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Evaluation of the RESIST-4 *K*-SeT assay, a multiplex immunochromatographic assay for the rapid detection of OXA-48-like, KPC, VIM and NDM carbapenemases

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Objectives: Accurate and fast identification of carbapenemase producers is essential for optimal patient management. Here, a new lateral flow immunochromatographic RESIST-4 *K*-SeT assay was assessed for the detection of carbapenemases in Enterobacteriaceae and non-fermenters.

Methods: The RESIST-4 *K*-SeT assay targets OXA-48-like, KPC, VIM and NDM, but not IMP carbapenemases. The assay was first evaluated using a collection of isolates with well-characterized resistance mechanisms to β-lactams ($n = 134$) and against an international external quality assessment carbapenemase panel ($n = 8$). The assay was then challenged prospectively using 345 consecutive, non-duplicate isolates including 279 Enterobacteriaceae and 66 non-fermenters (mostly *Pseudomonas* spp.) that were sent to the Belgian National Reference Centre for identification of the mechanisms related to carbapenem resistance.

Results: Globally, for the collection of retrospective and prospective clinical isolates ($n = 479$), the assay showed a sensitivity ranging from 99% for the detection of VIM to 100% for the detection of OXA-48-like, KPC and NDM carbapenemase-producing strains. The specificity was 100% for each carbapenemase and a perfect match in results was observed for the external quality assessment for the carbapenemases targeted by the assay.

Conclusions: The RESIST-4 *K*-SeT assay is a valuable alternative for detection and identification of carbapenemases from culture isolates compared with the more costly molecular assays, which may also further require skilled staff and dedicated facilities.

Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) have emerged and spread widely worldwide over the last decade and their correct and timely detection is essential for infection control as well as for optimal therapeutic management.[1,2]

Several rapid colorimetric assays or MS-based tests for the detection of carbapenemase activity in cultured bacteria are now commercially available.[3,4] These tests allow the detection of the presence of carbapenem-hydrolytic activity in 2 h, but they do not allow precise identification of the carbapenemases, which may be of therapeutic importance since last-resort antimicrobial drugs such as ceftazidime/avibactam are active against certain organisms producing class A and D enzymes, but not against MBL (class B)-producing organisms.[5]

Identification of the carbapenemase usually relies on molecular methods, which necessitate dedicated facilities and skilled personnel to implement in-house testing or costly instruments and/or commercial kits.[6]

Recently, a commercial multiplex lateral flow immunoassay (OKN; Coris BioConcept, Gembloux, Belgium) has been shown to have excellent performance for the detection of OXA-48-like, KPC and NDM carbapenemases from bacterial colonies.[7] One major drawback of this test was the absence of detection of the widespread VIM carbapenemase family.

Here, we present the evaluation of the RESIST-4 *K*-SeT assay, which fills this gap and allows the detection of the four major carbapenemases in one single test.

Materials and methods

Retrospective analysis external quality assessment (EQA)

A retrospective evaluation was performed using a collection of 134 Gram-negative isolates [Enterobacteriaceae ($n = 105$) and non-fermenters ($n = 29$)]. Since KPC and OXA-48 were previously extensively evaluated,[7,8] this retrospective collection included a larger proportion of VIM or NDM producers ($n = 39$ of each, alone or in association with other carbapenemases) as well as 57 control isolates [non-NDM, non-VIM carbapenemase

Table 1. Global performance of the RESIST-4 *K*-SeT assay and positive and negative predictive values of the RESIST-4 *K*-SeT assay

Target	*n*	sensitivity (95% CI)	specificity (95% CI)
Global performance of the RESIST-4 *K*-SeT assay for the collection of retrospective and prospective clinical isolates (*n* = 479)			
OXA-48-like	112	100 (95.9–100)	100 (98.8–100)
VIM	104	99 (94.0–99.9)	100 (98.7–100)
NDM	61	100 (92.6–100)	100 (98.9–100)
KPC	31	100 (86.3–100)	100 (99.0–100)
Other carbapenemases/non-carbapenemases	184		
Target	*n*	positive predictive value (95% CI)	negative predictive value (95% CI)
Positive and negative predictive values of the RESIST-4 *K*-SeT assay for the collection of prospective clinical isolates (*n* = 345)			
OXA-48-like	90	100 (94.9–100)	100 (98.2–100)
VIM	65	100 (93.4–100)	100 (98.3–100)
NDM	22	100 (81.5–100)	100 (98.5–100)
KPC	19	100 (79.1–100)	100 (98.5–100)
Other carbapenemases/non-carbapenemases	151		

producers ($n = 36$) and non-carbapenemase producers ($n = 21$)]; Table S1, available as Supplementary data at *JAC* Online).

