). One VIM-1-positive isolate of the species *Enterobacter cloacae* repeatedly showed very weak bands with a long time to positivity from MHA (15 min). Overall, the bands for NDM

Table 2 Sensitivity of the ICT depends on testing conditions; *MHA*, Mueller-Hinton agar; *SBA*, sheep blood agar; *n.d.*, not determined; *CI*, confidence interval

		MHA	MHA/ ertapenem	SBA	MHA+zinc
NDM	Sensitivity	96.6% (28/29)	100% (29/29)	100% (29/29)	100% (29/29)
	95% CI	82.8–99.4%	88.3–100%	88.3–100%	88.3–100%
VIM	Sensitivity	100% (43/43)	100% (43/43)	100% (43/43)	100% (43/43)
	95% CI	91.8–100%	91.8–100%	91.8–100%	91.8–100%
OXA-48-like	Sensitivity	100% (43/43)	n.d.	n.d.	n.d.
	95% CI	91.8–100%	n.d.	n.d.	n.d.
KPC	Sensitivity	100% (15/15)	n.d.	n.d.	n.d.
	95% CI	79.6–100%	n.d.	n.d.	n.d.

and VIM were the most difficult to read as they were faint in some isolates compared to OXA-48-like or KPC bands. This was more pronounced when isolates were tested from MHA or SBA and readability could be improved when tested from zinc-supplemented agar or when harvested from the inhibition zone of an ertapenem disk.

Discussion

The RESIST-4 O.K.N.V. K-SeT multiplex ICT can detect the four most common carbapenemases. In a collection of 169 molecularly characterized clinical isolates, the ICT performed well with a sensitivity of 99.2% when tested from MHA. As in previous versions of the assay, OXA-48-like and KPC showed excellent results with a sensitivity and specificity of 100% [3–5, 12]. The new monoclonal antibody for VIM worked equally well, with a sensitivity of 100% for all tested VIM variants. For NDM, the sensitivity was 96.6% from MHA; one NDM-1-producing *P. mirabilis* isolate gave a false negative result when tested from MHA. As shown for the previous version of the ICT with the same isolate [5], the RESIST-4 O.K.N.V. K-SeT tested positive when colonies picked from SBA, from zinc-supplemented MHA, or from the ertapenem inhibition zone were used (sensitivity 100% for NDM from these media). A similar improvement with stronger bands and a shorter time to positivity was observed for some VIM isolates, when tested from MHA/ertapenem or from zinc-supplemented MHA, even though the difference in time to positivity did not reach statistical significance ($p = 0.07$). A long time to positivity of 15 min was observed for a VIM-1-producing isolate of *E. cloacae*. This could be related to a low expression of VIM-1, documented by relatively low MICs for both imipenem (1 mg/L) and meropenem (4 mg/L).

A negative result for the metallo-β-lactamases NDM should be interpreted with caution if the isolate is tested from MHA; retesting using zinc-rich agars or from the inhibition zone of ertapenem is recommended to reliably exclude false negative results. In practice, retesting and further delay can be avoided since most laboratories using an automated susceptibility testing system perform a purity control of the bacterial inoculum, usually by plating on SBA or a chromogenic agar. Some laboratories even perform disk diffusion on MHA as purity control. If the purity control is plated on an agar with sufficient zinc content, the ICT can be directly performed from this agar at the same time as the result of the antibiogram is available. If disk diffusion is the primary susceptibility testing method (or used for purity control), the ICT can be performed from the inhibition zone of ertapenem.

The high sensitivity of the ICT for the detection of the different variants of OXA-48-like carbapenemases in Enterobacterales is important regarding the German epidemiology. According to the German National Reference Centre for Nosocomial Pathogens, the four carbapenemases covered by this ICT represent 98% of all carbapenemases. OXA-48-like carbapenemases are the most frequent (43.1%), followed by VIM (20.5%), NDM (17.5%), and KPC (16.9%) [6]. These carbapenemases are also the most prevalent in Enterobacterales worldwide. In contrast to previous tests, the new ICT includes VIM metallo-β-lactamases, which is the second most common carbapenemase in Enterobacterales and the most prevalent carbapenemase in German *Pseudomonas aeruginosa* isolates [6].

Several studies on other lateral flow multiplexed assays show comparably good results for the sensitivity and specificity for OXA-48-like, NDM, and KPC from solid media [3–5]. In addition, it has recently been shown that OXA-48-like, NDM, and KPC can be detected directly from positive blood culture by ICT with 100% sensitivity and specificity [12]. One study assessed the performance of another ICT (CARBA 5, NG Biotech) for VIM detection and a sensitivity of 100% was reported [13]. However, the effect of different test conditions on the performance was not investigated in this study. Recently, a study on the RESIST-4 O.K.N.V. K-SeT has been published [14]. However, in this study, a smaller selection of clinical isolates ($n = 69$) was investigated, with results on only 3 different VIM types (VIM-1, VIM-2, and VIM-4). Additionally, the impact of different culture conditions has not been studied. A recent study analyzed the impact of carbapenems on the sensitivity of the previous test version

RESIST-3 O.K.N. K-SeT [5]. An increase in sensitivity of the ICT was demonstrated when NDM-producing isolates were harvested at the inhibition zone of ertapenem, meropenem, and imipenem disks. Overall, ertapenem showed the best results. Similarly, in the present study on the new ICT, an improvement was noted when the inoculum was taken close to the ertapenem disk. Even though this was not analyzed in the present study, it is likely that other carbapenem disks also improve readability and time to positivity because of a higher expression of carbapenemases around the antibiotic disk.

