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Original Research Article

Prevalence and molecular characterization of carbapenem resistant gram-negative bacilli in a tertiary care hospital in Mumbai

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ABSTRACT

Background: Carbapenem *Resistance* (CR) among gram-negative bacteria is a worldwide problem. It is ranked in the highest priority category in the World Health Organization (WHO) global priority list of pathogens.It is responsible for therapeutic failure in both hospital and community-acquired infections. Identifying and monitoring the local epidemiology of the carbapenem resistant organisms, understanding specific mechanisms underlying the resistance and implementing measures to prevent their spread is the need of the hour.

The aim of the study was to determine the prevalence and molecular epidemiology of Carbapenem resistant Gram negative bacilli in this hospital.

Materials and Methods: A retrospective study was carried out in the Department of Microbiology of a tertiary care Hospital in Mumbai from January to December 2022. All Gram negative bacilli from various specimens were identified and antimicrobial susceptibility was reported using the VITEK2 automated system (BioMerieux, Durham, North Carolina) and interpreted as per CLSI 2022 guidelines. The resistant genes in the isolates were detected by Xpert Carba-R assay (Cepheid, Sunnyvale, CA) on request by a consulting physician.

Result: Out of 2,351 gram negative bacilli isolates, 624 were Carbapenem resistant (26.5%). *Klebsiella pneumoniae* (59.9%) was the most common CR isolate. Molecular testing was done for 190 of these 624 CR isolates. In which New Delhi Metallo beta lactamase (NDM (33.68%) was the most common resistant gene identified followed by NDM & OXA-48(32.63%).

Conclusion: With the prevalence of Carbapenem resistant isolates in the settings, adherence to infection control practices and stringent implementation of antimicrobial stewardship is crucial to curb the rate of infection.

1. Introduction

Gram negative bacilli are the causative agents of various infections including urinary tract, bloodstream, and lower respiratory tract infections.[1] Carbapenem group of drugs are often considered as the last therapeutic option for such infections.[2] A rise in resistance to carbapenems has been observed.[3] Infections due to Carbapenem-Resistant Organisms (CRO) result in longer hospital stays, higher healthcare costs, and increased mortality.[4]

Resistance to this group of antibiotics is mostly mediated enzymatically by production of carbapenemase. These enzymes hydrolyze the β lactam group of drugs. Loss or mutation of porin-encoding genes and overexpression of genes encoding efflux pumps are other non-enzymatic mechanisms for development of carbapenem resistance.[5] Since this resistance is plasmid mediated, the gene responsible for carbapenemase production can be

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easily transferred to other Enterobacteriaceae and Non-Enterobacteriaceae like *Pseudomonas spp*. and *Acinetobacter spp*. This increases risk of spread of resistance among susceptible isolates.[5] The high prevalence of ESBLs and limited options to treat MDR infections has led to an increase in consumption of carbapenems. Long term hospitalization, frequent use of invasive medical devices and medical tourism are other factors that have contributed to the rapid rise in carbapenem resistance.[6]

Carbapenem resistance is associated with resistance to other antibiotics like aminoglycosides and fluoroquinolones, which limits the treatment options for CRO infection.[7] Available options are limited by pharmacokinetic challenges, toxicity, and availability.[8]

Though the prevalence of multi drug resistant organisms varies amongst countries and institutions, the prevalence of CRO in India is an average of 13%.[9] To initiate antibiotic therapy and control CRO infections, early detection of carbapenemase production or other mechanisms of resistance is necessary.

With this background, this study was conducted to determine the prevalence and molecular characteristics of Carbapenem resistant gram-negative bacilli at Nanavati Max Super speciality Hospital.

2. Materials and Methods

A retrospective study was carried out in the Department of Microbiology at Nanavati Max Super Speciality Hospital, Mumbai. Ethics approval was obtained prior to the study. The gram-negative bacilli isolated during the period of 1 year i.e. from January to December 2022 from both IPD (wards and ICU) and OPD areas were included in the study.

Specimens like endotracheal secretion (456), urine (3564), pus (696), sputum (360), sterile body fluids (660) and blood (4896) were plated on MacConkey and Blood agar. Thegram negative bacilli were identified and antimicrobial susceptibility was performed using the VITEK2 automated system (bioMerieux, Durham, North Carolina) and interpreted as per CLSI 2022 guidelines.[10] To determine the mechanism of Carbapenem Resistance, Xpert Carba-R assay (Cepheid, Sunnyvale, CA) was used to detect the production of carbapenemases.[11]

3. Results

Out of the total 10,632 samples processed, gram-negative bacilli was isolated in 2,351 samples. This included *Klebsiella pneumoniae* (1006), *Escherichia coli* (858), *Pseudomonas aeruginosa* (335), *Acinetobacter baumannii* (80), Proteus spp. (55), *Salmonella* (7), *Citrobacter spp*. (3) and *Enterobacter spp*. (7). Out of the 2,351 isolates, 624 were Carbapenem resistant with a prevalence of 26.5% in the hospital.

