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# EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE IN A TERTIARY CARE TEACHING HOSPITAL IN ISLAMABAD AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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**Abstract**

*Enterobacteriaceae that produce extended-spectrum beta-lactamases pose a serious threat to public health. The rising rate of antimicrobial resistance caused by ESBL-producing Enterobacteriaceae is leading to treatment failure and economic burden on the Health System. This study aimed to determine the prevalence of Enterobacteriaceae that produce extended spectrum beta lipotoxins (ESBLs), the most common bacterial isolates from different clinical samples, and their antimicrobial susceptibility profile in a tertiary care hospital.*

*Materials and Methods: The study was carried out in the Microbiology section and was a retrospective descriptive cross-sectional study. In the Pathology Department of Dr. Akbar Niazi Teaching Hospital, Islamabad from July 2022 to December 2021. A total of 206 strains of Enterobacteriaceae isolated from different clinical specimens were included by non-probability convenient sampling technique.*

***Results**: Our results showed that the frequency of ESBL-producing isolates was 29.6% in our setup. The majority of the samples were received from the age group 61-70 years followed by the age group 31-40 years (16.4%), 51-60 years (14.8%), and 71-80 years (14.8%). Out of 61 positive isolates, most of them were from males (50.8%) than females (49.2%). The most common sample from which ESBL-producing isolates were obtained was urine (44.3%) followed by pus (21.3%). E. coli was the maximum common ESBL-producing pathogen (85.2%) followed by Klebsiella pneumoniae (11.5%).*

**Keywords:** *Common flora, Enterobacteriaceae, Extended-spectrum beta-lactamase, ESBL, Gram negative, Pathogen*

## INTRODUCTION

Enterobacteriaceae are facultative anaerobes that are gram-negative rods that do not form spores. Enterobacteriaceae is a family containing a large number of members (1). This group now comprises thirty genera and at least 100 species. The family Enterobacteriaceae is abundant and exists in several types of environments for example soil, water, vegetation, and animals (2). They are also present in the human colon as commensal flora. Enterobacteriaceae family includes different kinds of bacterial pathogens that cause infections that are acquired in both the community and hospitals. The members of Enterobacteriaceae include pathogens such as Escherichia coli, Klebsiella, Proteus, Enterobacter, Citrobacter, Providencia, Serratia, Salmonella, and Shigella. E. coli and K. pneumonia are more significant than others. They often colonize the gastrointestinal tract (GIT), but they can also result in severe infections of the blood,

gastrointestinal, urinary, and lower respiratory tracts (3). This family has a significant impact on the world's medical, public health, and veterinary communities than any other collective group of prokaryotic bacteria (5).

Infections caused by the members of Enterobacteriaceae are diagnosed in the laboratory by gram staining and culture of differential media such as MacConkey agar, Different biochemical tests such as the indole test, citrate utilization test, urease test, Triple Sugar Iron (TSI) test, and motility test is used for their identification (5). Escherichia coli is involved in diseases such as septicemia and urinary tract infections. Infections caused by Klebsiella include meningitis, pneumonia, and wound or surgical site infections. Citrobacter is involved in brain abscesses and abdominal sepsis. Salmonella enteric serotype typhi causes typhoid fever. Shigella causes shigellosis. These organisms have acquired different antibiotic resistance genes which confer resistance to multiple antibiotics (6).

Antibiotic resistance has become a major concern globally including in Pakistan. (7) This is due to misuse or inappropriate use of antibiotics. Antibiotic consumption is maximum in low and middle-income countries. By 2050, the world economy is projected to lose $100 trillion due to antibiotic-resistant diseases, which are predicted to kill more than 300 million people (8). Unfortunately, this issue is getting worse because no effective antibiotics are being developed to combat these illnesses. Only a limited number of treatments are available to treat infections caused by Multidrug-resistant (MDR) pathogens. Although using antibiotics for therapeutic purposes is highly recommended, some classes of antibiotics have also been used frequently in food-producing animals in sub-therapeutic doses as growth promoters and/or to stop disease outbreaks on farms, which has been leading to an increase in the selection of resistant bacteria in livestock. Given that human beings can acquire antibiotic resistance through the food chain; this poses a significant threat to public health. Due to the emergence of antibiotic resistance in food-borne illnesses and the increased use of antibiotics in food animal production, the difficulties posed by antimicrobial resistance have become more critical globally, particularly in developing countries (9). Infections caused by resistant bacteria are more difficult to treat and necessitate the use of hazardous and expensive medications. In rare circumstances, bacteria are no longer susceptible to any antibiotic. The use of several antibiotics for livestock growth as well as for medical and veterinary uses as well as other objectives exacerbates the problem of drug resistance (10). Comprehensive efforts are required to decrease the resistance among bacteria to various antimicrobial; agents. For this purpose, scaling up of multidisciplinary approaches is needed in healthcare facilities and agriculture sectors (11).

