Validation of Multiplex Lateral Flow Immunoassay NG-Test Carba 5 for the Rapid Identification of Carbapenemase-producing Organisms

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Introduction

Carbapenemase-producing organisms (CPOs) are a group of organisms that have acquired resistance to carbapenems. It poses a significant challenge in public health systems across the world due to its ability to spread rapidly. CPOs are spread via various transmission modes, including contact (person-to person, through contaminated surfaces and equipment), fecal-oral, and environment reservoirs. Carbapenemase-producing Enterobacterales (CPE) are able to spread their genetic materials to other Enterobacterales through various mechanisms of genetic exchange, hence introducing carbapenemase resistance to other organisms. In hospital settings, health care associated infection with CPOs limits treatment options, causes outbreak and increases mortality rate. Rapid diagnosis is required for optimum treatment and infection control strategies. The five carbapenemases, KPC, OXA-48-like, VIM, IMP and NDM are responsible to most of the nosocomial infections in hospitals.

In this validation, NG-Test Carba 5 (NG Biotech) was validated against the current clinical confirmation assay mCIM and Xpert® Carba-R (Cephid) used in Microbiology Laboratory, Nepean Hospital.

Aim

This study aimed to validate the performance of the NG-Test Carba 5 (NG Biotech) lateral flow immunoassay for rapid detecting carbapenemase production (KPC-, NDM-, VIM-, IMP -type and OXA-48-like) in common clinical bacterial isolates.

Methods

Twenty-six routine clinical isolates processed at Nepean microbiology with known antimicrobial patterns were tested using the NG-Test Carba 5. The mCIM test, and GeneXpert Xpress Carba-R (Cephid) result were used as the reference method for this validation. Seventeen isolates were known CPE, nine were known negative including two ESBL and one AmpC isolate. All archived isolates were tested retrospectively.

Modified Carbapenem Inactivation Method mCIM

A phenotypic assay to determine the presence or absence of a carbapenemase based on hydrolysis of a meropenem disc when incubated with a test organism.[4]

Xpert® Carba-R (Cephid)

A qualitative real time polymerase chain reaction (PCR) assay designed for the detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP} gene sequences associated with carbapenem resistance.

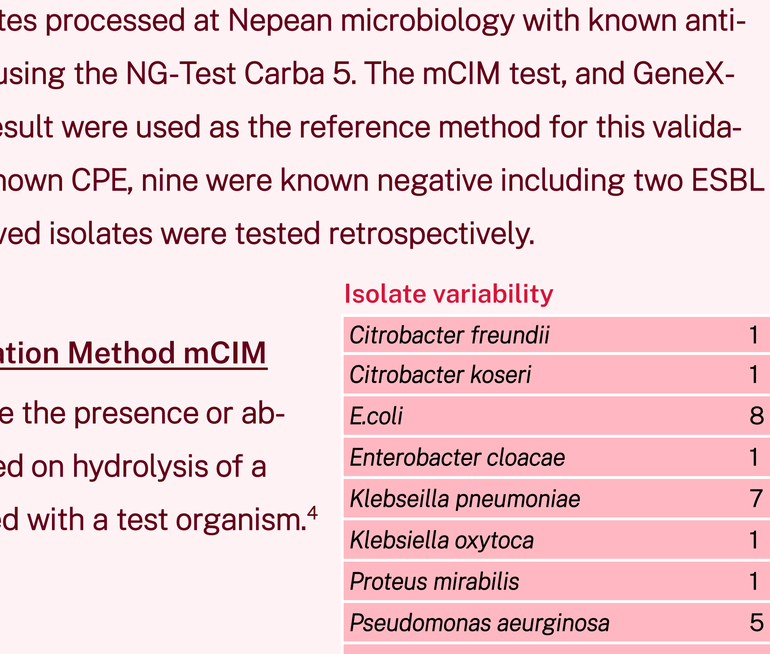
NG-Test Carba 5 (NG Biotech)

A qualitative multiplex immunochromatographic assay for the detection and differentiation of five common carbapenemase enzymes KPC, OXA-48-like, VIM, IMP and NDM.

Isolate variability

Citrobacter freundii	1
Citrobacter koseri	1
E.coli	8
Enterobacter cloacae	1
Klebseilla pneumoniae	7
Klebsiella oxytoca	1
Proteus mirabilis	1
Pseudomonas aeurginosa	5
Serratia marcescens	1

Breakdown of isolates and types of carbapenemase



Results

- In this study, Carba 5 successfully detected all isolates harbouring KPC, NDM, VIM, IMP-type, and OXA-48-like Carbapenemase with no false positives i.e. 100% specificity and sensitivity.
- Nine non-CPE isolates, including two ESBL, one AmpC and six susceptible isolates, tested negative on Carba 5.
- No cross reactions were observed on with ESBL.
- All isolates (95.8%) exhibited matching phenotypic mCIM results except for a single OXA-48 detected isolate, which mCIM was negative.

Carba 5 result	mCIM	
	POSITIVE	NEGATIVE
IMP	4	0
KPC	1	0
NDM	5	0
OXA-48	3	1
VIM	3	0
NEG	0	9

Discussion

Performance

The performance of Carba 5 is satisfactory. The sensitivity and specificity were 100% in this study. It also showed good detection to OXA-48 isolate. OXA-48 carbapenemases have low level of hydrolytic activity. It is often reflected in borderline or slightly elevated phenotypic resistance. Phenotypic methods have traditionally been less sensitive at identifying low level enzymatic activities including OXA-48. [5] Of the four OXA-48 isolates tested, one showed negative mCIM result.; where Carba 5 was able to detect OXA-48 in all four isolates.

Carba 5 did not show cross reaction with other resistance mechanisms like ESBL and AmpC. The highly specific antigen-antibody binding mechanism only targets the five carbapenemases and the result was satisfactory.

This study has a number of limitations: it was performed in a single centre only, with a limited number of isolates. However all five classes of carbapenemase were included and were detected.

Application

Cost-effective - It only requires basic laboratory equipment. No computer or analyser are needed. Maintenance and training are minimum comparing to the use of Xpert® Carba-R (Cephid).

Quick-turnaround - test preparation time is 5-10 minutes, and the result is ready in 15 minutes. mCIM is a grow-based phenotypic test that needs 22-24 hours for preparation and incubation.

Detection - mCIM detects the presence of many carbapenemase producers. However, it provides no identification of carbapenemase. Knowing the type of carbapenem resistance aids antimicrobial strategy, targeted infection control measures, surveillance and epidemiological study.

Conclusion

Carba 5 is a reliable, cost effective and quick turnaround assay for the detection of Carbapenemase. It facilitates efficient treatment decisions as well as rapid infec-

Reference

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