Section: Microbiology

Original Research Article

PHENOTYPIC DETECTION OF ESBL, MBL AND THEIR CO-OCCURRENCE AMONG MDR ENTEROBACTERIACEAE ISOLATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Antibiotic resistance poses a significant challenge for clinicians when treating infections. The detection of ESBL and MBL among the members of Enterobacteriaceae family guides us to use beta-lactam antibiotics carefully. Aim: The study was aimed to detect ESBL and MBL production in MDR Enterobacteriaceae isolates using phenotypic methods.

Materials and Methods: The hospital based cross sectional study was conducted over a period of 6 month from January 2023 to June 2023. A total of 226 Enterobacteriaceae isolates were identified from 8244 clinical samples received during the study period. Antimicrobial susceptibility testing was performed by using the Kirby Bauer disc diffusion method following Clinical and Laboratory Standard Institute (CLSI) guidelines. Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (MDR). Extended spectrum beta-lactamase production was detected by combined disc method using cefotaxime and cefotaxime/clavulanic acid discs. Similarly, metallo beta-lactamase production was detected by combined disc assay using imipenem and imipenem / ethylene diaminetetracetate discs.

Results: Out of a total of 8,244 clinical samples received for bacterial culture and sensitivity testing during the study period, 1,133 samples (13.74%) showed positive culture results. Among these, 226 (19.94%) were identified as Enterobacteriaceae isolates. Of the Enterobacteriaceae isolates, 46 (20.35%) were found to be multi-drug resistant. Out of 46 isolates, 11 isolates (23.91%) were confirmed ESBL producers and 29 isolates (63.04%) were confirmed MBL producers. The co-occurrence of ESBL and MBL was reported in 6 isolates (13.04%). Escherichia coli (54.54%) was the highest ESBL producer followed by Klebsiella oxytoca (27.27%) and Klebsiella pneumoniae (18.18%). Similarly, the highest producer of MBL was Klebsiella pneumoniae (48.27%) followed by Escherichia coli (31.03%), Klebsiella oxytoca (6.89%), Pseudomonas aeruginosa (6.89%), Citrobacter freundii (3.44%) and Citrobacter koseri (3.44%).

Conclusion: The study reveals an alarming rise in the prevalence of ESBL and MBL producing multidrug resistant Enterobacteriaceae. Therefore, it emphasizes the importance of indentifying multidrug resistant Enterobacteriaceae strains to ensure effective treatment for sever and mild bacterial infections.

Keyword: Enterobacteriaceae, MDR, ESBL, MBL, Co-occurrence.

INTRODUCTION

The Enterobacteriaceae is a large family of Gram negative bacteria that plays a significant role in causing both community and hospital acquired infections.[1] The infection caused by them often more concerning because of their higher tendency to develop multi drug resistance .MDR organisms are those organisms that show resistance to any one agent from three or more antibiotic classes.[2]

Beta lactam antibiotics are the most commonly prescribed treatments for Enterobacteriaceae infections. However the rising emergence of resistance to these drugs presents a significant challenge as it reduces effectiveness of current treatment options. Resistance to β-lactam antibiotics can arise through several mechanisms. These include the production of β-lactamases, enzymes that break down the antibiotic; alterations in penicillin-binding proteins (PBPs), reducing their affinity for β-lactams; overexpression of efflux pumps that actively remove the drug from the bacterial cell; and a decrease in membrane permeability, which limits the antibiotic's ability to enter the cell. Each of these strategies allows bacteria to evade the effects of β-lactam antibiotics and continue to proliferate. β-lactamases are enzymes that hydrolyse the β-lactam molecules and render antibiotics ineffective.[3]

There are more than 340 different types of β-lactamases, enzymes that confer resistance to β-lactam antibiotics. These include extended-spectrum β-lactamases (ESBLs), which hydrolyse a broad range of β-lactams; AmpC β-lactamases, which are often inducible and can degrade cephalosporins; and Carbapenemases, which target carbapenems, one of the most potent classes of β-lactam antibiotics.[4]

Extended-spectrum β-lactamases (ESBLs) are a major group of β-lactamases that confer resistance to a range of cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, and cefepime, as well as the monobactam aztreonam. However, ESBLs do not hydrolyse cephamycins or carbapenems. These enzymes can be effectively inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, which restore the activity of β-lactam antibiotics against resistant bacteria.[5]

