

Early Detection of Carbapenemases Producing Gram Negative Isolates by NG-Test CARBA 5: A Cross-sectional Study

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ABSTRACT

Introduction: Rapid and accurate detection of carbapenemase producing Gram negative bacteria is prerequisite for effective clinical management and infection control strategies. The limited susceptibility of carbapenemase producing bacteria to antibiotics leads to serious global health issues. Currently various molecular and phenotypic test are available for early screening and identification of carbapenemase producing organisms.

Aim: The aim of the present study was to detect various types of carbapenemase produced by multidrug resistant Gram negative bacterial isolates obtained from clinical samples during the study period and to compare the effectiveness of NG-Test CARBA 5 assay with TaqMan based Real-Time Polymerase chain reaction (RT-PCR) for the early detection of carbapenemase producing Gram negative isolates.

Materials and Methods: The present cross-sectional study was conducted at NKP Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India a Tertiary Care Hospital involving non-duplicate Gram negative carbapenem resistant isolates. NG-Test CARBA 5 was employed to detect carbapenemase production in different bacterial isolates. The study was conducted in duration of six months from August 2023 to February 2024. All the ertapenem resistant Gram negative bacterial isolates obtained from clinical samples during the study period were included in the study. Types of carbapenemase produced by multidrug resistant Gram negative bacterial isolates obtained from clinical samples by of NG-Test CARBA 5, by immunochromatographic assay

during the study period were documented. For 30 randomly selected isolates and a negative reference strain, the results obtained from NG-Test CARBA 5 were confirmed by TaqMan based multiplex RT-qPCR, for detection of carbapenemase genes, NDM, KPC, IMP, VIM, OXA. The test was performed as per the kit protocol. PCR results were considered as the gold standard for detection of genes. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for NG-Test CARBA 5 was calculated using Microsoft Excel 2016.

Results: Out of 277 Gram negative isolates, 75 (27.07%) isolates which were resistant to carbapenam were evaluated for presence of carbapenemase genes by NG-Test CARBA assay. The results revealed that 54 (72%) isolates were positive for one or more carbapenemase genes, whereas 21 (28%) isolates were confirmed to be negative. The negative control (*E. coli* ATCC 25922) showed appropriate results. To validate and evaluate the feasibility of the NG-Test CARBA 5 assay, RT-qPCR test was performed for 30 (20 isolates NG-Test CARBA 5 Positive and 10 isolates NG-Test CARBA 5 negative) randomly selected isolates, and a negative reference strain; all the isolates tested by RT-qPCR, showed presence of one or more genes and the reference negative strain was confirmed as a negative.

Conclusion: As per the present study findings, it is 64.5% sensitive and 100% specific, The PPV and NPV of NG-Test CARBA 5 is 100% and 8.4%. NG-Test CARBA 5 showed promise in detecting carbapenemase producing isolates although it needs further evaluation with large number of samples under Indian environmental conditions.

Keywords: Immunochromatography, Multiplex Real time quantitative polymerase chain reaction, Delhi Metallo-β-lactamase, Oxacillinase

INTRODUCTION

Enterobacteriales and *Pseudomonads* species are the most common cause of community as well as hospital acquired infections. Recently most of these isolates have been found to produce carbapenemase [1]. These Carbapenem-resistant Organisms (CROs) are considered as urgent threats by Centres for Disease Control and Prevention (CDC) [2]. Such carbapenemase producing bacteria are responsible for increased morbidity/mortality and high treatment costs [3,4]. The acquisition of Antimicrobial Resistance (AMR) in clinical bacterial pathogens is a natural phenomenon that occurs over time through changes at genetic level. The gene level changes occur because of environmental selection pressure, mainly by misuse and overuse of antibiotics to treat the infection.

There are mainly two mechanisms of acquiring resistance among CROs: Organisms which produce carbapenemase enzyme and

non-carbapenemase-mediated mechanisms, such as membrane permeability defects in combination with Extended Spectrum Beta Lactamase (ESBL) or AmpC Beta-lactamase production.

Carbapenemase producing Carbapenam Resistant Organisms (CP-CRO) have capability for transmission of carbapenemase genes to other Gram negative bacteria through the horizontal gene/plasmid transfer mechanism. Horizontally transmitted plasmids often carry additional AMR genes, which play an important role in the acquisition of multidrug-resistance in Gram negative bacterial isolates. As a result, the CP-CRO is considered as a one of the top global public health and development threats. Bacterial AMR is thought to have caused millions of deaths globally due to restricted treatment choices, misuse, and overuse of antimicrobials [5,6].