All isolates from this panel had been characterized previously for the presence of β-lactamase genes by PCR/sequencing.[7] The RESIST-4 *K*-SeT assay was also challenged using eight samples from the ESBL and carbapenemase 2017 QCMD panel (QCMD, Glasgow, UK).

Prospective analysis

From 1 August to 29 December 2017, all non-duplicate clinical isolates with decreased *in vitro* susceptibility to at least one carbapenem referred to the National Reference Centre were included. Carbapenemase resistance genes were sought for all tested strains by an in-house multiplex PCR targeting $bla_{\text{OXA-48-like}}$, bla_{NDM}, bla_{KPC}, bla_{VIM} and bla_{IMP},[9] and the amplicons were sequenced for allele identification using external Sanger sequencing services (Macrogen, Seoul, South Korea).

RESIST-4 K-SeT assay

The RESIST-4 *K*-SeT assay consists of two independent *K*-SeTs (one for the detection of OXA-48-like and KPC and a second for the detection of VIM and NDM). Both cassettes are provided in a single package and are to be used in parallel on the same bacterial lysis preparation. For this study, the strains were grown on Trypticase soy agar supplemented with 5% sheep blood (bioMérieux, Marcy-l'Étoile, France) for 16–24 h at 37°C and tests were performed according to the manufacturer's recommendations.

Statistical analysis

The sensitivity and specificity of the assay were calculated for all tested strains (retrospective collection and prospectively obtained isolates), whereas the positive and negative predictive values were calculated only for the prospectively obtained isolates. Upper and lower limits of the 95% CIs were calculated by means of the VassarStats software (http://www.vassarstats.net/).

Results

Retrospective evaluation of the RESIST-4 K-SeT assay

This retrospective validation confirmed the excellent results previously observed for the detection of OXA-48-like, KPC and NDM (100% sensitivity and 100% specificity). The assay was able to correctly detect all OXA-48-like producing isolates (including OXA-48, -162, -181, -204, -232 and -244) as well as those producing KPC (KPC-2, -3 and -4) and NDM (NDM-1, -4, -5 and -7) (Table S1). Isolates that co-produced KPC and OXA-48 or NDM and OXA-48 were also correctly detected by the assay. This validation also confirmed that OXA-405 and OXA-163, two ESBL variants of OXA-48 with no or only marginal carbapenemase activity, were not detected by the RESIST-4 *K*-SeT assay.[10] These antigens can be detected by a combination of specific antibodies not used in the RESIST-4 *K*-SeT assay.[11]

Regarding VIM producers, the RESIST-4 *K*-SeT assay was able to detect all VIM-producing isolates including two isolates that co-produced VIM-1 with KPC-2 or KPC-3. In three other isolates co-producing VIM-4 with OXA-48, OXA-23 or OXA-58, VIM was also well detected, along with the OXA class D carbapenem-hydrolysing enzyme. Nevertheless, the assay failed to detect one VIM variant in one NDM-1 and VIM-5-harbouring *Pseudomonas aeruginosa* isolate in which only NDM was detected. It should be highlighted that among the three isolates that expressed VIM-5 alone [*Escherichia coli* ($n = 2$) and *Pseudomonas putida* ($n = 1$)], the VIM signals were weaker than for other VIM variants, suggesting that this variant could be less well detected by the selected antibodies (not shown).

On the other hand, 10 CPE strains not belonging to any of the four targeted carbapenemases, as well as all 21 non-carbapenemase producers, yielded negative results with the test (Table S1). In the retrospective study part, the RESIST-4 *K*-SeT assay yielded 100% sensitivity for OXA-48-like, KPC and NDM, 97.4% sensitivity for VIM and 100% specificity for all carbapenemase types.

The RESIST-4 *K*-SeT assay also yielded 100% concordant results with PCR against eight strains of the ESBL and carbapenemase QCMD EQA 2017 panel, which included OXA-48-, OXA-48 + KPC-2-, OXA-244-, KPC-2-, VIM-1-, IMP-1-, NDM-1- and non-carbapenemase-producing strains.