Out of 624 CRO, *Klebsiella pneumoniae* (59.9%) was most commonly isolated followed by *Escherichia coli* (20.8%), *Pseudomonas aeruginosa* (12.2%), *Acinetobacter baumannii* (6.7%)and *Enterobacter* species (0.32%) (Figure 1).

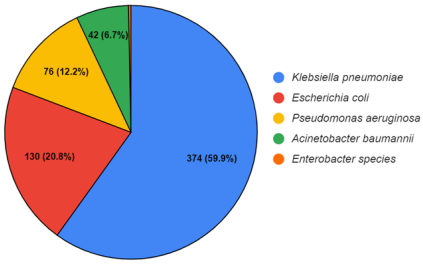

Fig. 1: Species wise distribution of CRO

CRO was isolated most commonly from urine samples (28.4%) followed by blood (23.7%), ET secretions (18.4%), tissue/ pus samples (13.7%), sputum (8.4%) and sterile body fluids (7.4%) in this study.(Figure 2)

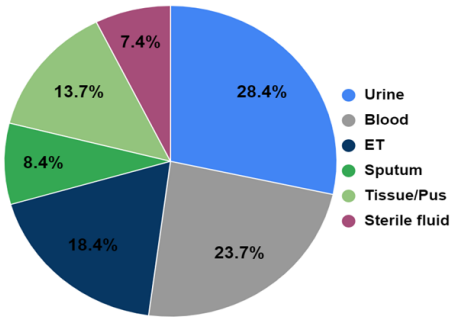

Fig. 2: Specimen-wise distribution of carbapenem resistant strains

Majority of the CRO isolates were from ICUs (57.7%) followed by wards (38.6%) and only 3.68% from outpatient departments (OPDs) i.e. from community settings. (Figure 3)

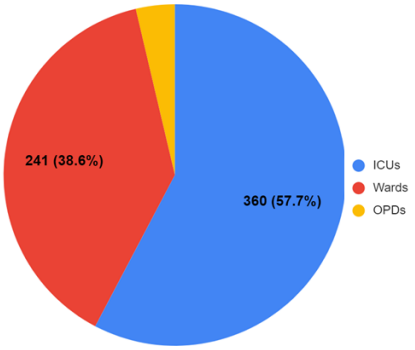

Fig. 3: Department Wise distribution of Isolates and carbapenem resistance

Table 1: Resistant gene wise distribution of carbapenem resistant isolates

Carbapenem Resistant gene	***Escherichia coli* (31)**	***Enterobacter* species (2)**	***Klebsiella pneumoniae* (134)**	***Pseudomonas aeruginosa* (13)**	***Acinetobacter baumannii* (10)**
NDM(n=64)	9	1	43	4	7
OXA 48(n=27)	6	0	19	2	0
NDM & OXA 48(n=62)	9	0	53	0	0
NDM & OXA 48 &VIM(n=4)	1	0	2	1	0
IMP(n=0)	0	0	0	0	0
VIM(n=1)	0	0	0	1	0
KPC(n=0)	0	0	0	0	0
NONE(n=32)	6	1	17	5	3

Xpert Carba-R assay was performed on 190 of these CR isolates to determine the mechanism of resistance. Resistance genes i.e. carbapenemase production was identified in 83.2 % of isolates, whereas in 16.8% isolates, none of the resistant genes were detected.

The most common resistant gene identified was NDM (33.68%) followed by NDM & OXA-48(32.63%), OXA-48 only (14.2%), NDM & OXA-48 &VIM (2.10%) and VIM only (0.52%); KPC and IMP were not detected amongst any isolates. (Table 1).

4. Discussion

In our study, 624 out of 2,351 gram negative isolates were Carbapenem resistant with an overall prevalence of 26.5%. In a similar study conducted from 2010-2015, the prevalence of Gram-negative bacilli was 37.9%, of which 4.2% were Carbapenem resistant.[12] The prevalence of the CR isolates has been rising over time as observed in the present study.

