VOLUME - 12, ISSUE - 05, MAY - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Clinical Microbiology

PREVELANCE OF CARBAPENEMASE PRODUCING ORGANISMS AND MOLECULAR RAPID DETECTION OF DIFFERENT GENES FROM VARIOUS CLINICAL ISOLATES IN PATIENTS ADMITTED AT TERTIARY CARE HOSPITALS OF VADODARA

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ABSTRACT

Introduction- Carbapenemase-producing organisms (CPO) have been identified as an urgent healthcare threat. The spread of carbapenemase-producing Enterobacterales (CPE) is a global health problem of great concern. Rapid detection of carbapenemase-producing organisms is clinically desirable for hospital infection control and antibiotic stewardship. Recently, an on-demand polymerase chain reaction (PCR) assay, namely, the Xpert Carba-R assay, that requires less than an hour of turnaround time, had been developed for CPO detection in clinical samples to identify and guide infection control programs to contain the spread of CPO within a hospital. **Objective-** Carba-R assay is a qualitative multiplex real-time PCR method that qualitatively detects and differentiates five common carbapenemase genes (*bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48, and *bla*IMP) directly from clinical samples or purified colonies within approximately 1 hour. This benefits hospitals and patients by facilitating timely Infection Prevention & Control measures, thereby reducing risk of exposure, transmission and bed-days lost. **Materials & Methods-** In this study, the Xpert Carba-R assay was evaluated for detection of the five carbapenemase genes (blaKPC, blaNDM, blaIMP, blaOXA-48, and blaVIM) in total of 40 non duplicate various clinical samples of admitted patients in tertiary care hospitals of Vadodara. **Result-** We performed Carba-R on 40 isolates: 18 blood samples, 06 urine, 05 rectal swabs, 03 Endotracheal secretion, 03 Pus, 02 wound discharge, 01 Bronchoalveolar lavage fluid, 01 sputum, and 01 ERCP stent isolates. 36/40 (90%) isolates had one or more carbapenemase genes. They were as follows: 19/40 (47.5%) both OXA48 and NDM , 12/40 (30%) NDM and 05/40(12.5%) OXA-48. There were 04/40 (10 %) isolates which were Carbapenem resistant on disc diffusion & VITEK but none of the resistant genes were detected possibly due to other resistant mechanism like efflux pump and porin channels. Klebsiella pneumoniae was the most common isolate with CR, 34/40 (85%). The most frequent genes encountered in Klebsiella pneumoniae were both OXA48 and NDM, 19/34 (55.88%), NDM 08/34 (23.53%) followed by OXA 48, 05/34 (14.71%) and Out of 34 Klebsiella isolates, 02 isolates failed to detect any of these five carbapenemase genes. **Conclusion-** Xpert Carba-R assay provides good reliable results for detection and differentiation of five carbapenemase genes in clinical isolates. Compared to bacterial culture followed by PCR identification of resistance genes from colonies, the Carba-R assay reduced turnaround time from 48 to 72 hours to less than 1 hour. Carbapenemase genes were detected by the Carba-R assay in *Klebsiella pneumoniae* (*34/40*), *Escherichia coli* (*5/40*), *Acinetobacter spp* (*1/40*). The Carba-R assay detected 19 both OXA48 and NDM (47.5%), 12 *bla*NDM (30% and 05 blaOXA-48 (12.5%) genes. Laboratory detection of these genes may help improve patient outcomes by tailoring therapy. This study was conducted for understanding the molecular epidemiology of Carbapenemase producing Enterobacteriaceae in a tertiary care hospital. The combined use of the Xpert Carba-R assay and culture produces rapid and reliable results for the active surveillance of CPO in patients.

KEYWORDS : CPO, CPE, Xpert Carba-R

INTRODUCTION

The past 10 years have brought a formidable challenge to the clinical arena, as carbapenems, until now the most reliable antibiotics against *Klebsiella pneumoniae, Escherichia coli*, and other Enterobacteriaceae, are becoming increasingly ineffective. Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) pose a serious threat to hospitalized patients. Moreover, CRE often demonstrate resistance to many other classes of antibiotics, thus limiting our therapeutic options. Furthermore, few new antibiotics are in line to replace carbapenems. This public health crisis demands redefined and refocused efforts in the diagnosis, treatment, and control of infections in hospitalized patients.[1] Adequate treatment and control of CRE infections is predicated upon their accurate and prompt diagnosis from patient samples in the clinical microbiology laboratory.[2] Carbapenems were developed in the 1980s and are derivatives of thyanamycin. In the 1990s, resistance began to occur in *Enterobacteriaceae* to cephalosporins, which used to be the first-line of defense against nosocomial infections. As a result of these incidences of resistance, the use of carbapanems significantly increased.[3]

Carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often used as "last-line agents" or "antibiotics of last resort" when patients with infections become gravely ill or are suspected of harboring resistant bacteria.[4,5,6,7] Several recent studies clearly show that resistance to carbapenems is increasing throughout the world.[8] Carbapenemase producing organisms are rapidly disseminating worldwide, and their presence in tertiary care hospitals poses a significant threat to the management of nosocomial infections.[9] Multi-drug resistant bacteria have become a healthcare problem around the world.[10,11,12,13] One of the greatest antimicrobial resistance challenges for the safe provision of healthcare & infection prevention control professionals is from carbapenemase producing enterobaterales(CPE).[10] Recently, carbapenemase producing organisms (CPO) such as Pseudomonas aeruginosa and Acinetobacter baumannii have become some of the most problematic antibiotic-resistant bacteria in hospital environments.[14] Furthermore, carbapenem-resistant Enterobacteriaceae (CRE), including bacteria such as Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae, are now commonly detected in the community.[15]

CRE arises from one or a combination of following four mechanisms, carbapenemase-producing CRE (CPCRE), production of ESBLs and/or AmpC in combination with porin loss/deficiency non-carbapenemase-producing CRE (non-CP-CRE), carbapenem efflux or mutations in penicillin binding proteins(PBPs)[16], among which CP CRE is the most problematic due to higher level antimicrobial resistance and plasmid localisation of many carbapenemase encoding genes, potentiating the possibility of horozontal gene

transfer.[17,18] Through this mechanism, CP-CRE has been linked to outbreaks of infectious antibiotic resistance in healthcare facilities.[20] A multi-faceted strategy is needed to minimize the worldwide spread of CPO, whereas rapid identification and isolation could help prevent their transmission.[21] Of the several CPO detection methods reported to date,[22] culture-based methods have been most frequently used; however, they have limitations with respect to sensitivity and specificity and have a long turn around time of 24 to 48hours.[23] Molecular tests have been developed to overcome these limitations, and the Xpert Carba-R assay (Cepheid, Sunnyvale, CA) has recently been used for CPO detection.[24] This method is based on a multiplex real-time polymerase chain reaction (RT PCR) technique and has the advantage of detecting blaVIM, blaIMP, blaNDM, blaKPC, and blaOXA-48-like alleles.

- K. pneumoniae carbapenemase (KPC)
- New Delhi metallo-beta-lactamase (NDM)
- Verona integron-encoded metallo-beta-lactamase (VIM)
- Imipenemase metallo-beta-lactamase (IMP)
- Oxacillinase 48 (OXA-48).

Furthermore, the results could be obtained within an hour.[25]

MATERIAL AND METHODS:

A total of 40 clinically significant, consecutive, non-repetitive isolates of Enterobacteriaceae were included in this study. It is a type of cross-sectional study. The isolates were obtained from various clinical samples like blood, urine, rectal swab, Endotracheal secretion, Pus, wound discharge, Bronchoalveolar lavage fluid, sputum, and ERCP stent received for culture and sensitivity in department of microbiology at Unipath Specialty Laboratory LLP, Vadodara.

The specimens were inoculated on MacConkey agar, Nutrient agar and Blood agar and incubated overnight at 37°c. Isolated colonies were identified with characteristic culture growth on media, gram staining and confirmed by biochemical reactions. Few identification of isolated colonies were done by VITEK as per request of clinicians.

The antimicrobial susceptibility testing was performed on Muller Hinton agar by Kirby Bauer disc diffusion method. The result was interpreted as per CLSI (Clinical and Laboratory Standards Institute) guidelines. Organisms showing resistance to one or more carbapenem drugs were identified as carbapenem resistant and were subjected to molecular rapid detection of different carbapenemase genes by Xpert Carba-R.

Xpert carba R is qualitative in vitro test for rapid detection & differentiation of IMP-1, VIM, NDM, KPC, OXA 48. It contains Single use disposable cartridge with PCR reagents in it. It is Simple to perform with the minimal hands on time & short TAT of < 1 hour with Sensitivity of 100% for all genes and Specificity of 100% for KPC & OXA-48, 99.8% for IMP & 99.7% for NDM & VIM

RESULT-

During the study period 40 isolates from various clinical samples were taken from patients admitted at tertiary care hospitals of vadodara, which showed following results.

