ORIGINAL ARTICLE

Current trends of Carbapenemase Production Amongst Multi-drug Resistant Gram-Negative Bacteria in a Tertiary-Care Hospital in West Bengal

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Abstract

Background: Carbapemase production, being encoded on plasmids, can lead to transferable resistance and outbreaks with a community/hospital setup. **Objectives:** Our aim was to study the prevalence of Carbapenemase producing multi-drug resistant gram-negative bacteria in different zones of our hospital to aid in antimicrobial resistance surveillance. **Methods and Materials:** All clinical samples sent for culture and sensitivity study during the study period were followed up for detection of Carbapenemase production by phenotypic tests such as Carbapenem Inactivation tests and Carba NP test. Subsequently, molecular testing was done by PCR using five gene targets - bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48} . **Results:** The overall prevalence of Carbapenemase production among strains of *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii* and *Pseudomonas aeruginosa* was 45.25%. *Klebsiella pneumoniae* was found to be the most common Carbapenemase producer (45%). Metallo-β-lactamase was the predominant phenotypic expression and the most common gene was NDM (74%). **Conclusions**: The high prevalence of Carbapenemase producing bacteria in intensive care zones is a cause of alarm. Early detection of resistance, stringent infection prevention and control measures and antimicrobial stewardship are essential to preserve the usefulness of Carbapenems and improve therapeutic outcomes in patients.

Key Words

Carbapenem resistance, Carba NP test, Carbapenemase production, NDM, OXA-48.

Introduction

The global emergence of Carbapenemase producing organisms (CPOs), acquired mainly due to prolonged hospital stay, catheterization or following instrumentation, treatment has become very challenging. Moreover such strains may form biofilms and are usually Multi- drug or Pan drug resistant, decreasing the repertoire of antibiotics left to treat such patients in most tertiary-care hospitals. Other Indian studies conducted during this period reported that most of the infections studied were associated with gram-negative bacteria, especially *Acinetobacter baumannii* and *Klebsiella pneumoniae*.[1,2] Carbapenemase production, being plasmid-mediated can to lead to community or hospital outbreaks.[3,4] The three most clinically relevant carbapenemases are OXA-

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48, IMP / VIM / NDM and KPC.[5, 6] The early detection of cases and geographical niches is warranted to expedite proper isolation and treatment.

Material and Methods

This cross-sectional, analytical single-centre study was carried out in the Department of Microbiology at a tertiary care centre in Kolkata. Isolates from all clinical samples of all patients (admitted in various Inpatient departments (IPDs) and Intensive care units (ICUs) and those visiting various Outpatient Departments (OPDs)) from February 2020 to August 2021, were followed up to detect Carbapenem resistance. The study was performed under the Antimicrobial Resistance Surveillance Network and was approved by the Research Oversight Committee of our Institute. All clinical, non-repetitive samples (from blood, urine, cerebrospinal fluid, swabs, and other body fluids) received during the study period were followed up using conventional or automated culture methods. Conventional methods included the inoculation of samples onto appropriate culture media and aerobic incubation at 37^{o}C as per standard textbooks and ICMR 2019 guidelines.[7, 8] Conventional Antimicrobial Susceptibility Testing (AST) was done by Kirby-Bauer Disc Diffusion method on cation-adjusted Mueller Hinton Agar using Standard Antibiotic discs (HiMedia, India) following CLSI 2021 guidelines.[9] Automations used were BacT Alert 3D® (bioMérieux, USA) system for blood, CSF, other bodily fluids culture; Vitek-2 Compact System® (bioMérieux,USA) for growth identification and AST based on Minimum Inhibitory Concentration (MIC) values.

A total of 150 culture-proven Carbapenem - resistant isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (CR-K.p, CR-E.c, CRAB, CRPsA respectively), were included in the study as they are frequently isolated from severe infections and are resistant to several classes of antimicrobials. Resistance to at least one of the carbapenem discs (Imipenem (IMI) 10 mcg or Meropenem (MER) 10mcg. Disc diameter breakpoint for resistance is d $\leq$ 19 mm for IMI/MER for Enterobacterales; d $\leq$ 15 mm for IMI/MER for *P.aeruginosa* and d" 18mm - IMI, d $\leq$ 14mm- MER for *A.baumannii*) or using MIC breakpoints guiding tables of Vitek®(MIC e $\geq$ 16 mg/L: Resistant breakpoint for all four test isolates for Meropenem and Imipenem) has been considered for Carbapenem resistance in the present study.[9, 10] Relevant patient data, including clinical and present treatment history were extracted from the online Hospital Medical Information System. The 150 isolates of Carbapenem resistant organisms (CROs) were subjected to phenotypic testing either by Carbapenem Inactivation Methods or Carbapenem NP test for in-vitro detection of Carbapenemase production, as discussed below. The samples testing positive for Carbapenemase production were followed up by PCR for molecular categorization.

