ORIGINAL RESEARCH

Phenotypic detection of carbapenem resistant Enterobacterales in clinical isolates at a tertiary care hospital

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Received: 11 June, 2023 Accepted: 15 July, 2023

ABSTRACT

Background: In the past decade, there has been a global emergence of Carbapenem-resistant Gram-negative bacilli, especially Enterobacterales. Carbapenem resistance is attributed to the ability of the bacteria to produce Carbapenemases. The aim of the study is to detect Carbapenem-resistant Enterobacterales in different clinical isolates and study Carbapenemase production by phenotypic method in Carbapenem-resistant Enterobacterales. **Methods:** A total of 379 Enterobacterales were isolated from different clinical samples from patients attending OPDs and admitted in wards and ICUs. They were tested for Carbapenem resistance by Kirby-Bauer Disk-Diffusion Method, and then tested for Carbapenemase production by EDTA Disk Synergy Test and Modified Carbapenem Inactivation Method. **Results:** This study was conducted from February 2021 to August 2022. Out of 379 Enterobacterales, 70 (18.47%) were Carbapenem-resistant Enterobacterales (CRE). Maximum Carbapenem resistance was shown by *Klebsiella pneumoniae* at 23.53%. The maximum Carbapenem resistance was seen in age-group of 16 to 45 years and the most number of CRE isolates were from Intensive Care Units. Phenotypic test results indicated that 54.28% (38/70) isolates were positive for Carbapenemase production by either of the phenotypic methods. **Conclusion:** Carbapenem resistance in our study was 18.47% (70/379). This study highlights the use of phenotypic methods to detect Carbapenemase production in Carbapenem-resistant Enterobacterales, which is responsible for multi-drug resistance. This information is relevant for surveillance, to implement infection prevention and control practices and antibiotic policies

Keywords: Prospective study, Enterobacterales, Antimicrobial resistance**,** Carbapenem resistance, Carbapenemase production, Multi-drug resistance, Phenotypic tests, EDTA Disk Synergy Test, mCIM

INTRODUCTION

Infections caused by bacterial isolates that are resistant to antimicrobial agents are emerging as a critical challenge in healthcare. The issue of antimicrobial resistance is well known to be related to unfavourable outcomes for both healthcare facilities and patients. These outcomes include increased mortality, prolonged hospital stays, and costs. Delays in providing effective therapeutic regimes also contributes to antimicrobial resistance[1]**.**

Carbapenemases are enzymes which are able to hydrolyze Carbapenems, as well as other Beta-lactam agents. This renders the drug ineffective. Carbapenemases are associated with resistance to Beta-lactam antibiotics, as well as to other classes of antibiotics, such as Fluoroquinolones and Aminoglycosides. The genes related to Carbapenemase production, reside in highly mobile genetic elements i.e. plasmids. Due to this, their transfer from one microorganism to the other is frequent making them multidrug resistant[2]**.**

The aim of this study was to detect Carbapenem-resistant and Carbapenemase-producing Enterobacterales (CRE), in various clinical samples received in the department of Microbiology from patients attending OPDs and admitted in wards and ICUs at a tertiary care hospital.

METHODS

All clinical samples (blood culture, broncho-alveolar lavage, cerebrospinal fluid, endotracheal aspirate, pleural fluid, pus, sputum, urine, vaginal swab) received in the Department of Microbiology were recruited during the study period from February 2021

to August 2022. These clinical samples were submitted from OPDs, various wards and ICUs from patients of all ages and gender.

The bacteria were identified using standard laboratory method of microbiological culture, colony morphology, Gram's stain and biochemical reaction. A total of 379 Enterobacterales isolates were isolated from various samples during study period.

These isolates were tested for Carbapenem resistance by Kirby-Bauer Disk-Diffusion Method. Carbapenem-resistant isolates, were then tested for Carbapenemase production by EDTA Disk Synergy Test and mCIM Test as per CLSI 2021 guideline[3].

All the statistical analysis was performed using R 4.2.2 Statistical Computing for Windows in R studio 2022.07.0 user interface. The critical levels of significance of the results were considered at 0.05 levels i.e., $P < 0.05$ for statistical significance.

This prospective study was given ethical approval by the Institutional Ethics Committee, Netaji Subhash Chandra Bose Medical College & Hospital, Jabalpur, Madhya Pradesh, India (No. IEC/2020/46).

RESULTS

Among the 379 isolates of Enterobacterales, the maximum number of isolates belonged to *Klebsiella pneumoniae* at 44.85% (170/379), followed by, *Escherichia coli* at 44.06% (167/379), and lastly Klebsiella oxytoca at 11.08% (42/379). The total number of male patients at 56.73% (215/379), outnumbered female patients at 43.27% (164/379).

