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Original Research Article

Prevalence of carbapenem-resistant isolates from clinical samples in a tertiary care hospital Vadodara, Gujarat

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ABSTRACT

Background: Carbapenem resistance in Gram-negative bacilli is a major concern due to the increasing antibiotic resistance, treating infections caused by *Enterobacteriaceae* has become more challenging. Objective was to investigate the prevalence of blaNDM, blaOXA, blaKPC, blaIMP, blaVIM producing carbapenem-resistant *Enterobacteriaceae* and non lactose fermenter.

Methods: The prospective observational study was conducted from September 2025 to October 2025 at department of microbiology, Parul Institute of Medical Sciences and Research. Total 100 samples were identified by VITEK 2. A modified carbapenem inactivation method was performed to detect presence of carbapenemase. O.K.N.V.I resist-5 is a rapid in vitro multiplex immunoassay kit used for the phenotypic detection of blaKPC, blaOXA, blaVIM, blaIMP, blaNDM directly from bacterial colonies.

Results: A total 100 clinical samples were taken from that 60 (60%) *Enterobacteriaceae* and non lactose fermenter analysed by VITEK 2. Out of them 25 (41%) isolates were multidrug resistant carbapenem *Enterobacteriaceae*. From 25 (41%) samples 10 (40%) were urine samples, followed by 5 (20%) blood culture, 5 (20%) pus discharge, 2 (8%) drain fluid, 2 (8%) were ET, 1 (4%) CSF. Out of 25 MDR *Enterobacteriaceae* and non lactose fermenter 10 (40%) were *Klebsiella pneumoniae* isolated 4 blaNDM, 1 OXA, 5 blaNDM and blaOXA, followed by 6 (24%) *E. coli* isolated 1 blaNDM, 1 blaOXA, 4 blaNDM and blaOXA, 3 (12%) *Pseudomonas aeruginosa* isolated 1 blaVIM and 2 blaNDM, 4 (16%) *Acinetobacter baumannii* isolates 4 blaNDM, 1 (4%) Acinetobacter lwoffii blaVIM gene was detected.

Conclusions: Knowledge of the prevalence of genes responsible for carbapenemase in a particular region will help in antibiotic stewardship for the selection of empiric antibiotics.

Keywords: Carbapenem-resistant, *Enterobacteriaceae* and non lactose fermenter, OXA and NDM

INTRODUCTION

Carbapenem resistance in Gram-negative bacilli is a major concern in the management of antibiotics resistant infections. The mechanism of carbapenem resistance is most commonly mediated by carbapenemase. Due to the increasing antibiotic resistance, treating infections caused by *Enterobacteriaceae* and non lactose fermenter has become more challenging. Five main mechanisms are responsible for antibiotic resistance, namely, enzyme modification and inactivation, antibiotic target site modification, target site replacement, efflux pumps and reduced permeability.[1] Many types and subtypes of carbapenemase (blaIMP, blaVIM, blaOXA, blaKPC, blaNDM) have been recognized among *Enterobacteriaceae*; the arrival of blaNDM-1 and blaOXA is in the increasing antimicrobial resistance problem.[2] Carbapenemase-producing CRE is distributed throughout the world and the reason for such spread is because of horizontal blagene transfer since they are on the plasmid, a mobile genetic element.[3] The mobile genetic elements carry the drug resistant genes and hence they can easily transmit from person to person via the healthcare personnel hands or through contaminated medical equipment. High level of resistance to carbapenem and many other antimicrobial agents (fluoroquinolones and aminoglycosides) is caused by these genes.[4] The overexpression of bacteria-induced efflux pumps, absence of porins in the cell membrane of bacterial cells, and poor binding of carbapenem to Penicillin-binding proteins (PBPs) constitute other mechanisms of resistance.[5] Carbapenem-resistant *Enterobacteriaceae* and non lactose fermenter causes life-threatening infection among patients with the lower immune response which may be because of factors such as, patients with comorbidities and hospitalized in Intensive Care Unit (ICU).[6,7] Carbapenems are broad-spectrum β-lactam antibiotics often used as last-resort treatments against multidrug-resistant gram-negative bacteria. However, global carbapenem resistance has emerged.[8] Centre for Disease Control and Prevention (CDC) defines CRE as any family member of *Enterobacteriaceae* resistant to imipenem, meropenem, doripenem or ertapenem.[9] In view of limited treatment options available for treatment of infections such as urinary tract infections, pneumonia, bloodstream infections and skin and soft tissue infections caused by CRE and to prevent further spread of these organisms, healthcare systems across the world have measured the magnitude of the problem. CDC has described an increase of prevalence of CRE from 1 to 4% in 2013.[10] Keeping in mind the therapeutic challenge of infection by CRE, high morbidity, mortality and potential to spread in healthcare setting, measuring the magnitude of CRE becomes significant and thus present study was conducted to generate data on CRE from this part of Gujarat, India.[11] Researchers systematically categorized the carbapenemases into four groups: A, B, C, and D. Group A included most of the KPC strains; group B included many VIM, IMP, and NDM strains with carbapenemase activity detected from gram-negative bacteria; group C included AmpC β-lactamase, but it can play a role in the hydrolysis of carbapenemase only under special circumstances (osmotic abnormality); and group D included the OXA-48 type of Enterobacteriaceae bacteria (1). In summary, under normal conditions, classes A, B, and D are able to exert carbapenemase activity, thereby achieving resistance.[12] Several studies have shown that resistance to carbapenems is prevalent throughout India. In lower middle-income countries like India with a population of 1.25 billion.[13] The distribution of multi drug-resistant gram-negative bacteria varies in different regions in India and the magnitude is directly related to the use of particular antibiotic at that particular area. Carbapenems are the class of beta-lactam antibiotics which mainly act on penicillin binding proteins and leads to cell lysis and they are used to treat MDR GNB infections but with emergence of carbapenem resistant organisms and now there are challenging the antimicrobial therapy The most commonly used carbapenems are imipenem, meropenem, ertapenems and doripenems. There are many mechanisms which cause resistance but the important and most predominant one is carbapenemase production.[14]