A variety of acquired carbapenemase has been reported in strains of *Enterobacteriales*, *Pseudomonads*, *Acinetobacter baumannii*. Ambler classification classifies the carbapenemase families

according to their amino acid identity. There are four classes according to Ambler classification, KPC (Ambler class A), NDM, VIM and IMP (Ambler class B), Amp C gene (Ambler class C) and OXA-48-types enzymes (Ambler class D) [7,8].

Among the above mentioned types of carbapenemases, class B metallo beta-lactamases are resistant to the action of all clinically-available inhibitors, except newer carbapenamase inhibitors like avibactam, relabactam and vaborbactam, therefore differentiating type of carbapenamases will help in more judicious selection of carbapenemase inhibitor [9].

The screening of carbapenemases from cultured isolates normally involve the initial detection of decreased susceptibility to carbapenems followed by the broad detection of carbapenemase production by a phenotypic method. Phenotypic testing methods proposed by Clinical Standards Institute (CLSI), includes modified Carbapenem Inactivation Method (mCIM), and Ethylene Diamine Tetra Acetic acid (ETDA)-Carbapenem Inactivation Method (eCIM) [10-14]. These procedures have several limitations, such as time consuming, expertise, unstable reagents, costly equipment's etc., Various phenotypic tests which are rapid with improved performance and with sample-to-answer molecular approaches have been developed over the recent past. Although these methods have overcome the other limitations but they still have limitations, such as the inability to detect all carbapenemase variants and cost [15]. Therefore, in the present study, the attempt has been made to evaluate the efficiency and feasibility of a rapid immunochromatographic test, NG-Test CARBA 5, which might be used in near future to tackle the clinical problem raised because of AMR. Aim and objectives of the present study was to detect various types of carbapenemase produced by multidrug resistant Gram negative bacterial isolates and to compare the effectiveness of NG-Test CARBA 5 assay with TaqMan based real-time PCR for the early detection of carbapenemase producing Gram negative isolates obtained from clinical samples during the study period.

MATERIALS AND METHODS

The present Cross-sectional study was conducted at NKP Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital a tertiary care hospital in Nagpur, Maharashtra, India. The study was conducted from August 2023 to February 2024 (The study was time bound). The Institutional Ethical committee clearance vide NKPSIMS & RC and LMH/IEC/2/2023 Dated.28.06.2023, was obtained.

Inclusion and Exclusion criteria: All the non-duplicate, multidrug resistant, Gram negative clinical isolates which were resistant to ertapenem were included in the study and the Gram positive isolates were excluded from the study.

Study Procedure

Species level identification of all non-duplicate multidrug resistant Gram negative clinical isolates from various clinical samples (blood, urine and sputum) was performed by standard phenotypic tests [15]. Carbapenem resistance was screened by testing the isolates for susceptibility to 10 µg ertapenem disk, as per the guidelines by CLSI standards [16]. Among carbapenams, ertapenem has superior sensitivity for detecting most carbapenemase producers hence, it is preferred over imipenem or meropenem [17,18].

NG CARBA 5 test: The samples which met the inclusion criteria were cultured on validated media, including Luria Broth (LB), Trypticase Soya Agar (TSA), and Mueller Hinton agar (MHA). The sample was prepared by suspending three colonies in 150 µL extraction buffer, vortexing, and incubating. The test device was opened, and 100 µL of the sample mixture was added to the sample well. Results were read after 15 minutes, following the instructions for interpretation for beta lactamase enzymes for NG-Test/CARBA-5 [19].

Real time qPCR: PCR reactions were performed for 30 isolates Deoxy Ribonucleic Acid (DNA) sample. The tubes were briefly centrifuged at 6000 rpm for about 10 seconds. The tubes were then placed in the Real-time PCR machine, and the recommended PCR program was set. The initial denaturation step was conducted at 95°C for 10 minutes. This was followed by denaturation at 95°C for five seconds, and then annealing and extension at 60°C for one minute, with plate reading after each cycle. The PCR process consisted of 45 cycles. Sampling was done using FAM, HEX, Texas Red and Cy5. Finally, the reaction was held at 4°C. The data from the amplification plot was interpreted by observing the Ct values [20]. Due to financial constraints a single kit of 40 PCR reactions was issued hence only 30 isolates from total of 75 isolates could be compared with the NG-Test CARBA-5. The results of both the test were compared for 30 isolates and statistical analysis was done to find sensitivity, specificity, PPV and NPV using PCR as gold standard.

STATISTICAL ANALYSIS

The sensitivity, specificity, PPV and NPV for NG-Test CARBA 5 was calculated using Microsoft Excel 2016.

RESULTS

A total of 277 non duplicate multidrug resistant Gram negative bacilli were isolated during study period, all these isolates were screened for sensitivity to ertapenam. Out of 277, 156 (56.32%) isolates were sensitive and 46 (16.6%) isolates were intermediate sensitive to ertapenam. A total of 75 (27.07%) isolates from various clinical samples were resistant to ertapenam by disc diffusion test.

