



# Detection Of Multidrug-Resistant (Mdr) And Extended-Spectrum Beta-Lactamase (Esbl) Producing Gram Negative Enterobacteriaceae From Various Clinical Samples In A Tertiary Care Hospital

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**Abstract: Introduction:** Antimicrobial resistance is increasing and becoming a major public health problem worldwide. The continued emergence of multidrug-resistant (MDR) and extended-spectrum beta-lactamases (ESBL) producing Enterobacteriaceae strains lead to the higher rate of morbidity and mortality among hospitalized patients. Obtaining regional drug resistance data is important for developing guidelines for appropriate antibiotic use and to control the emergence of antibiotic resistance. In this background this study aims to determine the prevalence of MDR and ESBL producing Gram Negative Enterobacteriaceae.

**Materials and Methods:** The Present cross-sectional study was carried out in Department of Microbiology, P.D.U. medical college, Rajkot between February 2024 and June 2024. A total of 900 Gram-negative Enterobacteriaceae isolates from various clinical specimens were subjected to antibiotic susceptibility testing as per the Clinical and Laboratory Standards Institute guidelines. **Results:** Out of the total 900 Enterobacteriaceae isolates, ESBLs production was confirmed in 341 isolates with isolation rate of 37.87%. Out of these maximum isolates were Escherichia coli (43.18%) followed by Klebsiella pneumoniae (38.77%). Maximum number of cases 169 (49.56%) were observed in older age group. In our study, 522 (58%) isolates were found to be MDR isolates. Antibiotic sensitivity of ESBL producers was observed highest to Meropenem (100%) and Amikacin (84.75%). **Conclusion:** Isolation rate of MDR and ESBL producing Enterobacteriaceae strains was higher in hospitalized patients which indicates these isolates are vital in health care associated infections and requires proper infection control measures. These isolates have high rate of resistance against commonly used drugs of antimicrobial categories like cephalosporins and fluoroquinolones which indicates to formulate strict antibiotics prescription policy in health care settings.

**Index Terms** - Multidrug-resistant, Extended-spectrum beta-lactamase, Prevalence, Enterobacteriaceae.

## INTRODUCTION

The rapid increase and spread of multidrug-resistant (MDR) and extended-spectrum beta-lactamases (ESBL) producing Gram-negative bacteria (GNB) in hospitals and communities has become the world's greatest threat and this causes community-acquired or hospital-acquired infections [1]. Beta lactam ( $\beta$ -lactam) antibiotics are the most widely used antibiotics in the world. They are used to treat Gram-negative bacteria, especially Enterobacteriaceae. The persistent exposure of the bacterial strains to various  $\beta$ -lactams has led to increased production and mutation of  $\beta$ -lactamases in the bacteria dynamically and continuously.

ESBLs belong to molecular class A group in the Ambler classification and Functional group 2be in Bush Jacoby Medeiros classification [2]. ESBL hydrolyze penicillin, oxyimino-cephalosporins, and monobactams but not cephamycin or carbapenems [1]. ESBL are plasmid mediated enzymes. Because they are plasmid mediated, these enzymes spread rapidly among various bacteria and are important in infection control and in clinical and therapeutic interventions. Hence detection of ESBL production by the clinical laboratory is essential.

MDR is defined as acquired resistance to at least one antimicrobial agent in three or more antimicrobial categories [4]. Bacteria acquire the multidrug resistance either by accumulating resistance expressing genes or by over-expression of genes encoding for multidrug efflux pumps [4].

ESBL producers often develop resistance to antibiotics from various families, including fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, leading to the persistence of multidrug-resistant (MDR) Extended-spectrum beta lactamase producing strains in both clinical and community settings [3]. These enzymes are found mainly in *Escherichia coli* and *Klebsiella pneumoniae*, but are also found in other members of the Gram-negative bacilli [3]. The antimicrobial resistance (AMR) report has been reported that *Escherichia coli* and *Klebsiella* species had reached alarming levels of resistance in various parts of the world; showing resistant to third-generation cephalosporins and carbapenems of up to 54% [4].

