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Phenotyping of carbapenemase to guide Ceftazidime Avibactam and Aztreonam therapy in a tertiary care hospital in Bengaluru

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Abstract

This study examines carbapenemase prevalence in a Bengaluru tertiary care hospital over two years, identifying contributors to the increasing burden of Carbapenem-Resistant Enterobacteriaceae (CRE) in the Indian subcontinent. A 2-year prospective study was conducted using conventional cultures, the VITEK 2 COMPACT automated system, and the modified Carbapenemase Inactivation Method (mCIM) for phenotypic identification. Molecular testing via real-time polymerase chain reaction (RT-PCR) determined carbapenemase genes in 10% of isolates showing phenotypic resistance. The study revealed a 57% prevalence of carbapenem resistance among 6,370 Enterobacteriaceae isolates, with Class B carbapenemases being the most common. The NDM gene was prevalent in 81% of the isolates subjected to molecular testing. Varied resistance rates among clinical samples underscored diverse presentations in different healthcare settings. These findings highlight the urgency for tailored antibiotic strategies, supporting empiric therapy with Ceftazidime-Avibactam and Aztreonam for severe infections, withdrawing Aztreonam if Class B carbapenemases are absent, and reserving Polymyxins for severe, unresponsive sepsis associated with Class D carbapenemases or other resistant mechanisms. This study provides vital insights for effectively managing carbapenemase resistance in a dynamic healthcare landscape.

Keywords: Carbapenemase, Enterobacteriaceae, Antibiotic Resistance.

Introduction

The surge in carbapenemase prevalence is a concerning global phenomenon, burdening healthcare facilities with

heightened infection rates. The CDC refers to Carbapenem resistant enterobacteriacae (CRE) as an urgent threat that is claiming more than 1,000 deaths annually in the USA[1]. The rates of CRE in Tertiary Care Hospitals in India ranges between 18-31%[2], leading to a high burden for hospitals to manage. Countries in the Indian subcontinent have a high burden of CRE that is driven by the spread of NDM-producing strains, but prevalence data are scarce[3]. This paper delves extensively into the prevalence within a Tertiary Care Hospital in Bengaluru over 2 years, with a focus on the identification of carbapenemase that contribute to this growing burden. Carbapenemases are classified into four distinct classes based on their genetic and functional characteristics: Class A (ESBL, Serine Protease Beta Lactamase): This class includes enzymes inhibited by clavulanic acid, except for KPC. Class B (MBL): Metallo-β-lactamases are inhibited by aztreonam. Class C (Amp C, Serine Protease Beta Lactamase): This class includes enzymes resistant to clavulanic acid. Class D (Oxacillinases): Enzymes in this class are susceptible to clavulanic acid. The study culminates with a discussion on selection of antibiotics based on prevalence of resistant patterns in the patient population. This approach not only addresses the mounting challenge of carbapenemase resistance but also highlights the crucial significance of precision in effectively managing and countering these increasingly challenging infections. Treatment of choice for class A & C includes ceftazidime avibactam. Aztreonam is added for Class B strains. Polymyxins are needed for Class D strains. NDM is the most common carbapenemase in the Indian subcontinent (India, Pakistan, Bangladesh), and also possibly in some of the Balkan nations[4]. Identification of the type of carbapenemase is necessary to choose appropriate antibiotics. Identification by molecular methods like PCR is considered to be the gold standard method but the cost of this method is high. Phenotypic identification is less expensive and easy to integrate into the routine process of identification and reporting bacterial isolates from patient samples. This study aims to estimate the prevalence of type of carbapenemase enzymes in bacterial isolates from clinical samples by phenotypic method. The study also aims to estimate the frequency of de-escalation or changes in antibiotics based on the reported carbapenemase type.

Methodology

This study was conducted for a period of 2 years at a tertiary care hospital laboratory in a metropolitan city of India. It was a prospective cross sectional analytical study. Study population included all the samples submitted to the microbiology department for culture and susceptibility testing. The isolation of bacteria was done using the conventional cultures onto blood agar and MacConkey's agar. The identification and susceptibility testing was done using the automated system – VITEK 2 COMPACT. The inclusion criteria for the study was defined as a bacterial isolate belonging to the family Enterobacteriaceae showing resistance to one or more of the clinically used carbapenems. The criteria in Table 1 was used to categorize the isolate as Carbapenem Resistant Enterobacteriaceae (CRE):

Exclusion Criteria: Isolates from repeated samples from the same patient were excluded from the study.

Table 1: Showing criteria to be recognised as CRE

Drug	MIC
Imipenem	>4µg/m
Meropenem	>4µg/m

Ertapenem	>2µg/m

These isolates were subjected to modified Carbapenemase Inactivation Method (mCIM) using EDTA and Phenylboronic acid with meropenem disc (3). A difference of more than or equal to 5 mm in the zone size with the inactivator was considered positive. The interpretation and classification of carbapenemase is shown in Table 2.

Table 2: Interpretation of mCIM results

Result	Type of Carbapenemase
≥ 5 mm with EDTA	Class B (MBL producer)
≥ 5 mm with PBA	Class A or C (Serine protease)
≤ 5 mm with both EDTA & PBA	Class D (OXA) or other mechanisms of resistance like efflux pump. Porin loss etc.