Prospective evaluation of the RESIST-4 K-SeT assay

One or more carbapenemase genes were identified by PCR and sequencing in 198 (57.4%) of 345 consecutive Gram-negative

clinical isolates with decreased carbapenem susceptibility sent to the reference laboratory. By group of organisms, carbapenemase producers were found in 167 of 279 (59.9%) Enterobacteriaceae and in 30 of 65 (46.2%) *Pseudomonas* spp. isolates (Table S2).

The RESIST-4 *K*-SeT assay allowed detection of all isolates that produced one of the targeted carbapenemases, including 88 OXA-48-like, 65 VIM, 20 NDM and 19 KPC producers. Four isolates that produced a carbapenemase not targeted by the assay [IMI-2 ($n = 1$), GES-5 ($n = 2$) and IMP-13 ($n = 1$)] yielded a negative result, as expected, as did the 147 non-carbapenemase-producing isolates (Table S2). The positive and negative predictive values for each carbapenemase targeted by the assay was 100% (Table 1).

Global performance of the RESIST-4 K-SeT assay

Based on the overall results, the RESIST-4 *K*-SeT assay displayed a sensitivity of 99% for the detection of VIM and of 100% for the detection of OXA-48-like, KPC and NDM (Table 1). The specificity was 100% for each carbapenemase. The lower value of the 95% CI for the sensitivity ranged from 86.3% (for KPC) to 95.9% (for OXA-48).

Discussion

The RESIST-4 *K*-SeT assay is an immunochromatographic assay designed to detect the four carbapenemase families (OXA-48 like, KPC, VIM and NDM) that are most commonly found worldwide. Globally, the performance of the RESIST-4 *K*-SeT assay was excellent with only one VIM-5 variant being undetected, in one *P. aeruginosa* harbouring NDM-1 and VIM-5. In the prospective study, the assay allowed direct rapid detection of 196 (97.5%) of 201 carbapenemases and the use of additional molecular tests would have been required only for the detection of five carbapenemase-producing isolates (i.e. two GES-5-producing *P. aeruginosa*, one IMP-13-producing *P. aeruginosa*, one IMI-2-producing *Enterobacter cloacae* and one VIM-4 + OXA-23-producing *Acinetobacter pittii*).

Most notably in the prospective study, carbapenemases were detected by the RESIST-4 *K*-SeT assay in 166/167 (99.4%) CPE isolates, missing only one IMI-2-producing *E. cloacae* isolate.

The assay also proved very efficient for the detection of carbapenemases in *Pseudomonas* spp. and *Acinetobacter* spp. without any modification of the procedure, including lysis buffer or incubation time. These results are in line with those of a recent study performed in France by Kolenda *et al*.[12] Of foremost importance is that this test allows direct confirmation and identification of the involved carbapenemases without any requirement for dedicated facilities or for skilled trained personnel. Also, it can be used for the confirmation of the presence of a carbapenemase from different selective chromogenic culture media or from antimicrobial susceptibility testing Mueller–Hinton agar plates.[3,4]

The current format of the assay, nevertheless, presents some inconveniences. Firstly, the packaging is presented for each single test as two separate pouches, each of them containing a dual target cassette. Secondly, it does not detect IMP, though the latter is only rarely reported in Western countries.[13–15]

These two drawbacks could be overcome by another test recently launched on the market by NG Biotech, which allows the detection of KPC, OXA-48-like, VIM, NDM and IMP on a single device.[16,17]

Finally, both tests are dedicated for the detection and identification of carbapenemases from bacterial cultures and not from clinical samples even though studies have reported their possible application for direct detection of CPE from positive blood cultures or from urine samples.[18,19]

In conclusion, the RESIST-4 *K*-SeT assay (recommended purchase price of €15/test) provides a powerful tool to very easily detect carbapenemase producers in a local setting.

The speed and ease of integration of this multiplexed assay in the laboratory routine represents a significant technical advance and makes it particularly attractive as it would drastically decrease the need for molecular methods to confirm the results or referral of the isolate centrally to a specialized laboratory, which may also increase the turnaround time. Further developments are now awaited to improve the sensitivity of the technology in order to allow direct detection from clinical samples.

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Transparency declarations

Y. G., T.-D. H. and P. B. are co-inventors of a patent licenced to Coris BioConcept for the immunochromatographic detection of carbapenemase. S. E.: none to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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