1 modified Carbapenem Inhibition Test (mCIM) and EDTA- Carbapenem Inhibition Test (eCIM): mCIM is used for detecting carbapenemases in Enterobacterales & *P.aeruginosa*. (mCIM has > 99% sensitivity and > 99% specificity for detection of carbapenemase producing Enterobacterales).[11] eCIM is used together with mCIM to differentiate metallo β-lactamases (MBL) from serine carbapenemases in Enterobacterales . However, mCIM is not recommended in *Acinetobacter baumannii* complex as its sensitivity / specificity was found to be low, as evident in a study by Zhang et al. as 8.24% and specificity 40% respectively and accuracy 49.55%.[12, 13] Therefore CarbaNP was done to detect CRAB exclusively. Amongst the 150 CROs, 100 isolates (excluding CRAB) were tested using this method.

2 Carbapenem NP test (CNPt) by Rapidec® Carba NP, bioMérieux, USA : A total of 50 CRO isolates (including CRAB) were tested by CNPt test. Class A and class B Carbapenemases are identified with consistent precision by CNPt tests as per studies.[14, 15, 16] However, Class D, OXA-48 has low carbapenemase activity and susceptibility to broad spectrum cephalosporins, therefore, phenotypic methods cannot be relied upon for its detection.[14, 17] Also, the zone difference between mCIM and eCIM as well as a positive CNPt test is not sufficient to rule out the existence of a Serine Carbapenemase encoding gene.[18] Genotyping is needed to detect more than one gene expression that may be missed with only CNPt.

3 Genotyping by PCR using Xpert® Carba-R, Cepheid,USA : All phenotypically confirmed CPOs were tested by PCR. This is a real time PCR, the targets included in this assay are bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48} only.

It has shown high sensitivity and specificity (100% and 77%, respectively), with a positive predictive value and negative predictive value of 96% and 100%, respectively.[19, 20]

Results

The total number of isolates of *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* obtained as pure growths in the Microbiology laboratory during the study period was 221, of which, 150 isolates were found to be Carbapenem resistant (CR). Amongst the 150 CR, the most Multidrug Resistant organism was found to be *A.baumannii* 49.3% (74/150), but the highest Carbapenem resistant rate belonged to *K.pneumoniae* 40% (60/150), followed by *E.coli* 28% (42/150), *A.baumannii* 17.3% (26/150), and *P.aeruginosa* 14.6% (22/150). In phenotypic testing for Carbapenemase production, 100 out of 150 isolates gave positive results (100/150 isolates tested by CIM tests and 50/50 isolates by CNPt test). These 100 isolates were then followed up by PCR for molecular identification.

1 Association of Carbapenemase producing isolates with infective syndromes

From OPDs- A total of 22 CPOs were isolated from various OPD samples. The most common samples associated with a CPO were found to be urine and pus collected from various infected sites (10/22) each. It was found that four out of ten CPOs from Urinary samples were associated with complicated Urinary tract infection (cUTI). Most CROs isolated from pus samples were found to be collected from infected stitch sites.

From IPDs - A total of 33 CPOs were isolated from samples from various wards. Here too, the most common sample associated with a CPO was urine (13/33); and five out of the 13 cases were diagnosed as Catheter Associated Urinary Tract Infection (CAUTI) and eight out of 13 were cUTI. It was also found that four out seven wound swabs yielding CPOs were taken from Surgical site infections (SSI).

From ICUs - Amongst the 45 samples received from these zones, 51% (23/45) were endotracheal tube aspirates from Ventilator Associated Pneumonia (VAP) cases and the main isolate was CRAB (11/23). One-third of CPOs isolated from blood samples were associated with a Central line and Clinical Sepsis each. All the urinary CPOs were associated with CAUTI.

Overall, 15 / 17 total isolates of CRAB, 37.7% (17/45) of CR-*K.pneumoniae*, nine / 29 isolates of CR-*E.coli*, and four out of the nine total CRPsA were isolated from Intensive care zones. Overall, urine was the most common sample for CPO isolation from OPDs and IPDs and also the dominant sample type in CR-*E. coli* (10/19). Pus, being the second most common sample type from these zones, was the main sample type in CRPsA (three out of five). Bacteremia was caused by all the four species, with *K. pneumoniae*, 53.3% (eight out of 15) and *A.baumannii* (three out of 15), being the most dominant species. **Figure 1A and 1B** shows the percentage prevalence of CPOs in different zones (OPDs, IPDs and ICUs) and their distribution according to sample type respectively.