NG CARBA test was performed on all the ertapenam resistant, 75 isolates along with one negative control (Control strain carbapenem susceptible *E. coli* ATCC 25922) 54 (72%) isolates were positive for one or more carbapenemase genes and 21 (28%) isolates were negative. The negative control gave appropriate reaction [Table/Fig-1].

Result	Number n=75
Positive	54 (72%)
Negative	21 (28%)

[Table/Fig-1]: NG test results among all isolates. (n=75).

Out of 54 isolates which showed carbapenamases, majority of isolates were positive for NDM+OXA 48 like 28 (37.33%). IMP and VIM were not detected in any of the isolates by NG CARBA test [Table/Fig-2].

Types of carbapenemase enzymes	Total	<i>Klebsiella Pneumoniae</i>	<i>Acinetobacter</i>	<i>Pseudomonas</i>	<i>E.Coli</i>
KPC	1 (1.33%)	1 (1.33%)	0	0	0
OXA 48 Like	8 (10.66%)	5 (6.66%)	1 (1.33%)	1 (1.33%)	1 (1.33%)
IMP	0	0	0	0	0
VIM	0	0	0	0	0
NDM	17 (22.66%)	6 (8%)	1 (1.33%)	5 (6.66)	05 (6.66)
NDM +OXA 48 Like	28 (37.33%)	21 (28%)	0	1 (1.33%)	4 (5.33%)
Negative	21 (28%)	9 (12%)	6 (8%)	2 (2.66%)	04 (5.33%)

[Table/Fig-2]: Types of carbapenemase enzymes detected by NG Carba test (n=75).

Out of the 75 isolates, 30 isolates (10 card negative and 20 card positive), were selected by simple random sampling method. PCR test was done for these 30 randomly selected isolates and a negative reference strain (ATCC 25922). All the 30 isolates showed presence of one or more genes by PCR and the reference negative strain was negative by both the methods [Table/Fig-3,4].

Test	NDM	KPC	IMP	VIM	OXA
PCR positive (n=20)	18	15	3	4	18
Card positive (n=20)	13	1	0	0	14

[Table/Fig-3]: Relation of various gene detection by PCR and Positive NG Carba (n=20).

Test	NDM	KPC	IMP	VIM	OXA
PCR positive (n=10)	10	9	0	1	10
Card negative (n=10)	1	1	1	1	1

[Table/Fig-4]: Relation of various gene detection by PCR and Negative NG Carba and Negative control (n=11).

Findings of PCR test of the 20 isolates, which were positive by NG Carba 5 co-related with NDM and OXA 48, but card test failed to detect VIM in 4, IMP in 3 and KPC in 14 isolates.

All the 10 card negative isolates were positive by PCR test, nine isolates were positive for NDM+OXA 48+KPC, and one each was positive NDM+OXA 48 and NDM+OXA 48+KPC+VIM. The negative control *E. coli* ATCC 25922 was negative by PCR. Thirty isolates underwent PCR. All card positive and negative isolates except negative control were positive by PCR test [Table/Fig-5].

Test	PCR Positive	PCR Negative	Total
Card positive	20 (a)	0 (b)	20
Card negative	10 (c)	0 (d)	10
Total	30	0	30

[Table/Fig-5]: Relation between PCR and NG Carba.

Statistical analysis was done sensitivity, specificity, PPV and NPV was calculated. The sensitivity and Specificity of NG Carba 5 was found to be 64.5% and 100%. The PPV was 100% and NPV was 8.4%.

DISCUSSION

The carbapenemase genes are mostly located on self-conjugative plasmids so they have increased propensity for transmitting these genes not only within genus, but outside genus also. These plasmids carry other resistance determinants also, thus making organism multidrug resistant. Rapid identification of such carbapenemase producers is needed as a guide for antibiotic therapy and to prevent the development of outbreaks [21]. To aid clinicians in choosing appropriate carbapenemase inhibitor drug and for better infection control practices, we need rapid, reliable, accurate and cost-effective phenotypic test for detection of carbapenemase producing organisms. Since the last decade, a significant increase (45%) in carbapenem use has led to the emergence and global dissemination of CROs raising mortality and healthcare costs.

For the qualitative detection and differentiation of carbapenemases, in-vitro rapid and visual multiplex immunochromatographic assay NG-Test CARBA 5 is being manufactured in India in collaboration with Himedia labs Mumbai. It detects carbapenemases (KPC, OXA-48-like, VIM, IMP and NDM) from carbapenem non-susceptible pure bacterial colonies and aids in the rapid identification and infection control in the detection of carbapenemase producing Enterobacteriaceae.