Delayed reporting of these MDR and ESBL producing Enterobacteriaceae increase risk of prolonged hospital stays, higher rate of morbidity, mortality and higher medical costs [1]. Information on the antimicrobial resistance among infectious agents is helpful in making appropriate empirical treatment options by updating hospital antibiogram. Therefore, present study is designed to explore the distribution of Multidrug resistant and Extended-spectrum beta lactamase producing Gram Negative Enterobacteriaceae in different age groups and sex.

## MATERIALS AND METHODS:

The present cross-sectional study was conducted at Department of Microbiology, P.D.U. medical college, Rajkot between February 2024 and June 2024 after receiving approval from our Institutional Ethics Committee (IEC). A total of 900 Gram-negative Enterobacteriaceae organisms were isolated and identified from various clinical specimens such as pus, blood, sputum, ear swab, urine, and body fluids. The isolates were confirmed by studying their morphology, colony characteristics and biochemical reactions. The antibiotic susceptibility pattern of these isolates was determined by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines [5].

Antimicrobial susceptibility testing was done with Ampicillin-sulbactam (10/10  $\mu$ g), Piperacillin/tazobactam (100/10  $\mu$ g), Gentamicin (10  $\mu$ g), Levofloxacin (5  $\mu$ g), Ciprofloxacin (5  $\mu$ g), Cotrimoxazole (1.25/23.75  $\mu$ g), Ceftazidime (30  $\mu$ g), Cefotaxime (30  $\mu$ g), Cefepime (30  $\mu$ g), Meropenem (10  $\mu$ g), Amikacin (30  $\mu$ g), Tetracycline (30  $\mu$ g) and Nitrofurantoin (300  $\mu$ g). Resistance to at least one antimicrobial agent in three or more antimicrobial categories was taken as MDR positive.

According to the CLSI guidelines, isolates were screened for ESBL production using ceftazidime (30 $\mu$ g) and cefotaxime (30 $\mu$ g). Screening positive isolates showing ceftazidime <22 mm and cefotaxime < 27 mm were subjected to Confirmatory Combined disc diffusion test by using two combinations of drugs [5]. First was Ceftazidime (30 $\mu$ g) alone and a disc of Ceftazidime in combination with clavulanic acid (30 $\mu$ g/10 $\mu$ g) and second was Cefotaxime (30 $\mu$ g) alone and a disc of Cefotaxime in combination with clavulanic acid (30 $\mu$ g/10 $\mu$ g). Disc of Ceftazidime alone and disc of Ceftazidime with clavulanic acid OR Cefotaxime alone and Cefotaxime with clavulanic acid were placed 25 mm apart, centre to center, on Muller Hinton agar plate. A zone difference of  $\geq 5$  mm around Ceftazidime and Ceftazidime-clavulanic acid OR Cefotaxime and Cefotaxime-clavulanic acid was taken as ESBL positive.

**RESULTS:**

In this study between February 2024 and June 2024, a total of 900 Enterobacteriaceae isolates were isolated from various clinical specimen and studied at our Microbiology department. *Klebsiella pneumoniae* (49%) was most common isolated organism followed by *Escherichia coli* (38.33%), *Proteus mirabilis* (10.67%), *Proteus vulgaris* (1.67%) and *Morganella species* (0.33%). Sample wise analysis showed maximum number of isolates were from pus (42.11%) followed by urine (28.67%), blood (14.67%), sputum (10%), swab (3.89%) and body fluids (0.67%) as shown in Table-1.

Table-1: showing sample wise distribution of isolates of Enterobacteriaceae family

Sample	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>	<i>Morganella species</i>	Total
Pus	170	114	81	11	3	379(42.11%)
Urine	88	151	15	4	0	258(28.67%)
Blood	86	46	0	0	0	132(14.67%)
Sputum	83	7	0	0	0	90(10%)
Swab	11	24	0	0	0	35(3.88%)
Body fluids	3	3	0	0	0	6(0.67%)

Table-2: showing MDR and ESBL producers among isolates of Enterobacteriaceae family

Organism	Total isolates	MDR isolates (Rate of detection)	ESBL producers (Rate of detection)
<i>Klebsiella pneumoniae</i>	441	302 (68.48%)	171 (38.77%)
<i>Escherichia coli</i>	345	218 (63.18%)	149 (43.18%)
<i>Proteus mirabilis</i>	96	2 (2.08%)	21 (21.87%)
<i>Proteus vulgaris</i>	15	0	0
<i>Morganella species</i>	3	0	0
Total	900	522 (58%)	341 (37.87%)

ESBL producing isolates were slightly higher in male (52.2%) than in females (47.8%). Maximum number of cases were observed 49.56% in the older age group (>51 years) followed by 38.42% in adult age group (15-50 years) and then 12.02% in children (<15years).

