ORIGINAL ARTICLE

Comparison of the NG Biotech NG-Test CARBA 5 and CORIS BioConcept RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in *Enterobacterales*

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ABSTRACT

Objective: To evaluate and compare the NG-Test CARBA 5 and RESIST-4 O.K.N.V. immunochromatographic lateral flow assays for the detection of carbapenemase enzymes in *Enterobacterales*. Both assays have the ability to detect four of the most widespread carbapenemases; OXA-48-like, NDM, KPC, and VIM. In addition, the NG-Test CARBA 5 is able to detect IMP types. The importance of rapid diagnostic tests in microbiology laboratories is becoming increasingly crucial with the emergence of Carbapenemase-producing *Enterobacterales* (CPE) in New Zealand as these isolates have limited treatment options.

Methods: NG-Test CARBA 5 and RESIST-4 O.K.N.V. were performed with 58 *Enterobacterales* isolates with reduced susceptibility to meropenem, including 45 CPE and 13 non-CPE.

Results: The respective sensitivity results for the NG-Test CARBA 5 and RESIST-4 O.K.N.V. were as follows: OXA-48-like – 94.7 (18/19) and 89.5% (17/19), NDM – 95.2% (20/21) and 90.5% (19/21), KPC – 100% (4/4) and 75.0% (3/4), VIM – 100% (2/2) and 100% (2/2). Additionally, the NG-Test CARBA 5 detected 80.0% (4/5) of IMP-types. Both assays produced 100% specificity .

Conclusions: NG-Test CARBA 5 and RESIST-4 O.K.N.V. detect the main carbapenemase types in *Enterobacterales*, as currently found in New Zealand. They are both highly specific assays and produced overall sensitivity values of 94.1% and 89.1% respectively. These products are robust, user-friendly kits that provide results within 20 minutes (including setup and incubation) indicating the presence or absence of singular or multiple carbapenemase type(s) detected.

Keywords: carbapenemase, *Enterobacterales*, immunochromatographic lateral flow assay, carbapenem.

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INTRODUCTION

Carbapenemase-producing *Enterobacterales* (CPE) confer resistance to carbapenem antibiotics via the ability to hydrolyse the beta-lactam ring in carbapenem structures. These enzymes are categorised into three major groups based on their molecular structures; Ambler class A carbapenemases including *Klebsiella pneumoniae* carbapenemases (KPCs), class B metallo-beta-lactamases (MBLs) including NDM, IMP, VIM carbapenemases and class D OXA carbapenemases (oxacillinases). The detection of carbapenemases is highly significant as their presence may result in a lack of effective antibiotics to treat infections caused by carbapenemase-producing bacteria (1). CPE may confer resistance to virtually all beta-lactam antibiotics as they have decreased susceptibility to carbapenems and are mostly, but not always, resistant to extended-spectrum cephalosporins. The majority of these enzymes are encoded by genes on transposable elements within plasmids, hence are an issue for infection control (2) as they have the ability to spread from one organism to another.

Other mechanisms of resistance to carbapenems in *Enterobacterales* include efflux pumps and ESBL or AmpC production coupled with porin loss (3). Differentiation as to whether it is carbapenemase-production resistance or another mechanism is optimal for laboratory analysis, results interpretation, clinical decision making, patient management and surveillance monitoring. Carbapenem resistance in *Pseudomonas* and *Acinetobacter* is also an emerging issue but are not discussed in this evaluation.

The EUCAST guidelines (July 2017) provide breakpoints for clinical treatment as well as breakpoints to indicate when screening for CPE should be carried out (2). Carbapenem MICs are set lower and disk diffusion zone breakpoints are set higher than the clinical breakpoints for *Enterobacterales*. Although some carbapenemase-producing organisms can have clinically susceptible breakpoints, it is still important to identify whether they harbour a carbapenemase in order to provide the best treatment options and to prevent the spread of resistance (4).