β-lactams are bactericidal antibiotics containing 4 major groups of antibiotics; penicillin's, carbapenems, cephalosporins, and monobactams. The β-lactam ring comprises of thiazolidine ring with three carbons and one atom of nitrogen. The dihydro thiazine ring and a β-lactam ring are combined in cephalosporins, whereas in carbapenems, the β-lactam ring is attached with a hydroxyethyl side chain, while the monobactams have no additional ring (12). The majority of antibiotic adjuvants used in clinical practice are β- lactamase inhibitors. They are used to overcome antibiotic β-lactam resistance. Despite their prolonged use, β-lactamase inhibitors continued to be the most effective antibiotic adjuvants (more than 70 years) (13). Beta-lactams usually act by inhibiting bacterial cell wall synthesis. The plasmid-mediated β-lactamases hydrolyze and deactivate beta-lactam antibiotics resulting in penicillin's, cephalosporins, and aztreonam resistance. However, beta-lactamase inhibitors like clavulanic acids and sulbactam inhibit β-lactamases. They are plasmid-mediated enzymes and their production is controlled by genes blaTEM-1, blaTEM-2, or SHV-1(14, 15). A rising worrisome trend of ESBL-producing Enterobacteriaceae has been reported. They are rapidly transmitted from one bacterium to another. Another alarming point is that in addition to penicillin and cephalosporin, these organisms are also resistant to fluoroquinolones, aminoglycosides, and sulphonamides in the majority of cases. Carbapenems, colistin, and tigecycline are the last resort antibiotics for such organisms, though carbapenem resistance has also been reported among ESBL-producing Enterobacteriaceae (16).

The increasing frequency of the antimicrobial resistance caused by ESBL-producing Enterobacteriaceae is leading to treatment failure and economic burden. To determine the prevalence of

ESBL-producing microbes, many studies have been conducted globally. Therefore, the goal of the current study was to determine the frequency of ESBL-producing Enterobacteriaceae and the most prevalent bacterial isolates from various clinical samples in Islamabad's tertiary care hospital. Additionally, the antimicrobial susceptibility profile of ESBL-producing isolates was also determined in this study. This would not only tell us about the frequency of ESBL production in our setup but also guide us regarding the treatment options available for these organisms. The objective of our study was to find out the frequency of Enterobacteriaceae that produce ESBL and determine the most common ESBL-producing bacteria isolates.

# MATERIALS AND METHODS

## STUDY DESIGN

The study Design for this study was a retrospective descriptive cross-sectional study conducted in the Microbiology Laboratory at the Pathology Department of Akbar Niazi Teaching Hospital Islamabad, Pakistan. The duration of this study was from July 2021 to July 2022.

The sample size of 206 is calculated using a 16.67% expected proportion of ESBL-producing Enterobacteriaceae, 95 percent confidence interval, and 5 percent margin of error. The following formula was used:

$X= Z2 \times p (1-p)/e2$

(Where, X= sample size, Z= standard normal varies (1.96), p= prevalence (expected proportion) e2=margin of error (0.05), X= (1.96) 2 ×0.16(1-0.16)/ (0.05)2)

This study used a non-probability convenient sampling technique for its sampling and was carried out on Enterobacteriaceae strains isolated from different clinical specimens of patients at Akbar Niazi Teaching Hospital, Islamabad.

## DATA COLLECTION

A total of 206 strains of Enterobacteriaceae isolated from different clinical specimens were included. The study was done after ethical approval from the Institutional Review Board (IRB). The clinical specimens included were urine, blood, pus/wound swab, high vaginal swabs (HVS), sputum, and body fluids such as ascetic fluid, pleural fluid, and CSF, the patient's demographic profile, isolated pathogens, and their antimicrobial susceptibility testing were documented on Proforma.