In our study 522 isolates were found to be MDR, showing resistance to at least one of the agents in three or more antimicrobial categories. Hence the prevalence rate of MDR isolates among Enterobacteriaceae isolates was 58% as shown in Table-2. Highest isolation rate of MDR producing isolates was seen among *Klebsiella pneumoniae* (68.48%) and *Escherichia coli* (63.18%). Both the ESBL production and MDR was higher among the clinical samples collected from inpatient department (78.01% and 75.1% respectively) than outpatient department (21.99% and 24.9% respectively) as shown in Table-3.

Table-3: showing MDR and ESBL producers in various clinical department

Department	MDR isolates (Rate of detection)	ESBL producers (Rate of detection)
Inpatient department (IPD)	392 (75.1%)	266 (78.01%)
Outpatient department (OPD)	130 (24.9%)	75 (21.99%)
Total isolates	522	341

Table-4: showing Antibiotic sensitivity of ESBL producing isolates of Enterobacteriaceae family

Antibiotic	Sensitive (%)	Resistant (%)
Ampicillin-Sulbactam (A/S)	55 (16.12%)	286 (83.88%)
Piperacillin/Tazobactam	216 (63.34%)	125(36.65%)
Cefepime (CPM)	10 (2.93%)	331(97.07%)
Meropenem (MRP)	341 (100%)	0
Levofloxacin	96 (28.15%)	245 (71.85%)
Ciprofloxacin	95 (27.85%)	246 (72.15%)
Amikacin (AK)	289 (84.75%)	52 (15.25%)
Gentamycin (GM)	219 (64.23%)	122 (35.77%)
Tetracycline (TE)	111 (32.55%)	230 (67.45%)
Cotrimoxazole (COT)	101 (29.62%)	240 (70.38%)
Nitrofurantoin (NIT) (Urine only)	61 (83.56%)	12 (16.43%)

ESBL producers among Enterobacteriaceae showed good sensitivity to Meropenem (100%), Amikacin (84.75%), Gentamicin (64.23%), Piperacillin-tazobactam (63.34%) & poor sensitivity to Cefepime (2.93%), Levofloxacin (28.15%), Ciprofloxacin (27.85%), Ampicillin-sulbactam (16.12%), Tetracycline (32.5%), Cotrimoxazole (29.62%) as shown in Table-4.

## DISCUSSION:

Enterobacteriaceae, which were only considered as environmental contaminants previously, have now become important nosocomial pathogens due to emergence and rapid spread of drug resistant Enterobacteriaceae worldwide. In our study, the majority of the total 900 Enterobacteriaceae isolates were *Klebsiella pneumoniae* (49%) followed by *Escherichia coli* (38.33%), *Proteus mirabilis* (10.67%), *Proteus vulgaris* (1.67%) and *Morganella* species (0.33%). Our result is comparable with study result by Shobha et al which reported highest isolation rate of *Klebsiella pneumoniae* (45.62%) [6]. Most of the studies reported that *Escherichia coli* was the most predominant isolate followed by *Klebsiella pneumoniae* among Enterobacteriaceae [7,8,9]. This may be because of different regional prevalence percentage of these organisms.

Maximum number of Enterobacteriaceae isolates were isolated from pus (42.11%) followed by urine (28.67%) which shows that most common infection caused by Enterobacteriaceae were soft tissue infection followed by urinary tract infection. Study by Shashwati et al (62.83%) and Thounaojam et al (67.8%) reported urinary tract infection as most common associated infection [7,9]. This discordance may be due to diverse nature of selection of samples in different institutes.