There are a variety of phenotypic methods currently available for the detection of CPE based on different principles. Combination disk testing methods, such as the D73C MASTDISCS® *Combi Carba plus* sets or ROSCO Neo-Sensitabs™, contain disks with meropenem +/- various carbapenemase inhibitors to detect the presence of CPE (2). Zones of inhibition are compared between disks containing meropenem alone and meropenem + inhibitors to determine if isolates produce carbapenemase activity. Colorimetric tests, such as the CarbaNP, utilise the principle of carbapenem hydrolysis indicated by a pH change, resulting in a colour change of the phenol red indicator (2). The Carbapenem inactivation method (CIM) and modified CIM also work by the principle of carbapenem hydrolysis. Following incubation of a meropenem disk in a broth inoculated with the test organism, the disk is placed on Mueller-Hinton agar inoculated with a carbapenem-susceptible indicator organism, incubated overnight and the zone of inhibition interpreted to determine whether meropenem has been hydrolysed (4).

Immunochromatographic lateral flow assays are a recent development, based on an antigen-antibody principle to identify the most widespread carbapenemases. In this study we evaluated two lateral flow assays, the NG-Test CARBA 5 and the CORIS BioConcept RESIST-4 O.K.N.V.

MATERIALS AND METHODS

A total of 58 *Enterobacterales* isolates were tested, including 45 previously characterised CPE and 13 non-CPE isolates harbouring other resistance mechanisms responsible for reduced susceptibility or resistance to carbapenems. Isolates used in this study were provided by the Institute of Environmental Science and Research Limited (ESR), Canterbury Health Laboratories and the RCPA. All non-CPE isolates tested negative for carbapenemase production by at least two phenotypic methods, including CarbaNP, together with one other method, such as the Carbapenem Inactivation Method (CIM), the modified CIM (mCIM), ROSCO MBL/KPC tabs or Cepheid Xpert® Carba-R PCR. Isolates were identified using the Vitek® MS matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry system (bioMérieux). The carbapenemase number and type of enzymes tested reflect those which are most commonly found in New Zealand to date (5), with a higher prevalence of OXA-48-like and NDM-type enzymes.

The RESIST-4 O.K.N.V. and NG-Test CARBA 5 assays utilise a nitrocellulose membrane cassette which is sensitised with antibodies directed against the different types of carbapenemases. A control capture reagent is incorporated to validate the test result. Tests were performed according to instructions provided by the manufacturers. Briefly, a bacterial test colony is homogenised in the provided buffer and a specified volume dispensed into the sample well of the cassettes and left to incubate at room temperature for 15 minutes. As the sample migrates through the well it produces a red line with the specific anti-carbapenemase antibodies if the isolate carries the corresponding carbapenemase.

The RESIST-4 O.K.N.V. kit has the ability to detect OXA-48-like, KPC, NDM, and VIM type carbapenemases with the respective detection limits of 0.125ng/ml, 0.625ng/ml, 0.25ng/ml and 0.23ng/ml (6). The NG-Test CARBA 5 is also able to detect OXA-48-like, KPC, NDM, VIM, and in addition IMP type carbapenemases with the respective detection limits of 300pg/ml, 600pg/ml, 150pg/ml, 300pg/ml and 200pg/ml (7) .

RESULTS

The results are displayed in Table 1. Both the NG-Test CARBA 5 and the RESIST-4 O.K.N.V. produced 100% specificity results as there were no false positive results. The overall sensitivity for each kit was calculated by considering each enzyme detectable to be an individual test. The NG-Test CARBA 5 therefore had a total of 290 tests (58 isolates tested against five enzymes), producing an overall sensitivity of 94.1%. The RESIST-4 O.K.N.V. had a total of 232 tests (58 isolates tested against 4 enzymes), producing an overall sensitivity of 89.1%.

The sensitivity for detection of OXA-48-like by the NG-Test CARBA 5 was 94.7% (18/19), due to one *Escherichia coli* OXA-48 producer being undetected. The same *E. coli* OXA-48 producer was also undetected by the RESIST-4 O.K.N.V. which also failed to detect OXA-48 in a *Klebsiella pneumoniae* co-producing NDM-1. These false negative results generated a sensitivity of 89.5% (17/19) for the detection of OXA-48-like enzymes. The sensitivity of NDM detection by the NG-Test CARBA 5 test was 95.2% (20/21), with one NDM-1-producing *Proteus mirabilis* undetected.