Carbapenems are used as the drug of choice to treat infections caused by ESBL-producing bacteria. However past few years carbapenem resistance has been reported all over the world. Carbapenem resistance is primarily mediated by metallo-β-lactamases (MBLs), a subgroup of β-lactamases classified as type B enzymes. MBLs require bivalent metal ions for activity and can hydrolyse nearly all β-lactam antibiotics, except monobactams. Notably, MBLs are not inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. These enzymes can be either chromosomally encoded or plasmid-mediated, contributing to the potential spread of resistance through horizontal gene transfer among Gram-negative bacteria. This makes MBL-mediated resistance a significant concern for treatment options and the control of resistant infections.[6]

The prevalence of ESBL & MBL producing Enterobacteriaceae is on the rise, contributing significantly to the spread of multidrug-resistant (MDR) bacteria in both healthcare and community settings worldwide. These pathogens pose a serious challenge to treatment options, as they are capable of resisting the effects of many commonly used antibiotics, complicating efforts to manage infections effectively. Monitoring of clinical isolates is crucial, particularly in tertiary care hospitals, where the potential for cross-transmission to other patients can have a significant impact on public health. Early detection and tracking of resistant strains in these settings are key to preventing widespread outbreaks and safeguarding the broader community. Thus the present study aimed to detect ESBL & MBL production among the clinical isolates of MDR Enterobacteriaceae in a tertiary care hospital.

Objectives

1. To detect multi drug resistance in Enterobacteriaceae isolates
2. Detection of Extended Spectrum Beta Lactamase (ESBL) production among clinical isolates of MDR Enterobacteriaceae
3. Detection of Metallo Beta Lactamase (MBL) production among clinical isolates of MDR Enterobacteriaceae.

MATERIALS AND METHODS

This hospital based cross sectional study was conducted in the Department of Microbiology at Jorhat Medical College and Hospital for a period of 6 month duration from January 2023 to July 2023. A total of 226 consecutive Enterobacteriaceae isolates, identified from 8,244 clinical samples received during the study period, were included in the analysis.

The samples were processed by using standard microbiological techniques. After overnight incubation of the samples in appropriate culture media, colonies were identified with the help of conventional biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method following Clinical and Laboratory Standard Institute (CLSI) guidelines. Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (MDR). Extended spectrum beta-lactamase production was detected by combined disc method using cefotaxime and cefotaxime/clavulanic acid discs. Similarly, metallo beta-lactamase production was detected by combined disc assay using imipenem and imipenem/ethylenediaminetetracetate discs.

ESBL detection: The principle of this method is to measure the inhibition zone around a disk of cephalosporin and around a disk of the same cephalosporin plus clavulanate. Both Cefotaxime (30mg) and Ceftazidime (30mg) disc alone and in combination with clavulanic acid (10mg) were used in this test. The two disks were placed on MHA plate incubated overnight at 37oC. An increase in zone diameter of ≥ 5 mm for cephalosporin with clavulanic acid as compared with the cephalosporin alone was considered as ESBL positive. Klebsiella pneumonia ATCC 700603 (ESBL positive) was used as quality control for ESBL test.

MBL detection: A 10-µg imipenem disk and an imipenem-EDTA disk were on the surface of the agar plate. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after overnight incubation at 37°C. An increase in the zone size of ≥5 mm around the imipenem-EDTA disc as compared to the imipenem disc alone was considered as MBL positive.

RESULTS

During the study period, a total of 8,244 clinical samples were received for bacterial culture and sensitivity testing. Out of these, 1,133 samples (13.74%) yielded positive culture results. Among the positive cultures, 226 samples (19.94%) were identified as Enterobacteriaceae isolates. Notably, 46 of these Enterobacteriaceae isolates (20.35%) were found to be multidrug resistant.

Among the MDR Enterobacteriaceae isolates the most common was Klebsiella pneumoniae (39.13%), followed by Escherichia coli (36.95 %), Klebsiella oxytoca (13.04%) and Pseudomonas aeruginosa (6.52%). Other organisms isolated were Citrobacter koseri (2.17%) and Citrobacter freundii (2.17%) (Figure 1).