Table 1: Age-group-wise distribution of Carbapenem resistance in Enterobacterale isolates

Age group (In years)	Total Enterobacterale isolates	Carbapenem-resistant Enterobacterale isolates (%)
Infants (up to 1 year)	42	15 (21.43%)
1 to 15	30	7 (10.00%)
16 to 45	208	26 (37.14%)
46 to 60	70	18 (25.71%)
Above 60	29	4 (5.71%)
Total	379	70

Out of 379, 70 (18.47%) were Carbapenem-resistant Enterobacterales, the maximum resistance was seen in the age-group of 16 to 45 years at 37.14% (26/70),followed by 46 to 60 years at 25.71% (18/70). and then infants (up to 1 year) at 21.43% (15/70). Minimum resistance was seen in the age-group of above 60 years at 5.71% (4/70) (Table 1).

Table 2: Gender-wise distribution of Carbapenem Resistance in Enterobacterales

Gender	Carbapenem-resistant Enterobacterale isolates (%)	Carbapenem-sensitive Enterobacterale isolates (%)	Total Enterobacterale isolates (%)
Male (%)	34 (15.81%)	181 (84.19%)	215 (56.73%)
Female (%)	36 (16.74%)	128 (59.53%)	164 (43.27%)
Total	70	309	379

Table 3: Distribution of Carbapenem-resistant Enterobacterales in different locations of the hospital

Location of sample	Number of Carbapenem-resistant Enterobacterales (%)	Total number of Enterobacterales (%)
Ward (%)	25 (14.45%)	173 (45.65%)
ICU (%)	39 (33.33%)	117 (30.87%)
OPD (%)	6 (6.74%)	89 (23.48%)
Total	70	379

Carbapenem resistance was higher in females at 21.95% (36/164) than in males at 15.81% (34/215) (Table 2). Maximum number of Carbapenem-resistant Enterobacterales isolates were from ICU at 33.33% (39/117), followed by ward at 14.45% (25/173), and then by OPD at 6.74% (6/89) (Table 3).

Table 4: Distribution of Carbapenem-resistant Enterobacterales

Particulars	Enterobacterale isolates	Total number of Enterobacterale isolates	Carbapenem-resistant Enterobacterale isolates
Carbapenem-resistant Enterobacterales (n=70)	*Klebsiella pneumoniae*	170	40 (23.53%)
	Klebsiella oxytoca	42	7 (16.67%)
	Escherichia coli	167	23 (13.77%)
	Total	379	70

The maximum number of Carbapenem-resistant Enterobacterales isolates were *Klebsiella pneumoniae* at 23.53% (40/170), followed by *Klebsiella oxytoca* at 16.67% (7/42), and lastly *Escherichia coli* at 13.77%

(23/167) (Table 4). These Carbapenem-resistant isolates showed multi drug resistance (resistance to one or more drugs of more than three classes of antimicrobials).

Table 5: Phenotypic detection methods of Carbapenemase production in Carbapenem-resistant Enterobacterale isolates

Enterobacterales	Total number of Carbapenem-resistant isolates	Isolates MBL positive by EDTA Disk Synergy Test alone	Isolates positive for Cabapenemase production by mCIM alone (Carbapenemase positive)	Isolates showing Carbapenemase production by both test (EDTA DST and mCIM)
Klebsiella pneumoniae	40	0	4	16
Escherichia coli	23	0	7	8
Klebsiella oxytoca	7	1	2	0
Total	70	1	13	24

In our study, out of total 70 Carbapenem-resistant Enterobacterales. 54.28% (38/70) isolates were positive for Carbapenemase production by either of the phenotypic methods. 34.29% (24/70) isolates were positive for Carbapenemase production by both phenotypic methods. 18.57% (13/70) isolates were positive for Cabapenemase production by mCIM alone and 1.43% (1/70) isolates were Metallo-Beta-lactamase (MBL) positive by EDTA Disk Synergy test alone (Table 5).

DISCUSSION

In our study, based on the data generated from the various clinical samples included in the study, there is a representation of both male and female patient demographics in the region, in a wide range of age-groups comprising of individuals belonging to the age-group of infants, going up to over and above 60 years. The data also has a representation of a wide-range in the type of patient's biological samples and also, the various department of the hospital from where their samples were received.

In our study the prevalence of Carbapenem-resistant Enterobacterales (CRE) was 18.47% (70/379). Similar findings of Carbapenem resistance in Enterobacterales have been reported in the studies by Bhatt et. al.[4], Namitha et. al.[5] at 18.54%, Mohan et. al.[6] at 18.10%. The study by Gupta et. al.[7] reported figures similar to ours, at 22.16% and 17.32%, specific to Meropenem and Imipenem respectively. In the study by Fatehpuria et. al.[8] CRE prevalence was 15.44% which is similar to our study, as this study was done in Gwalior Region of Madhya Pradesh, similarity might be due to similar geographical region. Dissimilar findings have been reported by Nair et. al.[9] at 12.26 %, Rizzo et. al.[10] at 3.2%, Kumar et. al.[11] at 73.1%.