Resistance to Carbapenems in the present study was seen most commonly among *Klebsiella pneumoniae* (59.9%) followed by *Escherichia coli* (20.18%) and then the other non-fermenters. However, studies in Southeast Asia report carbapenem resistant non-fermenters as being the most common CRO followed by a relatively lower prevalence of CREs.[13] It has been reported that 82.3% of all CROs were *A.baumannii* or *P. aeruginosa*, whereas only 17.7% were *K. pneumoniae* or *E. coli*.[14] Similarly, Cai et al. reported that 45% of *A. baumannii* and 19% of *P. aeruginosa* were CR, compared to only 1% of Enterobacteriaceae.[12]

We observed that 0.32% of *Enterobacter* species also showed carbapenem resistance, which is in contrast to the study conducted in another tertiary care hospital in Mumbai, where such resistance has not been reported. In *Salmonella* species, CR is not known, which is similar to the finding in our study.[14]

Carbapenem resistance among pathogens also depends on the site of infection. In our study, the majority of CROs were isolated from urine (28.4%) followed by blood (23.7%). Whereas in other studies CR isolates were more common in urine and pus samples, with lesser prevalence in blood.[14,15]

57.7% of isolates were from ICUs, 38.6% from wards and 3.68% from OPDs which correlates with other studies that also showed a similar pattern.[14,15] A multicentric study conducted across hospitals in India reports that Carbapenem resistance is common in organisms causing infections in Healthcare settings.[16] However, dissemination of CRO in the community is also noticed. Since carriage is a potential risk for the spread of CRE in healthcare settings, routine screening for identification of CRE carriers is needed.[17] Richter et al. Recommend that a hospital policy for routine screening for CRE carriage should be made based on the institution's epidemiology and resources.[18]

Among 190 CR isolates tested by the Xpert Carba-R assay, 83.15% harbored carbapenemase gene.The resistant gene detected most commonly was NDM (33.68%) which is in concurrence with other studies.[19–21] However, 0.5% VIM and 2.14 % co-existence of NDM, OXA-48 and VIM was detected unlike studies which observe absence of VIM gene in their isolates.[9,19,20,22] KPC was not detected in any of our isolates, while studies in the South Asian subcontinent reported the most prevalent carbapenemase gene among *K. pneumoniae* isolates was KPC.[20,23] IMP is commonly responsible for CR in *Acinetobacter* and Pseudomonas, while we did not detect IMP in our isolates.[20,22] Discrepancy was also seen in the prevalence of OXA-48. In contrast to our observation, in *Escherichia coli* it has been reported as the most common gene detected and found to be completely absent in non-fermenters.[20,22] We observed a co-existence of NDM & OXA-48(32.6%), which is much higher compared to a study in South India wherein only 12.5% CR isolates were co-producers of OXA-48 and NDM.[24] Another study conducted in Mumbai reported the Minimum Inhibitory Concentration (MIC) of carbapenems to be greater than 32 μg/mL more commonly in isolates with dual carbapenemase producers. This has clinical implications in countries where colistin is used in combination with carbapenems.[25] No carbapenemase gene was detected in 16.8% isolates, suggesting a

possibility of different mechanisms of resistance other than carbapenemase production. This is much lower compared to a study in which resistant gene was not detected in 30% of isolates.[21]

The exact reason for discrepancy in molecular epidemiology of CR isolates is unclear, but probably the empirical antibiotics used in different regions and the time period of the studies conducted could account for this. Most studies in the Indian subcontinent commonly report and monitor CRE isolates, but we have observed a rising trend of Carbapenem resistance among other non-fermenters also. Lack of molecular testing due to cost refrains, could also account for insufficient data in some regions.[15,16] However, all studies conclude that the prevalence and the trend of carbapenem resistance changes over time and region. Hence, periodic monitoring of the carbapenemase genes helps to optimize antibiotic therapy and to control CRO infections.

5. Conclusion

Carbapenem resistance among the Gram negative bacilli is on the rise and should be monitored closely. A cohort study including a network of hospitals across India would provide a better understanding regarding the changing trend in the CRO prevalence and antibiotic susceptibility patterns. In the absence of new antibiotics and limited therapeutic options, it is important to implement proper antibiotic policy and antimicrobial stewardship so that the antibiotics are used prudently. Also infection control practices should be followed strictly in the hospital to limit the emergence and spread of these multidrug-resistant bacteria.

6. Ethics Approval

Obtained through Letter no.BNH/0248/2023 dated 15^{th} February 2023.

7. Source of Funding

None.

8. Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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