Table 1 Gender Wise Distribution

GENDER	NO. OF PATIENTS (in %)
MALE	(22/40) 55%
FEMALE	(18/40) 45%

Table 2 Organism Wise Distribution

TYPE OF ORGANISM	NO. OF ORGANISM (in %)
Klebsiella pneumoniae	(34/40) 85%
Escherechiae coli	(5/40) 12.5%
Acinetobacter spp.	(1/40) 2.5%

Table 3 Sample Wise Distribution

TYPE OF SAMPLE	NO. OF SAMPLE (in %)
Blood	(18/40) 45%
Urine	(6/40) 15%
Rectal swab	(5/40) 12.5%
ET Secretion	(3/40) 7.5%
Pus	(3/40) 7.5%
Wound discharge	(2/40) 5%
BAL (Broncho-alveolar lavage)	(1/40) 2.5%
ERCP Stent	(1/40) 2.5%
Sputum	(1/40) 2.5%

Table 4 Gene Wise Distribution

Name of Gene	Number of positive gene (in %)
NDM	(31/40) 77.5%
OXA-48	(24/40) 60%
NDM + OXA 48	(19/40) 47.5%
Only NDM	(12/40) 30 %
Only OXA 48	(5/40) 12.5 %
IMP-1	0 /40
VIM	0 /40
KPC	0 /40
Not detected	(4/40) 10 %

Table 5 Distribution according to Antimicrobial susceptibility to Carbapenems

Carbapenems	No. of Resistant organisms (in %)
Meropenem	(40/40)100%
Ertapenem	(40/40)100%
Imipenem	(40/40)100%
Doripenem	(38/40) 95%

Table 6 Distribution of Outcomes

Outcome	No. of patient (in %)
Discharge	(28/40) 70 %
Death	(11/40) 27.5%
DAMA	(1/40) 2.5%

DISCUSSION-

The main purpose of this study was to demonstrate the performance of Xpert Carba-R assay for the determination of carbapenemase genes in Enterobacteriaceae. Included in the study were the detection rates obtained by testing samples that had been previously confirmed by the disc diffusion test. The final results show that Xpert Carba-R assay performs a 100% accuracy, which justifies that it is a well-suited method for the detection of carbapenemase genes. Ko, Y.J., Kim, J., Kim, HN. *et al.concluded that* The combined use of the Xpert Carba-R assay and culture produces rapid and reliable results for active surveillance of CPO.[26] Hou-He Li in his study concluded that Xpert Carba-R could work as a new clinical diagnostic tool and the gold standard.[27] D. Sheth also found that in his study Klebsiella pneumoniae and OXA 48 were the most frequent isolate and carbapenemase gene.[28]

Mohanty S, Gajanand M, Gaind R in their study concluded that out of 387 isolates (214 K. pneumoniae, 173 E.Coli) tested, 93 (24.03%) were found to be CRE.[29]

Filgona, J., Banerjee, T., & Anupurba, S. conducted a study at tertiary care hospital Varansi, found 159 isolates; comprising of 64 *E. coli* and 75 *K. pneumoniae,* of the multidrug resistant isolates were identified as carbapenem resistant enterobacteriaceae. 50/159(31.4%) isolates were positive for NDM-1 and 44/159(27.7%) for OXA-48, while 17/159(10.7%) co-harboured NDM-1 and OXA-48 like genes.[30]

CONCLUSION-

Carbapenems serve as the 1st line drugs for the treatment of drug resistant gram-negative bacilli (GNB) infections. However, carbapenem resistance (CR) among GNB is quite common in India. CR can be due to enzymes called as

Carbapenemases e.g. (New Delhi Metallo-beta-lactamase (NDM-1), Klebsiella pneumoniae carbapenemase (KPC), oxacillin-hydrolysing β-lactamases (oxacillinases) OXA-48, Verona Integron-Mediated Metallo-β-lactamase (VIM), Imipenemase (IMP) or due to porin channels or efflux pumps. With the availability of novel beta-lactam/beta-lactamase inhibitors (BL/BLIs) specifically acting in presence of some carbapenemase e.g., Ceftazidime avibactam for OX48 producers, laboratory detection of these genes may help improve patient outcomes by tailoring therapy. This study was conducted for understanding the molecular epidemiology of Carbapenemase producing GNB in a tertiary care hospital

- The emergence and global spread of carbapenemase-producing organisms (CPO) is of great concern to health services worldwide
- These bacteria are often resistant to all beta-lactam antibiotics and frequently co-resistant to most other antibiotics, leaving very few treatment options
- Healthcare facilities need the ability to test high-risk patients and get an accurate result quickly during or prior to the admission process for better patient and bed management
- Traditional enriched culture methods are laborious, taking up to 72 hours for a result
- To address this infection control challenge, it is desirable to have a rapid, accurate, and easy to use on-demand PCR test
- A comprehensive test that detects and differentiates the most prevalent carbapenemases gene families (KPC, NDM, VIM, IMP-1 and OXA-48, now covering OXA-181 and OXA- 232) is important for a successful infection control program

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