This NDM-1-producing *Proteus mirabilis* was also undetected by the RESIST-4 O.K.N.V., which also failed to detect a NDM-producing *Providencia stuartii* isolate, giving a sensitivity of 90.5% (19/21). All four KPC producers included in the study were detected by the NG-Test CARBA 5 kit resulting in a sensitivity of 100% (4/4). One KPC-2 enzyme produced by a *Klebsiella pneumoniae* was undetected by the RESIST-4 O.K.N.V. kit resulting in a sensitivity of 75.0% (3/4). Both kits detected the two VIM producers included in the study giving sensitivity results of 100% (2/2). The NG-Test CARBA 5 kit produced a sensitivity of 80.0% (4/5) for detection of IMP type carbapenemases, with one IMP-14-producing *Klebsiella pneumoniae* undetected. Although the RESIST-4 O.K.N.V. kit does not include detection for IMP types, the isolates were still tested in this study as all isolates were tested blindly.

Six of the isolates tested were co-producers of OXA-48-like and NDM-type carbapenemases, therefore these results have been included in the analysis of OXA-48 and NDM individually. Due to the frequency of CPE that co-produce carbapenemases it is important to recognise that both kits are able to detect multiple carbapenemases produced by the same isolate. Of the dual-producing isolates in this study, the only carbapenemase undetected by the RESIST-4 O.K.N.V. was a OXA-48 carried by a *Klebsiella pneumoniae,* which also produced an NDM.

Also of note is that there were two isolates which, although detected by both kits, produced a significantly weaker result with the RESIST-4 O.K.N.V. than the NG-Test CARBA 5. These isolates were a VIM-producing *Klebsiella pneumoniae* and a NDM-producing *Providencia stuartii*.

DISCUSSION

Global studies clearly suggest the emergence of CPE infections will continue to increase, impacting further on testing algorithms in diagnostic testing laboratories and requirements from health professionals for additional information on such isolates (8). Rapid confirmation reporting of CPE allows for more suitable patient management, including optimised treatment and implementation of contact precautions in order to minimise the risk of further spread between patients. The New Zealand National Antimicrobial Susceptibility Testing Committee (NZ NAC) has recently published guidelines for the “Minimum laboratory requirements for the detection of CPE from clinical samples and screening specimens” (9). This document provides laboratories in New Zealand clear cut instructions on how to identify *Enterobacterales* isolates that are suspicious for carbapenemase production , when further confirmatory testing should be performed and which isolates are required to be sent to ESR for confirmation and typing.

When performing phenotypic carbapenemase detection tests and a negative result is obtained it is crucial to consider patient risk factors and clinical information, with a low threshold for referring isolates to ESR for further testing, as indicated in the NZ NAC guidelines (9). As the NG-Test CARBA 5 and RESIST-4 O.K.N.V. lateral flow assays are specific to the kit profile carbapenemases, an option could be to perform a second phenotypic detection method when a negative result occurs and there is still high suspicion of carbapenemase. This may be useful if the isolate possesses a rare type, such as IMI or GES, or for detection of types with lowered sensitivity results from the lateral flow assays. Examples of such tests include the mCIM or the CarbaNP.

Meropenem MICs were performed alongside the NG-Test CARBA 5 and RESIST-4 O.K.N.V. cassettes. Those isolates with false negative carbapenemase results had meropenem MICs above the EUCAST screening cut-off for CPE (>0.125 mg/L). The decreased meropenem susceptibility indicates the carbapenemases were still active at time of testing therefore ruling out deterioration of the carbapenemase-bearing-plasmid as the reason for the false negatives. A possible explanation for these CPE not being detected may be due to potential low expression levels of the carbapenemases below the detection limits specified in the kit inserts.