In our study, the maximum number of Carbapenem-resistant Enterobacterales isolates belonged to Klebsiella species, followed by *Escherichia coli*. This is dissimilar to the finding of Namitha et. al.[5].

In our study, the maximum Carbapenem resistance was seen in the age-group of 16 to 45 years at 37.14% (26/70), similar to the study by Namitha et. al.[5], in which the predominant age group for Carbapenem resistance was 21 to 40 years (36.25%).

In this study, Carbapenem resistance was higher in females at 21.95% (36/164) than in males at 15.81% (34/215) which is similar to study by Mate et. al.[12].

Our study showed maximum Carbapenem resistance in admitted patients (ICUs and wards) and maximum 42 isolates belonging to Klebsiella species which was 24.41% (42/172). *Kumari et. al.*[13] also reported the highest Carbapenem resistance in admitted patients in Klebsiella species. *Nair et. al.*[9] reported highest Carbapenem-resistant Enterobacteriaceae isolates in wards, followed by ICUs.

In this study, the most common Enterobacterale isolate from OPDs was *Escherichia coli* (n=49). The prevalence of Carbapenem resistance in OPD was 6.74% (6/89), which is similar to Namitha et. al.[5], and the most common Carbapenem-resistant Enterobacterale isolate from OPDs was *Escherichia coli* (n=3) as well as *Klebsiella pneumoniae* (n=3).

In our study, most common Enterobacterale isolate from wards was *Escherichia coli* (n=74). The prevalence of Carbapenem resistance in wards was 14.45% (25/173) and the most common Carbapenem-resistant Enterobacterale isolate from wards was *Klebsiella pneumoniae* (n=11).

In our study, most common Enterobacterale isolate from ICUs was *Klebsiella pneumoniae* (n=64). This is similar to the study by Gunasekaran[14]. The prevalence of Carbapenem resistance was maximum in ICUs at 33.33% (39/117). This is similar to the study by *Fatehpuria et. al.*[8] and Mate et. al.[12].

Most common Carbapenem-resistant Enterobacterale isolate from ICUs was *Klebsiella pneumoniae*, followed by *Escherichia coli*. This is similar to the study by *Saseedharan et. al.*[15] and *Ramasubramanian et al.*[16] both of which reported the same trend.

With regards to the issue of antimicrobial resistance, there is a clear and critical need of antimicrobial stewardship policies, which need to be created and strictly adhered to. The World Health organization expresses this concern at a global scale[17]. Additionally, there are several studies on this topic, such as those by Carlet[18] and Vadala[19] that also highlight it.

CONCLUSION

In conclusion, the overall prevalence of Carbapenem resistance in Enterobacterales isolates at a tertiary care hospital was found to be 18.47% (70/379). The maximum number of Enterobacterale isolates belonged to *Klebsiella pneumoniae* at 44.85% (170/379). Maximum number of Enterobacterale isolates from OPDs and wards *Escherichia coli* while maximum isolates from ICUs was *Klebsiella pneumoniae*.

Carbapenem resistance was higher in females at 21.95% (36/164) than in males at 15.81% (34/215). Maximum Carbapenem-resistance was seen in the age-group of 16 to 45 years at 37.14% (26/70). Maximum Carbapenem-resistance was seen in ICU patients. Maximum number of Carbapenem-resistant Enterobacterale isolates were *Klebsiella pneumoniae* at 23.53% (40/170). Most common MBL producer was *Klebsiella pneumoniae* at 40.00% (16/40). Most common Carbapenemase-producing organism based on a positive mCIM result, was *Escherichia coli* at 65.22% (15/23). Of the two methods EDTA-DST is easier to perform than mCIM as it is simpler in process, doesn't require a standard ATCC strain, and allows for MBL production screening along with routine Kirby-Bauer Disk Diffusion Method, which is time-efficient in resource-constrained settings. High incidence of multi-drug resistance was observed in Carbapenemase-producing organisms, which pose a major threat to other admitted patients and as well as to the community, as these organisms might spread and can cause outbreaks in community.

CLINICAL SIGNIFICANCE

This study highlights the production of Carbapenemases by various Enterobacterales. Our study provides results for the local geographical region, in context of demographic factors. This information indicates a necessity of following antimicrobial stewardship program guidelines, judicious use of antimicrobial agents and containment of antimicrobial resistance

FINANCIAL SUPPORT

None

LIST OF ABBREVIATIONS

CLSI- Clinical and Laboratory Standards Institute
CRE- Carbapenem-resistant Enterobacterales
EDTA-DST- Ethylenediamine tetraacetic acid Disk Synergy Test
ICU- Intensive Care Unit
MBL- Metallo-Beta-lactamases
mCIM- Modified Carbapenem Inactivation Method
OPD- Out patient department

ACKNOWLEDGEMENT

None.

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