# Evaluation of a multiplex immunochromatographic assay for the rapid detection of carbapenemase-producing *Enterobacteriaceae* from culture colonies

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## ABSTRACT

Introduction: the increasing worldwide spread of multidrug resistant bacteria, in particular of carbapenemase-producing *Enterobacteriaceae* (CPE), represents a serious clinical and public health concern. An accurate and fast detection of infected patients or colonized carriers is thus mandatory.
Aim of this study was to assess the performance of a multiplex immunochromatographic assay (NG-Test CARBA 5, NG Biotech, Guipry, France) for the rapid detection of carbapenemases directly from pure bacterial colonies.
Methods: seventy-five non-replicated *Enterobacterales* isolates with decreased susceptibility to carbapenems, including 71 *Klebsiella pneumoniae,* 3 *Escherichia coli* and 1 *Enterobacter cloacae*, were analysed with NG-Test CARBA 5. At the same time the combination disk test (CDT) was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) indications, while confirmation of carbapenemase production was achieved by polymerase chain reaction (PCR).
Results: PCR assay could find 66 CPE strains, including 64 *Klebsiella pneumoniae* [53 producing *Klebsiella pneumoniae* carbapenemase (KPC), 5 New Delhi metallo-β-lactamase (NDM), 2 class D oxacillinases (OXA-48), 1 Verona integron-encoded metallo-β-lactamase (VIM) and 3 co-producing NDM and OXA-48] and 2 *Escherichia coli* (2 NDM+OXA-48) while 9 isolates were found as non-carbapenemase producing: 7 *Klebsiella pneumoniae,* 1 *Escherichia coli,* 1 *Enterobacter cloacae*. CDT allowed us to consider those 9 strains as extended spectrum β-lactamase (ESBL) or AmpC β-lactamase producers. NG-Test CARBA 5 successfully identified 66/66 CPE showing 100% sensitivity and 100% specificity. Unlike NG-Test CARBA 5, CDT was not able to correctly identify 5 strains co-producing NDM and OXA-48 carbapenemases.
Conclusion: NG-Test CARBA 5 is a reliable assay that can be useful in settings requiring a rapid identification of CPE directly from culture colonies. Moreover, this test is an easy-to-use option that could avoid misidentification of carbapenemases co-producers strains.

## INTRODUCTION

The increasing worldwide spread of multidrug resistant bacteria, especially of carbapenemase-producing *Enterobacteriaceae* (CPE), represents a serious clinical and public health concern because these strains are responsible for many hospital-acquired infections that are difficult to treat, resulting in longer hospital stay, higher costs and high mortality rates (1,2).

Carbapenemases are β-lactamases that hydrolyze penicillins, mostly cephalosporins and, to various degrees, carbapenems and monobactams, conferring resistance to practically all β-lactams. Frequently CPE also carry mechanisms conferring resistance to other antimicrobial classes, leaving very few treatment options (3,4). Carbapenemase enzymes include class A carbapenemases (e.g. *Klebsiella pneumoniae* carbapenemase - KPC), class B or metallo-β-lactamases (MBLs) (e.g. Verona integron-encoded metallo-β-lactamase - VIM, New Delhi metallo-β-lactamase - NDM and IMP enzymes), and class D oxacillinases (e.g. OXA-48-like enzymes). In addition,

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resistance to carbapenems in *Enterobacterales* can be due to a qualitative and/or quantitative deficiency of porine expression that cause a decreased antibiotic absorption, in association with overexpression of β-lactamases that possess very weak affinity for carbapenems, such as AmpC and extended-spectrum-β-lactamase (ESBL) (1).

The association of multiple resistance determinants and their ability to spread rapidly among several bacterial species require an accurate and fast detection of CPE infected patients or colonized carriers for both therapeutic management and infection control purposes (4,5).

Phenotypic detection of CPE can be challenging due to a high variation of minimal inhibitory concentrations (MICs) of carbapenems. It is important to consider that some isolates show low MICs for these molecules and routine susceptibility tests may not correctly detect the mechanisms of resistance (4,6). For these reasons, during the last years, several phenotypic tests have been developed for detection of carbapenemases in *Enterobacterales*; most of them are however time-consuming because they require 2 to 24 hours after the first result of the screening susceptibility tests to obtain reliable results (4). The combination disk test method (CDT) was the first phenotypic assay available (4,7). Successively other methods based on the detection of carbapenem-hydrolysing activity were developed such as biochemical (colorimetric) assays (e.g. CarbaNP), the carbapenem inactivation method and the application of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (8-10).

