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Comparison of two multiplex immunochromatographic assays for the rapid detection of major carbapenemases in Enterobacterales

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Objectives: Two commercially available lateral flow immunochromatographic assays (ICAs) for detection of the major carbapenemases were prospectively assessed for the detection of carbapenemases in Enterobacterales: RESIST-4 O.K.N.V. (Coris BioConcept) and NG-Test CARBA 5 (NG Biotech).

Methods: These two assays were performed prospectively on consecutive Enterobacterales suspected of producing a carbapenemase that were referred to the Belgian National Reference Center for Monitoring Antimicrobial Resistance in Gram-Negative Bacteria between March and June 2018. The intensity of the band corresponding to a carbapenemase for each test was compared using ImageJ software.

Results: Of the 161 isolates tested, a carbapenemase was detected in 91 (60 OXA-48-like, 15 VIM, 9 KPC, 5 NDM, 1 IMP and 1 IMP + OXA-48); in the remaining 70, no carbapenemases were detected. For both tests, the results were 100% concordant with the results of the PCR-sequencing reference method. Two IMP producers were only detected by NG-Test CARBA 5 as IMP is not targeted by RESIST-4 O.K.N.V. The mean intensity of the OXA-48, VIM and NDM bands displayed by NG-Test CARBA 5 was 3 to 3.7 times higher than for RESIST-4 O.K.N.V., while the KPC band was on average 1.7 times more intense with RESIST-4 O.K.N.V.

Conclusions: RESIST-4 O.K.N.V. and NG-Test CARBA 5 are two efficient assays for identification of the major carbapenemases. NG-Test CARBA 5 offers the advantage of detecting IMP, which remains rare in Western countries.

Introduction

The worldwide spread of carbapenemase-producing Enterobacterales (CPE) represents a major public health concern. WHO recommendations for fighting CPE include the development of new accurate and rapid diagnostic tools.[1]

Several rapid diagnostic tools for CPE are currently available on the market, including colorimetric assays (RAPIDEC® CARBA NP, bioMérieux; β CARBA™, Bio-Rad; Neo-Rapid CARB Kit and Rapid CARB Blue Kit, Rosco Diagnostica), tests based on MS (MBT STAR-Carba Kit, Bruker Daltonik)[2,3] or nucleic acid amplification assays.[4] Recently, two multiplex immunochromatographic assays (ICAs) were also commercialized, providing direct results from clinical isolates or positive blood culture broth in a maximum of 15 min.[5–10] RESIST-4 O.K.N.V. (Coris BioConcept, Gembloux, Belgium) is able to identify OXA-48-like, KPC, VIM and NDM carbapenemases,[11] while NG-Test CARBA 5 (NG Biotech, Guipry, France) provides the additional identification of IMP carbapenemase.[12] Here, we compared the performance of these two ICAs on 161 successive and non-duplicate CPE collected at the Belgian National Reference Center for Monitoring Antimicrobial Resistance in Gram-Negative Bacteria.

Materials and methods

Prospective analysis

From March to June 2018, all non-duplicate clinical isolates with decreased susceptibility to at least one carbapenem referred to the Belgian National Reference Center for identification of a carbapenemase were included. Carbapenemase resistance genes were sought in all tested strains by two in-house ISO 15189-certified multiplexed PCRs targeting $bla_{\text{OXA-48-like}}$, bla_{NDM}, bla_{KPC}, bla_{VIM} and bla_{IMP},[13] and the amplicons were sequenced for allele identification using external Sanger sequencing services (Macrogen, Seoul, Korea).