Molecular testing was done for 350 (10%) of the isolates showing phenotypic resistance to carbapenem. Real time polymerase chain reaction (RTPCR) method was used to determine the genes responsible for carbapenemase enzyme production. A multiplex PCR kit from Bruker company (Carbaplex IVD PCR lit) was used to detect genes coding for carbapenemase – OXA-48 LIKE, VIM, NDM, KPC, IMP. Open system PCR machine CFX-96 was used to run the test. A Ct value less than 35 was considered as positive for the presence of gene.

Results

A total of 39682 samples were received in microbiology lab for culture and susceptibility testing from different sections of hospital like ICU, wards, OPDs. 11904 samples yielded bacterial growth out of which 6370 organisms were from Family Enterobacteriaceae. Table 3 shows the frequency of isolating Enterobacteriaceae from different patient samples.

Table 3: Frequency of Enterobacteriaceae in different patient samples

Clinical Sample	Percentage
Urine	83%
Exudate	55%
Blood	67%
Body fluids	54%
Sputum	37%
Miscellaneous (ET secretions, BAL etc.)	41%

3631 bacterial isolates (57%) were found to be resistant to one of the carbapenems. Table 4 shows the frequency of isolating carbapenem resistant organisms from different clinical samples.

Table 4: Frequency of carbapenem resistant organism in different clinical samples

Clinical Sample	No. of CRE	Percentage
Urine	1587	44%
Exudate	1213	33%
Blood	415	11%
Body fluids	164	5%
Sputum	125	3%
Miscellaneous (ET secretions, BAL etc.)	127	3%
Total	3631	100%

Figure 1 shows the type of carbapenemase detected using the mCIM in 3631 isolates.

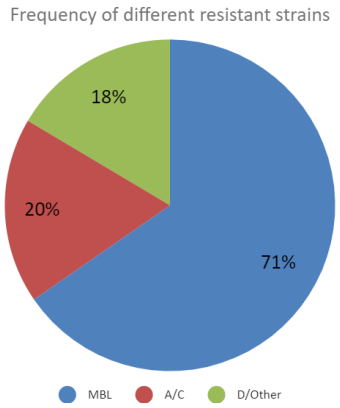

Figure 1: Chart depicting the type of carbapenemase in 3631 isolated detected using the mCIM.

The results of molecular testing is shown in figure 2.

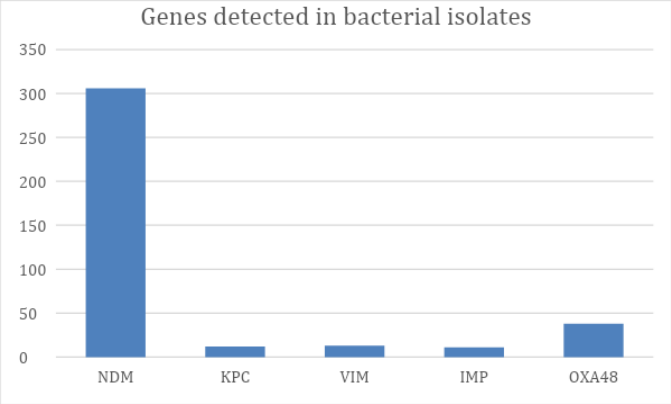

Figure 2: The results of molecular testing via RTPCR to isolate genes responsible for resistance

In 350 isolated tests for carbapenemase genes, 81% of organisms had the NDM gene. 30 isolates had more than 1 gene which is responsible for resistance. The result is consistent with the findings via the Kirby Bauer Disk Diffusion method.

Discussion

In our study the prevalence of carbapenem resistance strains was 57%, indicating that there is heavy burden prevalent in the hospital. In a similar study by Dr Aishwarya Govindaswamy et al. in 2019, 103 e.coli samples were taken to be tested for carbapenemase producing strains in a tertiary care hospital. 94 out of 103 samples showed resistance by the disc diffusion method (91.26%).

In a study by L Sumitra Devi in 2020, 1275 E. coli and K. pseudomoniae were isolated over a 3-year period in rural settings in India. Here 773 (60.6%), 102 (8%), and 28 (2.2%) isolates were detected as ESBL, carbapenemase and MBL producers, respectively. Similar high levels of resistance rates were found in our study which took place in an urban center where the total rate of resistance among the samples over a 2-year period is 57%.

A similar study by Joshi DN et al in a tertiary health center in South India analysed 164 CRE samples were analysed, of which 105 were blood culture (64%), 20 were urine samples (12.2%), 15 were tracheal aspiration (9.1%), 10 were purulent discharges (6.1%) and 14 were collected from other body fluids. Klebsiella pneumoniae ended up being the the most common pathogen isolated, being found in 152 samples (92.64%), followed by E.coli in 10 samples (6.09%), and 2 samples grew Enterobacter(1.21%). When compared to our study, which was done in a similar healthcare setting, it shows the varied presentation of resistance among different types of samples collected, as our study shows a higher burden isolated from urine and purulent body fluid samples.

Conclusion

Given the prevalence of carbapenemases being approximately 57% in all the samples positive for Enterobacteriaceae, empiric therapy with Ceftazidime-Avibactam and Aztreonam in serious infections like sepsis is justified. Aztreonam can be withdrawn from therapy after confirmation that class B carbapenemases are not present. Polymyxins are useful in 18% of patients

who produced either class D carbapenemase or have other mechanisms of resistance. Hence this group of drugs can be considered as reserve drugs/drugs of last resort for severe sepsis not responding to the CAZ-AVI + ATM combination.

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