Today, molecular detection of carbapenemases by polymerase chain reaction (PCR) is considered the gold standard but it is still expensive, requires a high level of expertise to obtain accurate results and is not suitable for all clinical microbiology laboratories worldwide. For this reason, the recently introduced immunochromatographic lateral flow tests (ICT), able to detect carbapenemase-specific epitopes using monoclonal antibodies directly from cultured strains taking only around 15 minutes, represents a rapid alternative. Previous studies about these methods demonstrated high sensitivity and specificity for OXA-48-like, KPC, and NDM carbapenemases variants (11, 12).

The aim of this study was to assess the performance of a multiplex immunochromatographic assay (NG-Test CARBA 5, NG Biotech) for the rapid detection (15 minutes) of KPC, NDM, VIM, IMP and OXA-48 carbapenemases directly from pure colonies compared with molecular characterization performed by real time PCR reactions and with phenotypic characterization carried out by CDT.

## METHODS

### Bacterial strains

Seventy-five non-replicated *Enterobacterales* isolates, detected in clinical specimens delivered to our laboratory and showing reduced susceptibility to carbapenems [meropenem and/or ertapenem MIC screening cut-off value: >0.125 mg/L, as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (4)] were evaluated. This panel of strains included 71 *Klebsiella pneumoniae,* 3 *Escherichia coli* and 1 *Enterobacter cloacae*. MIC values for carbapenems were detected with broth microdilution method using Sensititre™ ITGNEGF panel (ThermoScientific, TREK Diagnostic Systems).

### Molecular analysis

All the isolates were characterized to define the presence of carbapenemases by using the Xpert CARBA-R assay (Cepheid) as illustrated by Kost et al (13). The Xpert CARBA-R assay is a qualitative, *in vitro*, automated real-time PCR-based assay designed for the rapid detection of *blaKPC, blaNDM, blaVIM, blaIMP,* and *blaOXA-48* carbapenem resistance genes.

### Phenotypic assays

The complete set of strains was analysed with NG-Test CARBA 5. One colony of overnight growth harvested from Mueller Hinton agar plates was tested according to the manufacturer's instructions. Migration takes 15 minutes and, subsequently, the appearance of one or more red lines in the test region of the cassette, corresponding to the five carbapenemases KPC, NDM, VIM, IMP and OXA-48, was recorded. If the test was performed correctly, a control line appears (Figure 1).

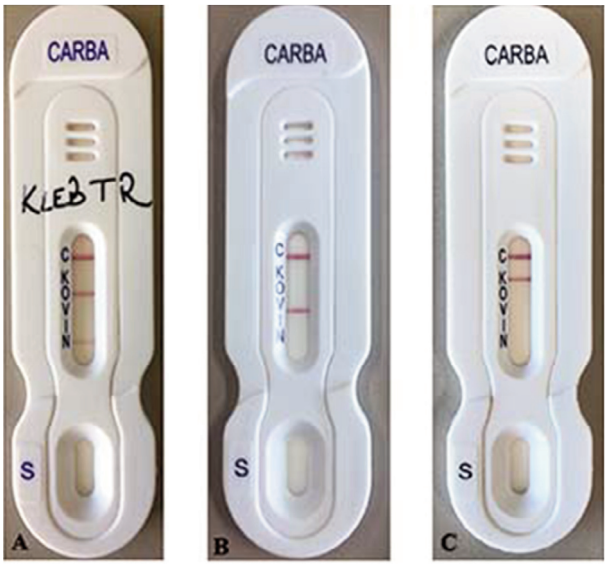


**Figure 1**
*Examples of a positive test for a co-producing class D oxacillinases (OXA-48) and New Delhi metallo-β-lactamase (NDM) strain (A), a Verona integron-encoded metallo-β-lactamase (VIM) producing strain (B) and a Klebsiella pneumoniae carbapenemase (KPC) producing strain (C) by NG-Test CARBA 5. C: control, K: KPC, O: OXA-48 like, V: VIM, I: IMP, N: NDM.*