RESIST-4 O.K.N.V. and NG-Test CARBA 5

Both tests are lateral flow assays. RESIST-4 O.K.N.V. was developed by Coris BioConcept for the detection of OXA-48-like and KPC carbapenemases on

one cassette and NDM and VIM carbapenemases on a second cassette. Both cassettes are provided within the same package. NG-Test CARBA 5 is a product of NG Biotech and detects KPC, OXA-48-like, IMP, VIM and NDM carbapenemases on a single cassette. The strains were grown on trypticase soy agar supplemented with 5% sheep blood (Becton-Dickinson, Erembodegem, Belgium) for 16–24 h at 37°C and then used according to the ICA manufacturer's recommendations. In brief, either a single colony or a few colonies were suspended in a few drops of a lysis buffer specific to each manufacturer. The specific number of drops defined by each manufacturer were then loaded on the sample pad of the cassette and the results were read after 15 min by two independent technicians. To compare the two assays, the intensity of each band corresponding to a carbapenemase was measured using the open-source ImageJ software (https://imagej.nih.gov/ij/) developed by the NIH (Bethesda, MD, USA). The software transformed each detected band into a peak representing the pixel count and the AUC expressed the intensity of the band.

Results

Prospective evaluation of the RESIST-4 O.K.N.V. assay and the NG-Test CARBA 5 assay

One hundred and sixty-one consecutive Enterobacterales isolates with a decreased susceptibility to at least one carbapenem were received from 49 different Belgian laboratories between March and June 2018. They included *Klebsiella pneumoniae* ($n=73$), *Enterobacter cloacae* ($n=27$), *Escherichia coli* ($n=21$), *Citrobacter freundii* ($n=15$), *Klebsiella oxytoca* ($n=11$), *Klebsiella aerogenes* ($n=5$), *Enterobacter asburiae* ($n=3$) and various other species ($n=5$). PCR and sequencing identified at least one carbapenemase in 91 isolates (56.5%) (Table S1, available as Supplementary data at *JAC* Online).

Both assays were able to detect all of the following carbapenemases: OXA-48-like [OXA-48 ($n=59$), OXA-244 ($n=1$)]; KPC-3 ($n=9$); NDM [NDM-1 ($n=4$), NDM-5 ($n=1$)] and VIM [VIM-1 ($n=12$), VIM-4 ($n=3$)]. As IMP was not targeted by RESIST-4 O.K.N.V., only NG-Test CARBA 5 was able to detect the IMP-8 expressed by two *K. pneumoniae* isolates, including one co-producing an additional OXA-48 (which was correctly detected by both tests).

The 70 non-carbapenemase-producing isolates did not yield any signal and thus were negative by RESIST-4 O.K.N.V. and NG-Test CARBA 5.

Hence both tests presented a sensitivity and specificity of 100% for detection of the claimed targets. However, the positive and negative predictive values for carbapenemase detection were 100% and 100%, respectively, for NG-Test CARBA 5 and 100% and 97.22%, respectively, for RESIST-4 O.K.N.V., which did not detect IMP-8 in two isolates.

Intensity of the signal

Representative examples of the results are shown in Figure S1. The intensity of each band, corresponding to the detection of a carbapenemase, was evaluated by calculating the AUC (converted number of coloured pixels) acquired using the ImageJ software (results not shown). For OXA-48, VIM and NDM, the signal appeared 2.2 to 3.2 times more intense with the NG-Test CARBA 5 test than with RESIST-4 O.K.N.V. (Table 1). This overall strengthened intensity of the signal provided improved comfort for visual reading. For the nine KPC producers, the signal was on average 1.7 times more intense with the RESIST-4 O.K.N.V. test (Table 1). The intensity of the signal could not be correlated to the specific type of carbapenemase.

Table 1. Mean ± SD of the signal (arbitrary units) observed with RESIST-4 O.K.N.V. and NG-Test CARBA 5 as measured by ImageJ software and the ratio of the mean signal for NG-Test CARBA 5 compared with RESIST-4 O.K.N.V.

	RESIST-4 O.K.N.V.	NG-Test CARBA 5	Signal ratio C5/R4
OXA-48	309±105	1002±274	3.2
KPC	574±211	344±175	0.6
VIM	348±204	862±180	2.5
NDM	126±118	381±36	2.2

C5, NG-Test CARBA 5; R4, RESIST-4 O.K.N.V.