METHODS

The prospective observational study was conducted from September 2025 to October 2025 at department of microbiology, Parul Institute of Medical Sciences and Research after getting ethical approval from PU-IECHR. The approval number for this study is PUIECHR/PIMSR/00/081734/9220.

Inclusion criteria

We have included isolates which are carbapenem resistant Enterobacteriaceae confirmed by Vitek-2. Samples which come during our study period. We have included the samples from IPD and OPD.

Exclusion criteria

Polymicrobial growth, *Enterobacteriaceae* that were susceptible to carbapenem, bacteria other than *Enterobacterales*.

Total 100 clinical samples were collected and cultured on nutrient agar, MacConkey agar and Blood agar. Out of these 100 clinical samples, 60 (60%) *Enterobacteriaceae* and non lactose fermenter were isolated. After 24-hour incubation isolated colonies on agar were picked up and subjected to vitek 2 for ID and AST. Out of 60 confirmed *Enterobacteriaceae and* Non lactose fermenter, 25 (41%) isolates were multidrug resistant carbapenem *Enterobacteriaceae* confirmed by Vitek. These confirmed CRE were again subjected to O.K.N.V.I. resist- 5 to detect prevalent gene for carabpenem resistant *Enterobacteriaceae* and non lactose fermenter. It is a rapid in vitro multiplex immunoassay test kit used for the phenotypic detection of blaKPC, blaOXA, blaVIM, blaIMP, blaNDM directly from bacterial colonies.

O.K.N.V.I Resist-5 rapid test

Principle

These tests are ready to use and are based on a membrane technology with colloidal gold nanoparticles. Aimed to detect and identify the carbapenemases from bacterial colony isolate of *Enterobacteriaceae* or non lactose fermenting gram negative bacteria growing on agar plate. O.K.N.V.I. resist-5 is a rapid in vitro multiplex immunoassay for the phenotypic detection and differentiation of five common carbapenemase families (blaKPC, blaOXA-48-like, blaVIM, blaIMP, and blaNDM) directly from bacterial colonies.