Concurrently, CDT was performed according to EUCAST indications (4), using the KPC/metallo-β-lactamases confirmation kit (Rosco). This commercial kit is made of 4 tablets containing: meropenem (tablet A), meropenem and dipicolinic acid (MBL inhibitor) (tablet B), meropenem and cloxacillin (AmpC inhibitor) (tablet C), meropenem and boronic acid (KPC inhibitor) (tablet D). The results were evaluated as follows: the zone of inhibition of tablet A is compared to the inhibition zone of each carbapenem-plus-inhibitor tablets (B, C, and D). An inhibition zone showing a diameter difference above 5 mm indicates the presence of the enzyme activity. Each tablet indicates one specific resistance mechanism: tablet B points to MBL activity; tablet D reveals KPC activity, the association of tablets C and D shows AmpC activity coupled with porin loss (7). Negative results of all synergy tests are presumptive of an OXA-48 or ESBL activity. When an isolate presenting this phenotypic pattern associated to a negative result by PCR and ICT was detected, a CDT for the detection of ESBL by using disks or tablets containing the cephalosporin alone (cefotaxime and ceftazidime) and in combination with clavulanic acid (BBL Sensi-Disc, BD) was also performed. In this method the inhibition zone around the cephalosporin disk or tablet combined with clavulanic acid is compared with the zone around the disk or tablet with the cephalosporin alone. The test is considered positive if the inhibition zone diameter is ≥5 mm larger with clavulanic acid than without it (4).

### Control strains

ATCC 700603 (*Klebsiella pneumoniae* SHV-18 ESBL producing) and NCTC 13438 (*Klebsiella pneumoniae* KPC producing) were used as negative and positive control, respectively.

## RESULTS

PCR assay allowed to find 66 CPE strains, including 64 *Klebsiella pneumoniae* (53 producing KPC, 5 NDM, 2 OXA-48, 1 VIM and 3 co-producing NDM and OXA-48) and 2 *Escherichia coli* (2 co-producing NDM and OXA-48), while 9 isolates were found as non-carbapenemase producing: 7 *Klebsiella pneumoniae*, 1 *Escherichia coli* and 1 *Enterobacter cloacae*. Similarly, NG-Test CARBA 5 successfully identified 66/66 CPE (100% sensitivity and 100% specificity). For the strains that had negative results both with PCR and with NG-Test CARBA 5, CDT allowed to detect the AmpC production only for the *Enterobacter cloacae*, whereas for 7 *Klebsiella pneumoniae* and 1 *Escherichia coli* the test showed a possible production of ESBL or OXA-48 like enzymes. On these 8 strains we also performed CDT for detection of ESBL in order to discriminate the previous result, obtaining an inhibition zone diameter ≥5 mm, putative for ESBL production. In addition, unlike NG-Test CARBA 5, CDT was not able to correctly identify the 5 strains co-producing NDM and OXA-48 carbapenemases. All the results are summarised in Table 1.

## DISCUSSION

Carbapenemases disseminated in Europe from the second half of the 1990s representing a source of concern because they may confer resistance to virtually all β-lactams and are rapidly transferable (1,4). The variable level of expression of these enzymes and their frequent association with other resistance mechanisms result in a wide range of phenotypical patterns among CPE, which sometimes are difficult to identify correctly (4). Actually, nucleic acid amplification technology represents the gold standard since it can detect the presence of a specific carbapenemase gene with high performance and it is useful when it is necessary to obtain a rapid identification of the mechanisms of resistance (e.g. for rapid screening of faecal carriage of CPE). Unfortunately, this method is not yet available in all laboratories because of the high costs and the need of properly trained personnel. On the other hand, more accessible methods, such as CDT, despite having rather good performance, require at least 24 hours to obtain a result from the first colony growth that has to be evaluated for carbapenemase production.

Lateral immunocromatography, performed directly from culture colonies or from clinical sample (e.g. rectal swab), could represent a rapid and cost-effective alternative to culture and molecular tools (11,14,15). Our results show that NG-Test CARBA 5 is a reliable assay, with 100% of sensitivity and specificity, useful in settings requiring a rapid identification of CPE directly from culture colonies. Furthermore, this test is an easy-to-use option that allows avoiding misidentification of carbapenemases co-producers strains. Actually, CDT can miss detection of strains with enzyme co-production. For example, in our set we found 5 strains co-producing NDM and OXA-48 that CDT was not able to correctly identify in contrast to ICT that permitted to detect the presence of the two enzymes. Moreover, it is important to consider that combination of different phenotypic methods, able to distinguish different mechanisms, can be accessible in term of cost and useful for microbiological laboratory routine, allowing a high level of precision in typing. In our collection, the inclusion of CDT for ESBL with NG-Test CARBA 5 and CDT was able to discriminate between ESBL or AmpC producers among carbapenemases non-producer strains. A correct identification of the resistance mechanisms is useful not only for infection control and public health purposes (4), but it can be relevant also for the therapeutic approach, for example in the choice of ceftazidime/avibactam combination that is highly active against KPC but not against MBLs producers (15). Finally, we consider that it would be important to extend the evaluation to a greater number of CPE strains, such as IMP producers that have not been included in this study and have been reported difficult to detect by other authors (16), in order to obtain further confirmation of assay efficiency.