Discussion

RESIST-4 O.K.N.V. and NG-Test CARBA 5 are two ICAs detecting the major carbapenemase families (OXA-48 like, KPC, VIM and NDM for both tests and additionally IMP for NG-Test CARBA 5). The RESIST-4 O.K.N.V. assay is designed on two separate cassettes, one for the detection of OXA-48 and KPC and the second for the detection of VIM and NDM. For NG-Test CARBA 5, the five carbapenemases are detectable within a single cassette. The visual reading and result interpretation was clear and straightforward for both tests. Overall, the performance of both tests achieved 100% sensitivity and specificity for detection of the carbapenemases targeted by each of the assays. Our study confirmed the recently published excellent performance of both tests for the detection of OXA-48, KPC, VIM and NDM (sensitivity >97.6%).[14]

Most notably, all 92 carbapenemases from the 91 CPE isolates were detected by NG-Test CARBA 5 while RESIST-4 O.K.N.V. missed one IMP-8 carbapenemase produced by a *K. pneumoniae* isolate and did not detect IMP-8 in an IMP-8- and OXA-48-co-producing *K. pneumoniae* isolate.

In our view, the signal observed for OXA-48-like, VIM and NDM carbapenemases was more intense with NG-Test CARBA 5 than with RESIST-4 O.K.N.V., while the KPC signal was slightly more intense with RESIST-4 O.K.N.V. The objective measurements in our study supported the general subjective impression that VIM and NDM signals occasionally appeared weak for RESIST-4 O.K.N.V. In the current study, both tests allowed easy and clear interpretation of the presence or absence of a carbapenemase and could replace the use of PCR being performed on colonies in most routine laboratories. These tests are very convenient and should be used for the confirmation of the presence of a carbapenemase in association with selective chromogenic screening media, antimicrobial susceptibility testing and/or carbapenem (or chromogenic derivative) hydrolysis-based tests.[14]

A quotation for each test was provided by the respective Belgian distributors to our laboratory as the National Reference

Center. For one kit of 20 tests, the NG-Test CARBA 5 was priced at €569.31 (€28/test) and RESIST-4 O.K.N.V. at €361.18 (€18/test) with all taxes, administrative costs and transport included. Major price differences may nevertheless occur according to the type of laboratory, country, specific local offers, etc. In our reference centre, these ICAs are used first on each clinical strain presenting reduced susceptibility to carbapenems or growing on CPE selective medium. If the result is negative by ICA, the absence of a carbapenemase is confirmed by a rapid chromogenic test such as β CARBA™ (around €5/test) or, in the case of doubtful results, with the MBT STAR-Carba kit, which is more time-consuming and expensive (around €15/test). These imipenem (or the undisclosed chromogenic substrate molecule of β CARBA™) hydrolysis-based tests allow the exclusion of a carbapenemase undetected by the ICA. The need for molecular testing methods for identification and confirmation of carbapenemases from bacterial culture is thus very limited and would be required only for a minority of isolates. Hence molecular testing by rapid PCR assay such as Xpert® Carba-R from Cepheid (around €50–55/test) appears too expensive for carbapenemase confirmation on isolates; the test was designed and should be used for carriage screening of rectal swabs in the case of nosocomial outbreak management. The rapid detection of a carbapenemase can shorten the time to implement infection control measures and may influence the choice of appropriate treatment regimens such as excluding the use of some antibiotics (e.g. exclusion of ceftazidime/avibactam for class B carbapenemase producers) or adjunction of additional drugs (combination therapy). Another particular advantage of these ICAs is their availability to work directly[8,9] or after a few additional procedures[7,10] on positive blood culture broths. This could provide important added value in the treatment of critically ill patients, especially in settings with a high prevalence of carbapenemase-producing organisms causing bloodstream infections.

Finally, neither test was designed for direct testing on clinical samples, although studies have reported the application of RESIST-4 O.K.N.V. and NG-Test CARBA 5 for direct (or after short enrichment or additional processing of the sample) detection of CPE from faecal samples[15] or from urine.[16]

In conclusion, both assays provide a powerful tool to very easily detect nearly all common carbapenemases in a routine microbiology laboratory without specialized technical skills or the need for characterization by nucleic acid amplification methods. 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

P.B. and T.-D.H. are co-inventors of a patent licensed to Coris BioConcept for the immunochromatographic detection of carbapenemase. All other authors: none to declare.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* Online

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