Procedure

Prepare one collection tube and add 11 drops of O.K.N.V.I. resist-5 assay buffer in the tube harvest bacteria by taking 3 colonies with a disposable bacteriological loop and dip the loop in the bottom of the tube containing the buffer. The same bacteriological loop can be used to collect the 3 colonies. Stir thoroughly before removing the loop. Vortex the preparation to homogenize. Place the nozzle on to the assay buffer tube. Place the tube 90 degree on the top of sample well and add 3 drops/100 µl sample into sample well. Allow to react for 15 minutes and read the result. TRURAPID® Positive results may be reported as soon as the test and control lines become visible. Do not take the appearance of new lines into account after the reaction time has passed. The result must be read on still wet strip.[15]

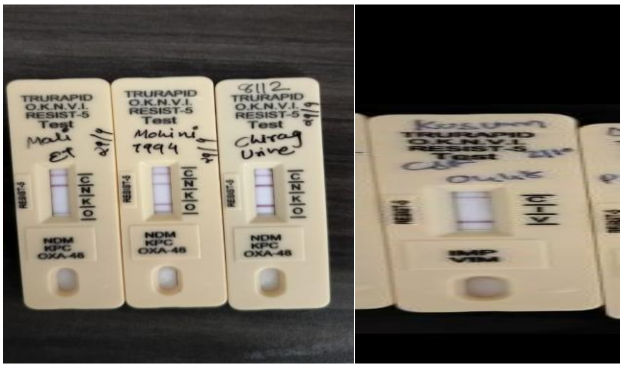

Figure 1: 1st kit shows blaNDM gene, 2nd kit blaOXA, 3rd kit blaNDM, blaOXA and 4th kit blaVIM gene detected.[15]

Interpretation

The results were to be interpreted as follows:

Negative test result: a reddish-purple line appears across the central reading window at the control line (C) position. No other line is present.

Positive test result: in addition to a reddish-purple line at the control line (C), a visible reddish-purple line appears at one of the test lines position ("N" or "K" or "O") on cassette labelled (i) NDM, KPC, OXA-48 or at one of the Test lines position ("I" or "V") on cassette labelled (ii) IMP and VIM. Intensity of the test line may vary according to the quantity of antigens as well as of the variant type present in the sample. Any reddish-purple test line (OXA-48, KPC, NDM, VIM and IMP), even weak, should be considered as a positive result. If a positive test line appears beside of the "O" mark, the sample contains OXA-48 or OXA 48-like variants. If it appears beside the "K" mark, the sample contains KPC variants; beside the "N" mark, the sample contains NDM; the "V" mark, the sample contains VIM; and beside of the "I" mark, IMP is present in the sample. Combinations of positive test lines can occur. In this case the sample contains several carbapenemases.

Invalid test result: the absence of a control line indicates a failure in the test procedure. Repeat invalid tests with a new test device. Note: during the drying process, a very faint shadow may appear at the test line positions. It should not be regarded as a positive result

RESULTS

Study was conducted from September 2025 to October 2025. A total of 100 clinical samples were taken from that 60 (60%) *Enterobacteriaceae* analysed by VITEK 2. Out of them 25 (41%) isolates were multidrug resistant carbapenem *Enterobacteriaceae*. From 25 samples 10 (40%) were urine samples, followed by 5 (20%) blood culture, 5 (20%) pus discharge, 2 (8%) drain fluid, 2 (8%) were ET, 1 (4%) CSF. In my study Out of 25 MDR *Enterobacteriaceae* 10 (40%) were *Klebsiella pneumoniae* isolated 4 NDM, 1 OXA, 5 NDM and OXA, followed by 6 (24%) *Escherichia coli* isolated 1 NDM,1 OXA, 4 NDM and OXA, 3 (12%) *Pseudomonas aeruginosa* isolated 1 VIM and 2 NDM, 5 (20%) *Acinetobacter baumannii* isolates 5 NDM, 1 (4%) *Acinetobacter lwoffii* VIM gene was detected. The highest gene resistant was seen for combination OXA and NDM followed by NDM and OXA.

Table 1: Total number of *Enterobacteriaceae* and non lactose fermenter. Represent multidrug resistant carbapenem-resistant *Enterobacteriaceae*, non lactose fermenter and gene detection rate.

Total number of samples	*Enterobacteriaceae* and non lactose fermenter (n=60)	Multidrug resistant carbapenem-resistant *Enterobacteriacea*, non lactose fermenter (n=25)	Gene detected (n=25)
100	60(60%)	25 (41%)	25 (41%)

Table 2: Total number of carbapenem-resistant *Enterobacteriaceae* and non lactose fermenter organisms and identified genes responsible for carbapenem resistance.