ESBL positive isolates, which are difficult to identify, pose a great risk due to their role in unnoticed transmission, especially within hospital. So, this study was conducted with the aim of understanding the occurrence of ESBL production among the Enterobacteriaceae. The prevalence of ESBL production among the Enterobacteriaceae in this study was 37.87% which is comparable with the study conducted by Metri et

al (32.1%) [10]. ESBL detection rate is lower than that which reported in studies conducted by Shashwati et al (48.27%) and Thounaojam et al (50.4%) [7,9]. Because of individual studies were done in different parts of the countries with different risk factors, different detection rates were reported in different studies.

Maximum ESBL production rate was detected in *Escherichia coli* (43.18%) followed by *Klebsiella pneumoniae* (38.77%). Our study is in concordance with the studies done by Shashwati et al, Gupta et al. and Thounaojam et al which reported higher ESBL production rate in *Escherichia coli* 50.14%, 44.1% and 52.35% respectively. [7,8,9]. ESBL producers were isolated higher slightly from male (52.2%) than females (47.8%). This result is comparable with studies by Kamlesh kumar et al & Roshan et al which reported higher ESBL isolation from male 56% and 66% respectively. [11,12]. Maximum ESBL producing isolates were observed 49.56% in the older age group (>51 years). Due to subnormal immune system in older age group, Olders are more prone to infections by drug resistant organisms which was also seen in our study. This result is similar to studies done by Kamlesh et al (36.88%) and Shah et al (48%) [11,13]

The prevalence rate of MDR isolates was 58% in this study which is comparable with study by Pooja et al (52.3%) and Ram et al (54.2%) [2,14] but lower than study by Thounaojam et al (76%) and Kamlesh kumar et al (96.84%) [9,11]. Due to non-compliance and incorrect use of antibiotics, there is increased MDR Enterobacteriaceae isolates. Other reason for higher prevalence rate of MDR isolates in developing countries such as India could be the increasing prevalence of diseases, poor diagnostic methods, poor infection control practices, and increasing health care cost. The spread of these MDR isolates among Enterobacteriaceae decrease treatment options. Highest prevalence rate of MDR was detected among *Klebsiella pneumoniae* (68.48%) and *Escherichia coli* (63.18%) in our study which was also reported in study by Thounaojam et al (73.86%) [9].

MDR and ESBL producing strains were isolated higher from inpatients department thus, pointing to the fact that MDR and ESBL are largely a problem of hospitalized patients who share numerous risk factors. More isolation of MDR and ESBL producing strains from inpatients department could be due to misuse of antibiotics in health institutes, prolonged hospital stays, and the presence of invasive devices. Our study result is similar to study by Thounaojam et al (77.6%) and Ram et al. (79.2%) [9,14]

ESBL producers among Enterobacteriaceae showed good sensitivity to Meropenem (100%) which correlates with the study by Shashwati et al. (87.5%) and Ram et al (92.6%) [7,14]. ESBL producing urinary isolates showed higher sensitivity for Nitrofurantoin (83.56%) which is similar to study by Shashwati et al. (82.5%) and Ram et al (86.4%) [7,14]. In present study, low sensitivity was observed for fluoroquinolones; Levofloxacin (28.15%) & ciprofloxacin (27.85%) which correlates with finding of study by Ram et al [14]. Higher resistance to fluoroquinolones may develop due to changes in the target enzymes (DNA gyrase and topoisomerases IV) and alteration in drug entry and efflux mechanism [14]. Broad-spectrum use of fluoroquinolones has been responsible for the rapid emergence of fluoroquinolones resistance. In our study, Poor sensitivity to Cefepime (2.93%), Levofloxacin (28.15%), Ciprofloxacin (27.85%), Ampicillin-sulbactam (16.12%), Tetracycline (32.5%), Cotrimoxazole (29.62%) was observed because Plasmid coding ESBL enzymes may carry co-resistance genes for these antibiotics.

## CONCLUSION:

Thus, to conclude, MDR and ESBL producing Enterobacteriaceae are emerging pathogen So it is important to perform routine screening of these organisms in diagnostic laboratories to decrease the rapid dissemination of these strains in hospital. High degree of antibiotic resistance among ESBL producing isolates indicate the need to rationalize the use of antibiotics in hospital settings and to develop a surveillance system for these organisms to develop empirical treatment guidelines.

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