Out of 46 isolates, 11 isolates (23.91%) were confirmed ESBL producers and 29 isolates (63.04%) were confirmed MBL producers. The co-occurrence of ESBL and MBL was reported in 6 isolates (13.04 %). Escherichia coli (54.54%) was the highest ESBL producer followed by Klebsiella oxytoca (27.27%) and Klebsiella pneumoniae (18.18%) (Table 1 & Figure 2). Similarly, the highest producer of MBL was Klebsiella pneumoniae (48.27%) followed by Escherichia coli (31.03%), Klebsiella oxytoca (6.89%), Pseudomonas aeruginosa (6.89%), Citrobacter freundii (3.44%) and Citrobacter koseri (3.44%) Table 1 & Figure 3). The ESBL and MBL co production was seen in 13.04% isolates and it was found to be maximum in Escherichia coli (33.33%) and Klebsiella pneumoniae (33.33%) followed by Klebsiella oxytoca (16.66%) and Pseudomonas aeruginosa (16.66%) (Table 1 & figure 4).

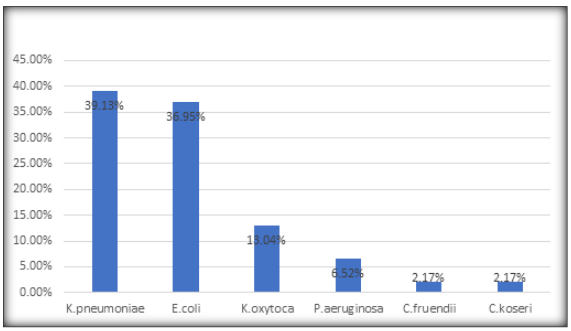

Figure 1: Distribution of MDR Enterobacteriaceae isolates

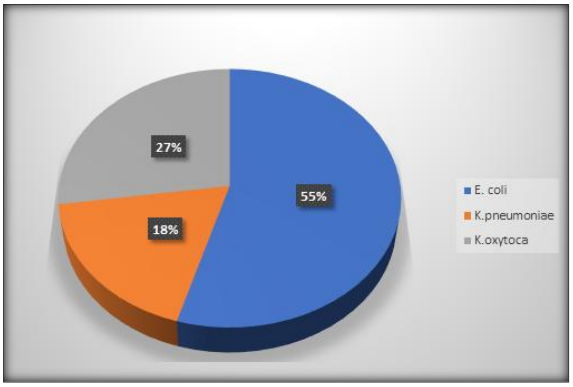

Figure 2: Distribution of ESBL producing isolates

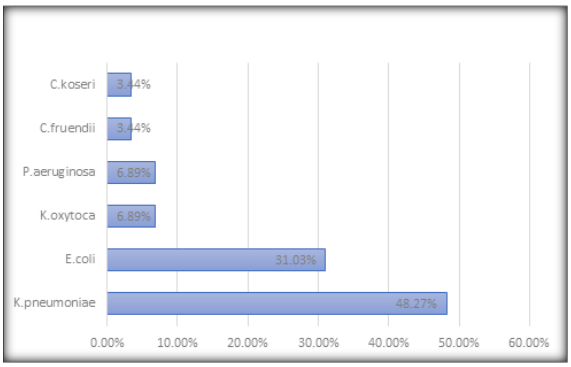

Figure 3: Distribution of MBL producing organism

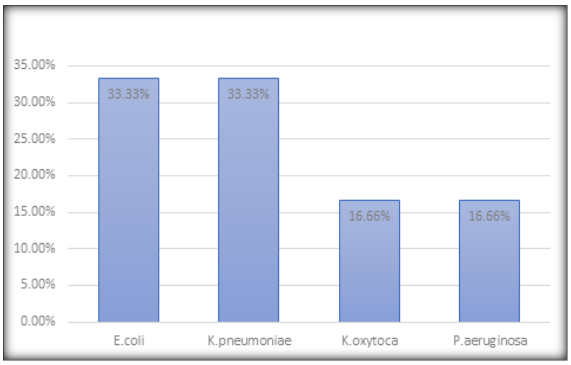

Figure 4: Distribution of ESBL MBL co-producing isolates

Table 1: Distribution of ESBL, MBL & their co-occurrence among the isolates

ISOLATES	TOTAL	ESBL	MBL	CO-OCCURRENCE (ESBL/MBL)
E.coli	17	6 (54.54%)	9 (31.03%)	2 (33.33%)
K.pneumoniae	18	2 (18.18%)	14 (48.27%)	2 (33.33%)
K.oxytoca	6	3 (27.27%)	2 (6.89%)	1 (16.66%)
P.aeruginosa	3	-	2 (6.89%)	1 (16.66%)
C.fruendii	1	-	1 (3.44%)	-
C.koseri	1	-	1 (3.44%)	-
TOTAL	46	11 (23.91%)	29 (63.04%)	6 (13.04%)

DISCUSSION

The rise of multidrug-resistant (MDR) Enterobacteriaceae, particularly those producing Extended-Spectrum Beta-Lactamases (ESBL) and Metallo-Beta-Lactamases (MBL), has important implications for clinical care and public health. The purpose of this investigation was to detect ESBL and MBL production, as well as their co-occurrence in MDR Enterobacteriaceae isolates from a tertiary care hospital. Our results show a significant prevalence of ESBL and MBL producers among MDR Enterobacteriaceae isolates. The identification of these enzymes emphasizes the vital need for ongoing surveillance and strong infection control methods.

In this study, the prevalence of β-lactamases among Enterobacteriaceae was found to be 20.35%. This is considerably lower compared to a similar study by Oberoi L et al. in Amritsar, which reported a prevalence of 70.69%.[7] Among the strains, 23.91% were identified as ESBL producers, and 63.04% were MBL producers. This aligns with other studies, such as the one by Laghawe et al., which reported 19.67% 8 ESBL producers, and Oberoi L et al., who found 35.16% 7 ESBL producers. In a separate study by Lakshmi N.Y., 32% of isolates were MBL producers,[9] while Bandekar et al. reported a lower percentage of 15.7%,[10] Additionally, a study by Thounaojam Salvia et al. reported 58% ESBL producers and 12.8% MBL producers.[11] Again ESBL producer is found to be 63.9% in a study conducted by B. Adane.[12]