Organisms	Total number of isolated-n=60 (60%)	Carbapenem resistant *Enterobacteriaceae* n=25 (41%)	Gene detected n=25 (41%)
Klebsiella pneumonia	22	10	4 NDM,1 OXA,5OXA and NDM
Escherichia coli	19	6	1 NDM,1 OXA,4 NDM and OXA
Acinetobacter baumannii	9	5	5 NDM
Pseudomonas aeruginosa	9	3	1 VIM,2 NDM
Acinetobacter lwoffii	1	1	1 VIM
Total	60	25	25

Table 3: Total number of carbapenem-resistant *Enterobacteriaceae* and non lactose fermenter organisms responsible for carbapenem resistance isolates from various Specimen.[17]

Organisms	Urine	Pus	ET	Drain fluid	CSF	Blood culture
Klebsiella	5	1	1	1	0	2
E. coli	3	1	0	1	0	1
Pseudomonas Aeruginosa	1	1	0	0	0	1
Acinetobacter baumanni	1	2	1	0	0	1
Acinetobacter lowffii	0	0	0	0	1	0

Table 4: Distribution of MDR carbapenem resistant *Enterobacteriaceae* and non lactose fermenter across ICU, ward and OPD.

	ICU	Ward	OPD
Total number of MDR carbapenem resistant *Enterobacteriaceae*	16 (64%)	8 (32%)	1 (4%)

Table 5: Age wise distribution of MDR carbapenem resistant *Enterobacteriaceae* and non lactose fermenter.

Age wise distribution	Number of patients MDR carbapenem resistant *Enterobacteriaceae* and non lactose fermenter
1-20	4
21-40	3
41-60	7
61 above	11

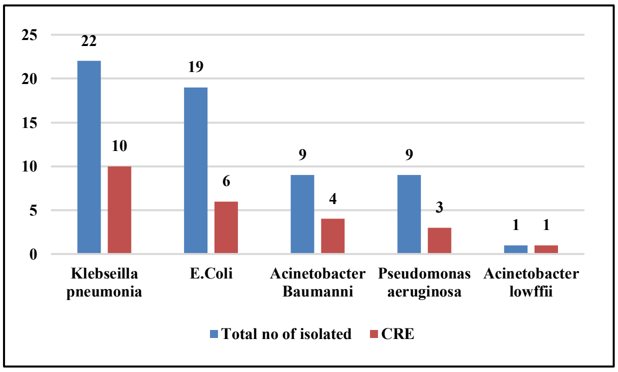

Figure 2: Highest carbapenem resistant *Enterobacteriaceae* and non lactose fermenter isolated from *Klebsiella pneumoniae*.

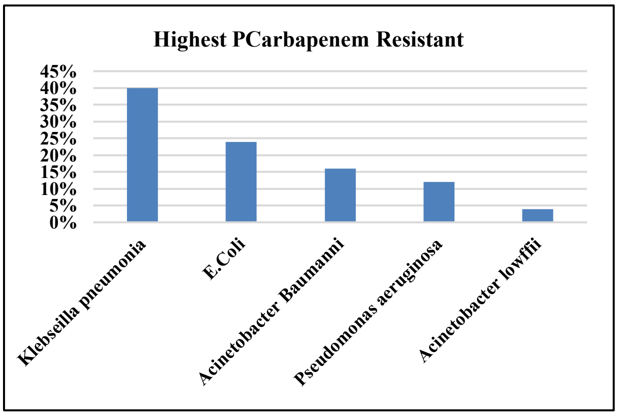

Figure 3: Percentage of carbapenem resistant *Enterobacteriaceae* and non lactose fermenter.

DISCUSSION

Early, rapid identification of the genes causing carbapenem resistance will help to choose appropriate antibiotics early in treating CRE infections. In this study, a total of 100 clinical samples were taken from that 60 (60%) *Enterobacteriaceae* isolated. From them 25 (41%) samples could identify the genes for carbapenemase production. In my study Out of 25 MDR *Enterobacteriaceae* 10 (40%) were *Klebsiella pneumoniae* isolated 4 NDM, 1 OXA, 5 NDM and OXA, followed by 6 (24%) *Escherichia coli* isolated 1 NDM,1 OXA, 4 NDM and OXA, 3 (12%) *Pseudomonas aeruginosa* isolated 1 VIM and 2 NDM, 5 (20%) *Acinetobacter baumannii* isolates 5 NDM, 1 (4%) *Acinetobacter lwoffii* VIM gene was detected. The highest gene resistant was seen for combination OXA and NDM Followed by NDM and OXA.