## SAMPLE INOCULATION

Three different agar plates—blood, chocolate, and MacConkey—were used to inoculate the clinical specimens. On Cystine-lactose-electrolyte-deficient (CLED) agar, a urine sample was inoculated. The plates were incubated for 24 to 48 hours at 35-37ºC. The isolates were identified by colony morphology, gram staining, and biochemical tests. The biochemical tests were the Indole test, citrate utilization test, urease test, triple sugar iron (TSI) test, oxidase, and motility test.

## ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing (AST) was performed by Kirby-Bauer disc diffusion method. Three to five isolated colonies of each strain were mixed in normal saline to make a suspension. The suspension was inoculated on a Mueller-Hinton agar plate. Antibiotic discs will be applied and plates will be incubated for 18 to 24 hours at 35 to 37°C. The zone diameters of antibiotics were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2021. The following antimicrobial discs were applied: amoxicillin-clavulanic acid, cefotaxime and ceftazidime (for detecting ESBL), amikacin, gentamicin, levofloxacin, ciprofloxacin, piperacillin-tazobactam, cefepime, cefoperazone-sulbactam, imipenem, meropenem, trimethoprim- sulfamethaxazole and minocycline. Additional antibiotics were applied: nitrofurantoin and fosfomycin (for urine samples), tigecycline (for pus/wound samples), and polymyxin and colistin for strains resistant to all other antibiotics.

## ESBL DETECTION

It was determined whether ESBL was present in Enterobacteriaceae. In the experiment, a disk containing amoxicillin-clavulanic acid was placed in the middle of a Mueller-Hinton agar plate, with ceftazidime and cefotaxime spaced 15 to 20 mm apart on either side. An increase in the zone towards the center was regarded as ESBL positive. ESBL production is detected by enhanced activity of ceftazidime and cefotaxime with clavulanic acid (Fig. 1).

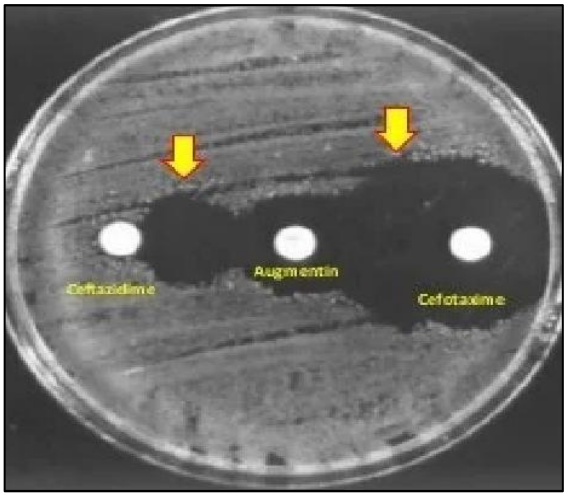


**Fig. 1.** ESBL positive isolate

## STATISTICAL ANALYSIS

Version 22 of the Statistical Package for Social Sciences (SPSS) was utilized to analyze the gathered data. Standard deviation and mean were used to express quantitative variables like age. Frequency and percentage were used to represent qualitative variables like department, ESBL positive, isolated pathogen, and gender. The correlation between ESBL-producing Enterobacteriaceae and various variables, including age, gender, and department, was assessed using chi-square analysis. If a p-value was less than 0.05, it was deemed statistically significant.

## ETHICAL CONSIDERATION

Written informed consent was taken from patients and adequate confidentiality of the research data was ensured.

# RESULTS

## FREQUENCY OF ESBL POSITIVE ISOLATES

Out of a total of 206 isolates of Enterobacteriaceae, 61(29.6%) were ESBL-producing isolates and 145 (70.4%) were non-ESBL-produCING ISOLATES AS SHOWN IN FIG. 2.