The co-occurrence of both ESBL and MBL producers in our study was observed in 13.04% of isolates. This is slightly higher than the 8.79% reported by Oberoi V et al,[7] and significantly higher than the 0.83% reported by Jena J et al,[13] The differences in the prevalence of ESBL and MBL producers across various studies could be attributed to factors such as geographical variation and differences in antimicrobial usage in different institutions.

Regarding the distribution of bacterial species, Escherichia coli was the most prevalent ESBL producer, followed by Klebsiella oxytoca. In terms of MBL production, Klebsiella pneumoniae was the most common producer, with Escherichia coli coming in second. These findings are consistent with a study by A. Shrestha et al., which also found that ESBL production was highest in E. coli followed by K. pneumoniae, and MBL production was most commonly observed in E. coli.[14] Similarly, Oberoi L et al. found that E. coli was the most frequent ESBL producer, but K. pneumoniae was the primary producer of MBL.[7]

CONCLUSION

This study highlights the alarming rise in the prevalence of β lactamases among the Multi-Drug Resistant Enterobacteriaceae isolates in clinical samples. To combat this, therapeutic use of antimicrobials should be monitored which should be based on current guidelines as well as the epidemiological data of the institution. Our findings underline the necessity of strong antimicrobial stewardship programs and infection control practices. Regular screening for ESBL and MBL production should be incorporated into normal diagnostic workflows to quickly detect and isolate infected patients, preventing nosocomial spread. Furthermore, administration of antibiotics guided by susceptibility testing, is critical to preventing the spread of these resistant bacteria.

Infection control practices such as continuous surveillance and implementation of strict hygiene measures in the hospital etc will go a long way in combating the multidrug resistant strains. The presence of ESBL and MBL producing Enterobacteriaceae considerably limits clinicians' treatment options. Carbapenems, which are frequently seen as the last line of defence, are rendered useless against MBL manufacturers. This demands the adoption of other therapy regimens, such as polymyxins and tigecycline, which may have higher toxicity and lower efficacy. The co-occurrence of ESBL and MBL exacerbates the problem, emphasizing the critical need for new antimicrobial medicines and combination therapy.

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