2 Prevalence of different carbapenemases in different zones

In this study, phenotypic tests showed the following prevalence of Metallo-β-lactamase producers (MBL)-CR-*K.pneumoniae* 64% (64/100), CR.*E.coli* 29.6% (19/64), CRAB 12.5% (8/64) and CRPsA 12.5% (8/64). Whereas, among Serine Carbapenemase producers, prevalence rates were as follows: CR-*K.pneumoniae* 44.4% (16/36), CR.*E.coli* 27.7% (10/36), CRAB 25% (nine/36) and CRPsA 2.7% (one /36).

Genotyping by Carba R, we got the molecular data of 95/100 isolates, due to the limited scope of the cartridge used. Out of these 96 isolates, it was found that 30.5% (29/95) harboured more than one gene for Carbapenemases. The following order of prevalences - NDM 76.8%(73/95) , OXA-48 37.9% (36/95) and OXA-48 & NDM 17.9%(17/95)- were observed during the study. KPC, VIM, IMP-1 and their combinations were found to be associated with the least number of isolates. The detailed distribution of genotypes among the study isolates is shown in **Figure 2.**

Amongst the 17 CRAB isolates, Carba R gave no molecular data for four isolates; and among those 13 isolates for which it did, nearly all (12/13) were found to harbour the NDM genotype. However, 71.1% (32/45) of CR-*K.pneumoniae* harboured the NDM genotype, followed by OXA-48, which was 42.2% (19/45). Also, among the CR-E.coli isolates, the predominant genotype was found to be NDM (23/28), followed by OXA-48 (14/28). For CRPsA isolates, NDM was the main genotype, isolated from seven out of nine isolates. The detailed molecular data of the test isolates is given in **Table 1.**

We also tallied the genotype results we got from Carba R with the phenotypic expression of the test isolates, and found that 13.8% (five out of 36) of the isolates phenotypically expressing a Serine Carbapenemase harboured genes from Ambler Class B MBL genes (NDM, VIM or IMP). An Ambler Class B MBL gene along with a Class A or D gene were present in 23% (22/95) of the isolates. From these 22 isolates with dual genotypes, 15/22 isolates were found to phenotypically

express MBL and seven out of 22 isolates expressed a Serine Carbapenemase phenotype.

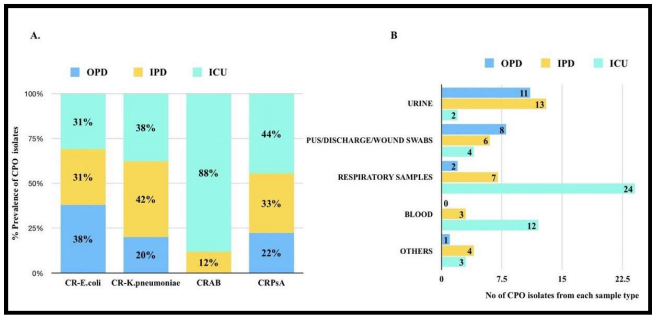

Fig 1: A - Distribution of CPOs in Different Zones (OPDs, IPDs and ICUs) B: Distribution of CPOs According to Sample Type From Different Zones (Respiratory Samples Include Sputum, Endotracheal Aspirates and Bronchoalveolar lavages. Others Include Body Fluids, Implants and Biopsies.)

Discussion

During this study, it was found that most samples were resistant to both Meropenem and Imipenem, and monotherapy with either would be met with clinical failure. While most strains show resistance via Carbapenemase production, some are innately resistant to carbapenems due to presence of efflux pumps and porin channels. Our study corroborated the fact that CPOs are mostly prevalent in the intensive care units of hospitals, where severe infections and invasive procedures predominate. However, the rising numbers of CRO isolation from patients attending various OPDs is a major cause of concern. Asymptomatic carriage of CPOs remains an impertinent issue requiring screening, treatment and isolation of such carriers.

In the present study, the most prevalent CPO in the Intensive care zones was found to be *Klebsiella pneumoniae*, which was also associated with higher Multidrug resistance. The main genotype overall was found to be NDM in this study, thus explaining the high failure rate with regimes comprising â-lactams, aminoglycoside and macrolides. Even though all the isolates tested by Carba R were found to be all Carbapenemase producers by phenotypic tests, four CRAB isolates gave no result by Carba R. This indicates that some other gene/genes, which was/were not included in the testing panel, may be present in such isolates. Other Indian studies that used a wider array of genes, showed the prevalence of Group D Carbapenemases *viz*. OXA-23-like, OXA-58-like, OXA-181 genes.[21, 22] CR-*K.pneumoniae*, harbouring the NDM gene, was found