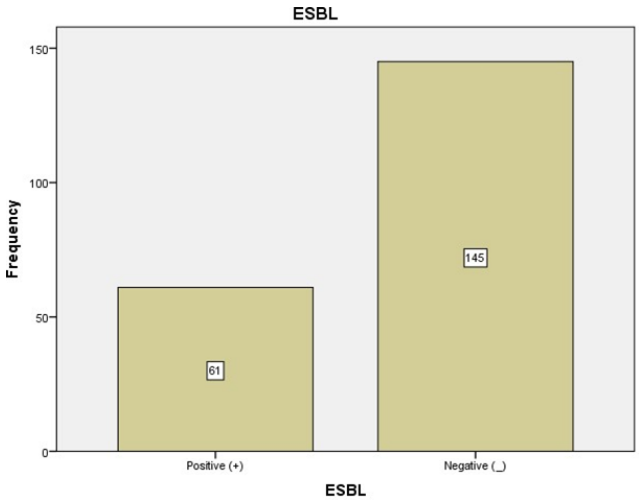


**Fig. 2.** This Graph Represents the Frequency of ESBL-positive isolates

## AGE-WISE DISTRIBUTION OF PATIENTS WITH ESBL-PRODUCING ISOLATES

The mean age of the patients from which ESBL-producing isolates were obtained was 5.15+2.286. The majority of ESBL-producing isolates were received from age group 61- 70 years (18%) followed by 31-40 years (16.4%) years. The age-wise distribution of the patients is shown in Table I.

**Table I.** This table represents age wise distribution of patients with ESBL-producing isolates

| Age of patient | Frequency | Percent |
|---|---|---|
| 1-10 | 3 | 4.9 |
| 11-20 | 7 | 11.5 |
| 21-30 | 7 | 11.5 |
| 31-40 | 10 | 16.4 |
| 41-50 | 3 | 4.9 |
| 51-60 | 9 | 14.8 |
| 61-70 | 11 | 18 |
| 71-80 | 9 | 14.8 |
| 81-90 | 2 | 3.3 |
| Total | 61 | 100 |

## GENDER-WISE DISTRIBUTION OF PATIENTS WITH ESBL ISOLATES

Out of 61 ESBL-producing isolates, 31 (50.8%) were obtained from males and 30 (49.2%) were obtained from females as shown in Fig. 3.

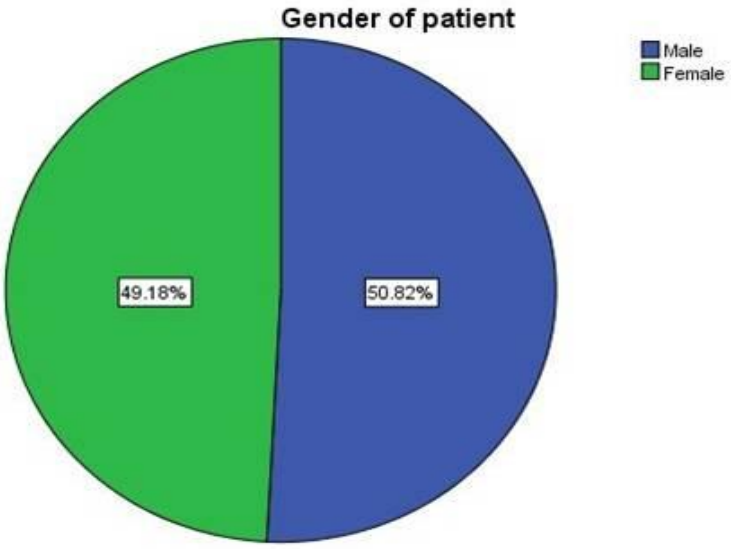


**Fig. 3.** This Graph represents Gender wise distribution of patients with ESBL-producing isolates

## CLINICAL SAMPLES WITH ESBL-PRODUCING ISOLATES

Among 61 ESBL-positive clinical samples, urine was the most common sample from which ESBL-producing isolates were obtained (44.3%). The second most common specimen was pus/wound swab (21.3%) followed by tips (14.8%) (Table II)

**Table II.** This Table represents Clinical samples with ESBL-producing isolates

| Clinical samples | Frequency | Percent |
|---|---|---|
| Urine | 27 | 44.3 |
| Pus/wound swab | 13 | 21.3 |
| Tip | 9 | 14.8 |
| Blood | 5 | 8.2 |
| HVS | 4 | 6.6 |
| Sputum and Tracheal secretions | 2 | 3.3 |
| Body fluid | 1 | 1.6 |
| Total | 61 | 100 |

## DEPARTMENT-WISE DISTRIBUTION OF ESBL-PRODUCING ISOLATES

The majority of the ESBL-producing isolates were received from the Medicine department (45.9%), followed by the Emergency department (21.3%) and the Medical ICU (14.8%). These results are shown in Table III.