The sensitivity of NDM detection was lower for both kits as they showed false negative results for an NDM-1 *Proteus mirabilis* isolate. Similarly, this has been shown in other studies where a *Proteus mirabilis* NDM-1 producer was undetected using the RESIST-4 O.K.N.V. (10) as well as the RESIST-3 O.K.N. kit (11). This was analysed further to find an explanation for the false negative result, concluding that to increase the

performance of the CORIS RESIST kits for NDM detection the isolate could be cultured onto a blood-containing media, using an increased inoculum, or taking the test isolate directly adjacent to a carbapenem disk from Mueller Hinton agar (MHA) as there is a higher expression of the carbapenemase in that area (10,11). Studies show that the sensitivity of detecting NDMs can be increased by supplementing MHA with zinc ions, as MBLs bind zinc at their active site resulting in increased enzymatic activity (10). Using MHA with the addition of zinc therefore may prove beneficial by producing a darker line on the kits as they will have a stronger reaction for those isolates in the study which produced weaker lines using the RESIST-4 O.K.N.V. test (*Klebsiella pneumoniae* VIM and a *Providencia stuartii* NDM producer).

The detection of OXA-48-like carbapenemases can be challenging as they commonly have weak resistance to carbapenems, therefore providing a test which is highly specific to OXA-48-like carbapenemases which is important for CPE detection in areas where OXA-48 are one of the most prevalent types (12). High sensitivity values for OXA-48 detection have been portrayed in global studies (13,14). In a study by Kolenda et al including 11 OXA-48-like CPE, the sensitivity was 100% using the RESIST-4 O.K.N.V. (13). Likewise, the validation of the NG-Test CARBA 5 detected all OXA-48-like CPE (n = 37) (14).

The co-expression of OXA-48-like and NDM in six of the CPE tested were correctly detected by the NG-Test CARBA 5 assay. The RESIST-4 O.K.N.V. kit failed to detect one OXA-48 enzyme in co-expression with a NDM, however, correctly detected the co-expression of the other five isolates. Recent studies by Greissl et al and Boutal et al show both assays producing 100% sensitivity for the detection of isolates co-expressing OXA-48-like and NDM types (10,14).

Although only a small number of KPC and VIM producing isolates were available for this study, there have been larger studies performed with comparable high sensitivity results for detection of KPC and VIM using both kits. A validation study of the NG-Test CARBA 5 kit produced 100% sensitivity for KPC (n = 22) and VIM (n = 17) (14). Likewise in a small study, the RESIST-4 O.K.N.V. kit has shown to have a 100% sensitivity for KPC (n = 10) and VIM (n = 34) (13).

The sensitivity of IMP detection by the NG-Test CARBA 5 kit was decreased due to a false negative result of IMP-14 *Klebsiella pneumoniae*. Similarly, IMP-14 carbapenemases (n = 2) were undetected in an evaluation study by Hopkins et al contributing to the decrease in sensitivity for detection of IMP variants (15).

Overall both kits achieved acceptable sensitivity in our study. However, the sensitivity of the NG-Test CARBA 5 assay was higher than the RESIST-4 O.K.N.V. for the detection of OXA-48-like, NDM and KPC-types. In addition, the NG-Test CARBA 5 is able to detect IMP types. Both kits achieved a sensitivity of 100% for the detection of VIM-types. This evaluation of the NG-Test CARBA 5 and RESIST-4 O.K.N.V. produced 100% specificity as there were no false positive results.

Limitations of the study include that only *Enterobacterales* were investigated, therefore further testing would be required to evaluate the use of these kits for other Gram-negative organisms that can produce carbapenemases, including *Pseudomonas aeruginosa* and *Acinetobacter* species.