**Table 1**
*Phenotypic and genotypic characteristics of isolates evaluated in the study.*

| Number of isolates | Species | Ertapenem MIC range (mg/L) | Meropenem MIC range (mg/L) | PCR | ICT | CDT |
|---|---|---|---|---|---|---|
| 53 | *Klebsiella pneumoniae* | > 1 | 2 - > 64 | KPC | KPC | KPC |
| 5 | *Klebsiella pneumoniae* | > 1 | 4 - 8 | NDM | NDM | MβL |
| 1 | *Klebsiella pneumoniae* | > 1 | 64 | VIM | VIM | MβL |
| 2 | *Klebsiella pneumoniae* | > 1 | > 64 | NDM + OXA-48 | NDM + OXA-48 | ESBL/ OXA-48 |
| 1 | *Klebsiella pneumoniae* | > 1 | 4 | NDM + OXA-48 | NDM + OXA-48 | MβL |
| 2 | *Escherichia coli* | > 1 | > 64 | NDM + OXA-48 | NDM + OXA-48 | MβL |
| 2 | *Klebsiella pneumoniae* | > 1 | 2 | OXA-48 | OXA-48 | ESBL/ OXA-48 |
| 7 | *Klebsiella pneumoniae* | > 1 | ≤ 0.125 - 2 | Undetected | Negative | ESBL/ OXA-48* |
| 1 | *Escherichia coli* | > 1 | 1 | Undetected | Negative | ESBL/ OXA-48* |
| 1 | *Enterobacter cloacae* | > 1 | ≤ 0.125 | Undetected | Negative | AmpC |

*MIC, minimal inhibitory concentration; PCR, polymerase chain reaction; ICT, immunochromatographic test; CDT, combination disk test; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-β-lactamase; MβL, metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase; ESBL, extended-spectrum-β-lactamase.*
**for these isolates combination disk test for ESBL was performed showing a positive result.*

## CONFLICT OF INTEREST

None.

## REFERENCES


1. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 2012;18:263-72.
2. Nordmann P, Cornaglia G. Carbapenemase-producing Enterobacteriaceae: a call for action! Clin Microbiol Infect 2012;18:411-2.
3. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2011;17:1791-8.
4. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0 July 2017. http://www.eucast.org/resistance_mechanisms/ (last accessed: January 2019).
5. Munoz-Price LS, Poirel L, Bonomo RA et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis 2013;13:785-96.
6. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2013;68:487-9.
7. Bartolini A, Frasson I, Cavallaro A, et al. Comparison of phenotypic methods for the detection of carbapenem non-susceptible Enterobacteriaceae. Gut Pathog 2014;6:13.
8. Dortet L, Agathine A, Nass T et al. Evaluation of the RAPIDEC. CARBA NP, the Rapid CARB Screen. and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2015;70:3014-22.
9. van der Zwaluw K, de Haan A, Pluister GN et al. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One 2015;10:e0123690.
10. Lasserre C, De Saint Martin L, Cuzon G et al. Efficient detection of carbapenemase activity in Enterobacteriaceae by matrix-assisted laser desorption ionization-time of flight mass spectrometry in less than 30 minutes. J Clin Microbiol 2015;53:2163-71.
11. Greissl C, Saleh A, Hamprecht A. Rapid detection of OXA-48-like, KPC, NDM, and VIM carbapenemases in

Enterobacterales by a new multiplex immunochromatographic test. Eur J Clin Microbiol Infect Dis 2019;38:331-5.
12. Hamprecth A, Vehreschild JJ, Seifert H et al. Rapid detection of NDM, KPC and OXA-48 carbapenemases directly from positive blood cultures using a new multiplex immunochromatographic assay. PLoS One 2018;14;13:e0204157.
13. Kost K, Yi J, Rogers B et al. Comparison of clinical methods for detecting carbapenem-resistant Enterobacteriaceae. Pract Lab Med 2017;18;8:18-25.
14. Fauconnier C, Dodemont M, Depouhon A et al. Lateral flow immunochromatographic assay for rapid screening of faecal carriage of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2019;74:357-9.
15. Tuon FF, Rocha JL, Formigoni-Pinto MR. Pharmacological aspects and spectrum of action of ceftazidime-avibactam: a systematic review. Infection 2018;46:165-81.
16. Hopkins KL, Meunier D, Nass T et al. Evaluation of the NG-Test Carba 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48 live, NDM, VIM and IMP carbapenemases. J Antimicrob Chemother 2018;73:3523-6.