While we studied a high number of Enterobacterales with different carbapenemases, a limitation of this study is that only carbapenemase-producing Enterobacterales were included. Therefore, results for *Pseudomonas* spp. or other nonfermenters should be investigated in future studies.

Overall, the new ICT shows a very good performance for the detection of the OXA-48-like, KPC, NDM, and VIM carbapenemases in Enterobacterales, which are by far the most common in Germany. With 17 different carbapenemases and 125 carbapenemase-producing clinical isolates included, a large variety of the four carbapenemase types targeted by this ICT was investigated in this study.

Conclusion

The advantage of rapid results and comparably low costs (~ 15€/test) makes this ICT a good choice for the confirmation of carbapenemases in medical laboratories, especially for smaller laboratories which cannot perform PCR. With the inclusion of VIM, about 98% of the carbapenemases present in Enterobacterales in Germany can be detected using this assay. The assay requires less equipment and hands-on time and is cheaper than most commercial PCR assays, e.g., the GenXpert Carba-R. However, for best performance, isolates should be harvested from agars rich in zinc and/or next to a carbapenem disk.

Acknowledgements The RESIST-4 O.K.N.V. K-SeT was supplied free of charge for evaluation by Coris BioConcept. We thank Stephan Göttig from the University Hospital of Frankfurt for providing further carbapenemase-producing isolates.

Funding information This study was supported by grants from the Faculty of Medicine, University Hospital of Cologne. AH was supported by the German Centre for Infection Research (DZIF).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study, formal consent is not required.

References

1. Nordmann P, Naas T, Poirel L (2011) Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 17:1791–1798. https://doi.org/10.3201/eid1710.110655
2. Giske CG, Martinez-Martinez L, Cantón R, Stefani S, Skov R, Glupczynski Y et al (2017) EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, pp 4–12. http://www.eucast.org/resistance_mechanisms. Accessed 15 September 2018
3. Glupczynski Y, Jousset A, Evrard S, Bonnin RA, Huang T-D, Dortet L et al (2017) Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48-like, KPC and NDM carbapenemases. J Antimicrob Chemother 72:1955–1960. https://doi.org/10.1093/jac/dkx089
4. Wareham DW, Abdul Momin MHF (2017) Rapid detection of carbapenemases in Enterobacteriaceae: evaluation of the Resist-3 O.K.N. (OXA-48, KPC, NDM) lateral flow multiplexed assay. J Clin Microbiol 55:1223–1225. https://doi.org/10.1128/JCM.02471-16
5. Saleh A, Göttig S, Hamprecht AG (2018) Multiplex immunochromatographic detection of OXA-48, KPC, and NDM carbapenemases: impact of inoculum, antibiotics, and agar. J Clin Microbiol 56:e00050–e00018
6. Pfennigwerth N (2017) Report of the German National Reference Centre (NRZ) for nosocomial pathogens. Epidemiol Bull 29:229–33. https://doi.org/10.17886/EpiBull-2018-034
7. Gruber TM, Gottig S, Mark L, Christ S, Kempf VAJ, Wichelhaus TA et al (2014) Pathogenicity of pan-drug-resistant Serratia marcescens harbouring blaNDM-1. J Antimicrob Chemother. https://doi.org/10.1093/jac/dku482. 2 [cited 2018 18]; https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dku482
8. Koroska F, Göttig S, Kaase M, Steinmann J, Gatermann S, Sommer J et al (2017) Comparison of phenotypic tests and an immunochromatographic assay and development of a new algorithm for detection of OXA-48-like carbapenemases. J Clin Microbiol 55:877–883
9. Gottig S, Hamprecht AG, Christ S, Kempf VAJ, Wichelhaus TA (2013) Detection of NDM-7 in Germany, a new variant of the New Delhi metallo- -lactamase with increased carbapenemase activity. J Antimicrob Chemother 68:1737–1740. https://doi.org/10.1093/jac/dkt088
10. Hamprecht A, Rohde AM, Behnke M, Feihl S, Gastmeier P, Gebhardt F et al (2016) Colonization with third-generation cephalosporin-resistant Enterobacteriaceae on hospital admission: prevalence and risk factors. J Antimicrob Chemother 71:2957–2963. https://doi.org/10.1093/jac/dkw216
11. Jazmati N, Hein R, Hamprecht A (2016) Use of an enrichment broth improves detection of extended-spectrum-Beta-lactamase-producing Enterobacteriaceae in clinical stool samples. J Clin Microbiol 54:467–470. https://doi.org/10.1128/JCM.02926-15
12. Hamprecht A, Vehreschild JJ, Seifert H, Saleh A (2018) Rapid detection of NDM, KPC and OXA-48 carbapenemases directly from positive blood cultures using a new multiplex immunochromatographic assay. PLoS One 13. https://doi.org/10.1371/journal.pone.0204157
13. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G et al (2018) A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 73:909–915. https://doi.org/10.1093/jac/dkx521
14. Kolenda C, Benoit R, Carricajo A, Bonnet R, Dauwalder O, Laurent F (2018) Evaluation of the new multiplex immunochromatographic O.K.N.V K- *S* et assay for the rapid detection of OXA-48-like, KPC, NDM and VIM carbapenemases. J Clin Microbiol. https://doi.org/10.1128/JCM.01247-18. 5 [cited 2018 21]; http://jcm.asm.org/lookup/doi/10.1128/JCM.01247-18