Table 1. Results

Resistance Mechanism	Organism	n*	Number carbapenemase detected	
Carbapenemase			**Carba5**	**RESIST-4 O.K.N.V**
OXA-48-like	*Escherichia coli*	10	9	9
	Klebsiella pneumoniae	3	3	3
OXA-48-like + NDM	*Escherichia coli*	3	3	3
	Klebsiella pneumoniae	3	3	2.5†
NDM	*Citrobacter freundii*	1	1	1
	Escherichia coli	6	6	6
	Klebsiella pneumoniae	4	4	4
	Klebsiella oxytoca	1	1	1
	Providencia stuartii	2	2	1
	Proteus mirabilis	1	0	0
KPC	*Klebsiella pneumoniae*	4	4	3
VIM	*Klebsiella pneumoniae*	2	2	2
IMP	*Escherichia coli*	2	2	0
	Klebsiella oxytoca	1	1	0
	Klebsiella pneumoniae	2	1	0
		45	**42**	**35.5**
Non-carbapenemase				
ESBL + AmpC (CIT Group)	*Escherichia coli*	2	0	0
pAmpC (ACC)	*Proteus mirabilis*	1	0	0
pAmpC (DHA)	*Klebsiella pneumoniae*	1	0	0
pAmpC (CIT Group)	*Citrobacter koseri*	1	0	0
	Escherichia coli	2	0	0
pAmpC	*Proteus mirabilis*	1	0	0
Hyper AmpC	*Enterobacter cloacae complex*	2	0	0
Hyper AmpC + porin	*Enterobacter aerogenes*	1	0	0
ESBL	*Escherichia coli*	1	0	0
None	*Escherichia coli*	1	0	0
		13	**0**	**0**

*n = number tested, † one co-producing isolate only NDM detected and OXA-48 not detected.

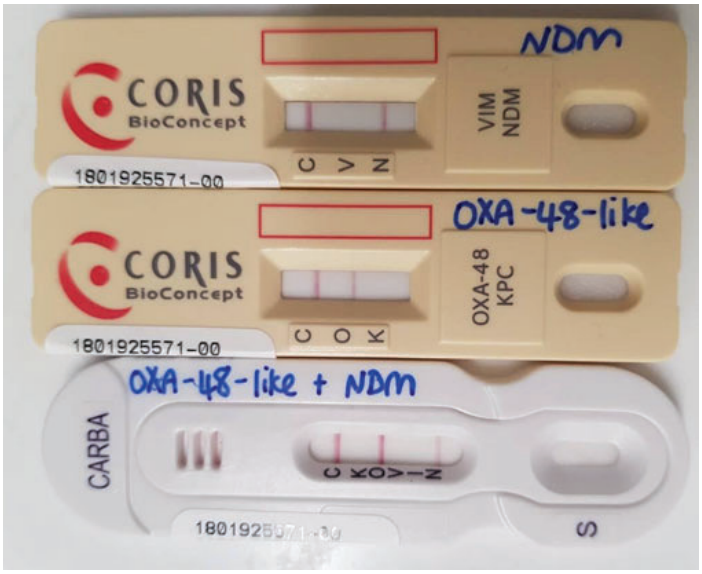

Figure 1. Biotech NG-Test CARBA 5 and CORIS BioConcept RESIST-4 O.K.N.V. detection of OXA-48-like and NDM co-expression.

The number of KPC, VIM, and IMP-type carbapenemases tested were limited, however, this reflects the current prevalence of carbapenemase-types in New Zealand. The fact that IMP-types are not detected by the RESIST-4 O.K.N.V. kit is a limitation. Although IMP-types are not commonly found in New Zealand, they are the highest reported type of carbapenemase detected in Australia (16). As carbapenemases have the ability to spread easily and with Australia being close geographically, New Zealand laboratories need to be vigilant and have methods suitable to detect IMP types.

Both kits tested were found to be robust as they can be stored between 4 - 30°C with a shelf life of up to 2 years, therefore suitable when testing volumes are low. They are user friendly, produced high specificity results and the sensitivity values produced for the assays were 94.1% for the NG-Test CARBA 5 and 89.1% for the RESIST-4 O.K.N.V. Results were rapidly produced within 20 minutes (15 minutes incubation plus set up of kit) indicating the presence or absence of singular or multiple carbapenemase type(s). These kits proved to be very straight forward to use, following a short number of steps required to prepare the isolates and no intensive training or specialised skills required to run the test.

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