In the present study, we tried to detect various types of carbapenemase produced by multidrug resistant Gram negative bacterial isolates obtained from clinical samples during the study period NG CARBA test was performed on all the ertapenam resistant, 75 isolates, 54 isolates (72%) were positive for one or more carbapenemase genes by NG Carba test [Table/Fig-1].

In study by Haji SH et al., 2021 in Iraq, 65/110 (59%) isolates were identified as carbapenemase producers [21]. In another study by Elbadawi HS et al., 2021 in Sudan, 58.7% isolates harboured one or more carbapenemase genes [22]. In India study by Prabhala S et al., 2023 carbapenemase resistance genes was identified in 83.2%

of carbapenam resistant isolates, many such studies have shown that carbapanamase production is increasing [23].

In our study, many isolates showed more than one resistance gene, maximum isolates showed presence of NDM +OXA 48 like carbapenemase enzymes 28 (37.33%) by NG Carba test IMP and VIM enzymes were not detected in any of the isolates [Table/Fig-2]. Indian Studies showed similar findings, in the study by Prabhala S et al., 2023 in which New Delhi Metallo beta lactamase (NDM (33.68%) was the most common resistant gene identified followed by NDM and OXA-48 (32.63%) [23]. In a study by Han R et al., 2021 from China most common resistsnce gene was KPC (51.6%), NDM (35.7%), and OXA-48-like carbapenemases (7.3%) [24]. In the study by Elrahem AA et al., 2023 [25] in Egypt carbapenemase-encoding genes NDM 15 (50%), followed by VIM 9 (30%), IMP 8 (26.7%), and KPC 7 (23.3%). Studies conducted in India by Pawar SK et al., 2020 Mohanti S et al., 2017 and Solankhi R et al., 2014 were similar to present study in respect to IMP and VIM gene detection [26-28].

IMP type MBL gene is endemic in Japan and Taiwan [29]. Similarly, VIM is more prevalent in Greece [8]. In the present study, due to financial constraints only 31 isolates could be compared with PCR. All the isolates except for the negative control showed presence of more than one genes by PCR. Out of the multiple genes detected in a single isolate, most common gene combination detected was NDM+ OXA +KPC followed by NDM+ OXA. In the present study, NG Carba-5 test as compared to PCR test, it is 64.5% sensitive and 100% specific. The PPV and NPV of NG Carba test is 100% and 8.4% (statistical analysis). In studies by various authors, sensitivity was between 92% to 100% [Table/Fig-3,4]. There is wide variation between the present study and studies done by various other authors. [Table/Fig-6] [24,30-34].

S. No.	Author	Sensitivity	Specificity
1	Zhu Y et al., 2021 [30]	100%	99.9%
2	Han R et al., 2021 [24]	100%	100%
4	Liu Z, et al., 2021 [31]	92.1%,	100%
7	Kon H et al., 2021 [32]	100%	98%
5	Saito K et al., 2022 [33]	99.1%	100%
6	Gu D et al., 2023 [34]	96.82%	100%
7	Present study 2024	64.5 %	100%

[Table/Fig-6]: Comparative studies by various authors for NG-Test CARBA 5 [24,30-34].

The reason for such discrepancy can be due to various factors: In all the studies cited above, the isolates tested for NG-Test CARBA 5 were previously tested for crbapenamase genes by PCR/X-pert Carba/mCiM but in our study the selection was based on resistance to ertapenam. Which may have included CRO due to non crabapenamases mediated mechanisms. In the literature search, there was no study in which isolates which were negative by NG-Test CARBA 5 were tested for PCR. Some False negative results could be attributed to low level of expression of carbapenemase genes. The present device was made and marketed in India in collaboration with Hi-Media Laboratories. We were unable to find any validated study for the present device.

Limitation(s)

Sample size was less because study was time bound. Only 31 isolates could be compared with PCR due to financial constraints a larger study is needed for further validation of device.

In the present study, Pseudomonads, Acinetobacter along with Enterobacterales were included. A separate study to test only Enterobacterales should be conducted.

CONCLUSION(S)

The present study has shown that NG-Test CARBA 5 is 64.5% sensitive and 100% specific as compared with RT-qPCR. The PPV and NPV of NG Carba test is 100% and 8.4%, respectively. NG-Test CARBA 5 showed promise in detecting carbapenemase producing isolates although it needs further evaluation with large number of samples under Indian environmental conditions.

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PLAGIARISM CHECKING METHODS:

- Plagiarism X-checker: Jun 10, 2024
- Manual Googling: Jul 24, 2025
- iThenticate Software: Jul 26, 2025 (7%)

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7

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