A similar lateral flow assay, Resist-4 O.K.N.V., developed by Coris BioConcept (Gem bloux, Belgium) (which detects KPC, NDM, OXA-48, and VIM enzymes), is yet to be cleared by the USFDA but has shown good performance for all carbapenemases except NDM enzymes.[17]

Firstly, the widespread use of carbapenems within hospital settings exerts substantial selective pressure, creating a favorable environment for the emergence and dissemination of resistant strains, including CRE. Additionally, the overuse and misuse of antibiotics in clinical practice, characterised by inappropriate prescription practices, incomplete treatment regimens, and antibiotic mismanagement, play a pivotal role in driving resistance development, fostering conditions conducive to the rise of CRE. Furthermore, suboptimal infection prevention and control measures within healthcare facilities contribute significantly to CRE transmission. Insufficient hand hygiene protocols, lapses in sterilization procedures, and overcrowding within healthcare settings collectively promote the dissemination of these resilient strains among patients. Lastly, the use of antibiotics in agricultural practices, particularly in livestock farming for growth promotion and disease prevention, further amplifies the selection and dissemination of antibiotic-resistant bacteria.[18] Similar findings in different study by Giri et al *Klebsiella pneumoniae* isolated OXA-48 (n=1) followed by NDM (n=5), NDM+OXA (n=14) *E. coli* isolated NDM (n=6), OXA-48 (n=2).[19] In the present study, the majority of carbapenem resistant isolates were obtained from general wards (367, 41.5%) followed by ICU (294, 33.2%), NICU/PICU (14, 1.5%) and OPD (209, 23.6%). Different study showed that *Klebsiella pneumoniae* OXA-48 (n=39) followed by NDM (n=18), *E. coli* (n=89), NDM (n=12), KPC (n=9), OXA-48 (n=6). *Klebsiella pneumoniae* isolated (92.64%) [152 out of 164]), *Escherichia coli* (6.09% [10 out of 164]).[6] Among the *Klebsiella* found to be a combination of OXA-48 and NDM [45.40% (69 out of 152)], followed by OXA-48 alone (42.00%) and NDM alone (11.20%).[16] The OKNV assay is an accurate and rapid assay for identifying OXA-48-like, KPC, NDM, and VIM carbapenemases produced by Enterobacteriaceae isolates.[20] These findings on CRE in the present study are highly significant and worrisome. Infections with CRE are difficult to treat as well as the treatment options are limited and costly, imposing additional financial burden on the patients 1 As per WHO Global report on antimicrobial resistance surveillance, two regions from 71 World Health Organisation (WHO) member states reported carbapenem resistance in *Klebsiella sp.* in excess of 50%.

Table 6: Justification of the study to select treatment.

Classification	Carbapenem drugs	Ceftazidime/ avibactam	Meropenem/ veborbactam	Imipenem/ cilastatin/relebactam	Cefiderocol
Class A Esbl (KPC)	Yes	Yes	Yes	Yes	Yes
Class B MBL (NDM, VIM, IMP)	No	Yes	No	No	Yes
Class C (AMPC beta lactmase)	Yes	Yes	No	No	Yes
Class D OXA	No	Yes	No	No	yes

CONCLUSION

The highest gene resistant was seen for OXA-48 and NDM. Knowledge of the prevalence of genes responsible for carbapenemase in a particular region will help in antibiotic stewardship for the selection of targeted antibiotics. This emphasizes the need for control of CRE spread in the community. Early identification and isolation of CRE patients with infection control practices and a strict implementation of antimicrobial stewardship programme with restricted use of carbapenems are of paramount importance in view of prevention of further increase in carbapenem resistance.[21] Research shows that CRE has been widely studied, especially in the context of nosocomial and community-acquired infections. Classified as a critical priority on the WHO list of priority bacterial pathogens, CRE is being intensively researched for its potential impact on global health. Development of new antibiotics is of great importance in the fight against CRE.[22] The most prominent members of the order *Enterobacterales* responsible for community-acquired and healthcare-associated infections are *Klebsiella pneumoniae* and *Escherichia coli*.[23] In conclusion carbapenem resistant gram negative clinical isolates are increasing in rapid rate because of gene transfer throughout the world. In a country like India with huge population, in order to control the spread of these microorganisms, serious steps need to be taken which include contact precautions, hand hygiene, proper medical waste disposal.[6] This study help to clinician to selection of different antibiotics is as a targeted therapy specific multidrug resistant gram negative bacterial infections.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee approval number PUIECHR/PIMSR/00/081734/9220

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