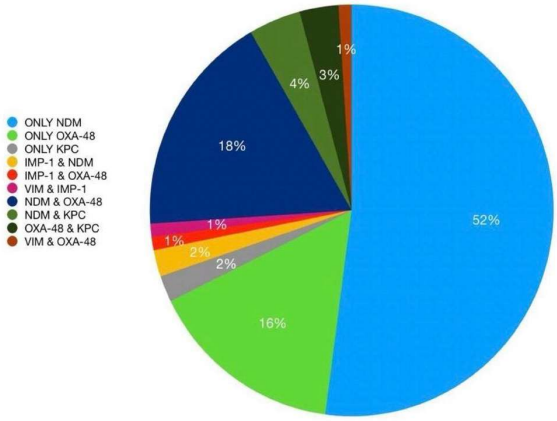

Figure 2: Pie Chart Showing Different Percentage Prevalences of Genes (either singly or in combination) Associated with Carbapenemase Production. NDM: New Delhi Metallo-Beta-Lactamase; OXA-48: Oxacillinase-48; KPC: Klebsiella pneumoniae Carbapenemase; IMP-1: Imipenemase Metallo-beta-lactamase-1; VIM: Verona Integron-encoded Metallo-beta-lactamase.

to be the predominant isolate from isolates from various OPDs and wards in this study. Such isolates are termed as "superbugs" because they are resistant to hydrolysis by almost all clinically available Beta-Lactam antibiotics and are notorious for producing biofilms.[23] The mainstay of treatment of NDM producing strains of Enterobacterales is now a combination of Ceftazidime-Avibactam with Aztreonam [24], which is not always available in many Indian health-care settings. In a few cases, CR-*K.pneumoniae* showed presence of two Carbapenemase types, and in such patients, Ceftazidime-Avibactam with Aztreonam treatment would be particularly beneficial. Avibactam neutralises the Extended Spectrum Beta-Lactamase activity of the organism, allowing Aztreonam to retain its bactericidal effects against NDM producers.[25] The enormous numbers of NDM isolation in this study only shows the widespread dissemination of this gene in our Institute. However, ours was a single centre study and our findings may not be representative of the prevalence of Carbapenemase producers elsewhere. Nonetheless, it was valuable in helping us update our institutional infection prevention and control measures and formulate antibiotic policy.

Even though this was an exhaustive study, there remained some notable limitations that needed to be mentioned. Firstly, since we used only a limited number of Vitek® cards and only routine antibiotics were tested,

Table 1: Distribution of Different Genotypes of Carbapenemase Producing Isolates

	CR-K.p (n=45)	CR-E.c (n=29)*	CRAB (n=17)^	CRPsA(n=9)
NDM	20	13	12	5
OXA-48	7	5	1	2
NDM + OXA-48	8	9	0	0
KPC	2	0	0	0
KPC + NDM	4	0	0	0
KPC + OXA-48	3	0	0	0
OXA-48 + VIM	1	0	0	0
NDM + IMP-1	0	1	0	2
OXA-48 + IMP-1	0	0	0	0

Shows the molecular distribution of CPOs. * Out of 29 CR-E.c isolates, only one isolate got no result on Carba R. ^ Out of 17 CRAB isolates, four gave no results on Carba R.

our results cannot be extrapolated to all currently available cards and antibiotic discs. Secondly, since we tested only four of the more common Gram-negative clinical isolates, there were other Carbapenem resistant isolates as well that need to be considered in future studies. Such organisms include Gram-positive bacteria that were found lesser in numbers and were associated with varying severity of infections. Correlation studies for some 'drug-bug' combinations also need to be investigated to determine the performances of drugs against the prevalent CPOs available in India now. Thirdly, since the study was done during the COVID-19 pandemic, it was affected by several factors that came up during that time, but its effects on different parameters of the study were not included in the scope of the study. The decreased patient footfall post-lockdown, stringent infection control protocols and different comorbid conditions and treatments of patients, resulted in varying isolation of CPOs, and a separate dedicated study is warranted to understand the pattern of Carbapenem resistance post pandemic. Due to the vast numbers of routine clinical samples followed up daily to detect Carbapenem resistance, we could not attempt to resolve discrepancies and investigate the possible reasons behind them through repeat AST or molecular testing.

It is crucial to determine the mechanism of resistance in severe illnesses by molecular testing to analyze the epidemiology of CPOs and design the most effective antibiotic therapy. Diagnostic stewardship is currently the only possible way out of this stark crisis of untreatable infections. Coordination between hospital administration, clinicians and microbiologists is the need of the hour. Moving forward, regular monitoring of the prevalent Carbapenem resistant bacteria at a multicentric level is needed for a better understanding of emerging patterns.

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