**Table III.** Department-wise distribution of ESBL-producing isolates

| Ward of patient | Frequency | Percent |
|---|---|---|
| Medicine | 28 | 45.9 |
| ER | 13 | 21.3 |
| Medical ICU | 9 | 14.8 |
| Surgery | 6 | 9.8 |
| Pediatric ICU | 5 | 8.2 |
| Total | 61 | 100 |

## ESBL-PRODUCING MEMBER OF ENTEROBACTERIACEAE

The most frequent ESBL-producing member of Enterobacteriaceae was Escherichia coli (85.2%). Other ESBL-producing positive pathogens were Klebsiella (11.5%), Salmonella (1.6%), and Proteus (1.6%).

**Table IV.** ESBL producing members of Enterobacteriaceae

| Members of Enterobacteriaceae | Frequency | Percent |
|---|---|---|
| Escherichia coli | 52 | 85.2 |
| Klebsiella | 7 | 11.5 |
| Salmonella | 1 | 1.6 |
| Proteus | 1 | 1.6 |
| Total | 61 | 10 |

## ANTIBIOTICS' SENSITIVITY AND RESISTANCE PATTERNS ACCORDING TO THEIR FREQUENCY AND PERCENTAGE

Out of a total of 61 ESBL-positive clinical samples, the isolates showed 98.4% sensitivity to imipenem and meropenem. The isolates also showed better sensitivity zones to amikacin (88.5%), cefoperazone-sulbactam (82%), and piperacillin-tazobactam (75.4%). Out of a total of 27 urine isolates, nitrofurantoin and fosfomycin sensitivity was shown by 26(96.3%) isolates. Only 1(3.7%) strain was resistant to nitrofurantoin and fosfomycin. Out of 61 clinical samples, only one strain was pan-resistant for which polymycin and colistin were applied. The strain was sensitive to both polymyxin (100%) and colistin (100%). Tigecycline was applied in 13 pus/wound swabs and all the strains were sensitive to tigecycline (100%). The sensitivity pattern of isolates to various antibiotics is shown in Table V.

**Table V.** Sensitivity Pattern of ESBL-producing isolates to various antibiotics

| Antibiotic | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| Amikacin | 54 (88.5%) | 2 (3.3%) | 5 (8.2%) |
| Gentamicin | 32 (52.5%) | 4(6.6%) | 25 (41%) |
| Levofloxacin | 6(9.8%) | 0(0%) | 55(90.2%) |
| Ciprofloxacin | 8 (13.1%) | 0(0%) | 53(86.9%) |
| Piperacillin-Tazobactam | 46 (75.4%) | 1 (1.6%) | 14 (23%) |
| Cefepime | 11 (18%) | 0(0%) | 50 (82%) |
| Cefoperazone-Salbactam | 50 (82%) | 2(3.3%) | 9 (14.8%) |
| Imipenem | 60 (98.4%) | 0(0%) | 1 (1.6%) |
| Meropenem | 60 (98.4%) | 0(0%) | 1(.6%) |
| Minocycline | 31 (50.8%) | 1 (1.6%) | 29 (47.5%) |
| Trimethoprim-Sulfamethaxazole | 13 (21.3%) | 0(0%) | 48(78.7%) |
| Nitrofurantoin | 26(96.3%) | 0(0%) | 1(3.7%) |
| Fosfomycin | 26(96.3%) | 0(0%) | 1(3.7%) |
| Polymycin | 1 (100%) | 0(0%) | 0(0%) |
| Colistin | 1 (100%) | 0(0%) | 0(0%) |
| Tigecycline | 13(100%) | 0(0%) | 0(0%) |

# DISCUSSION

One of the most concerning issues in the field of infectious diseases is the generation of ESBL in Gram-negative bacilli. The bacteria typically exhibit cross-resistance to many of the routinely used antibiotics, which makes the synthesis of this enzyme problematic from a therapeutic standpoint. Although beta-lactam antibiotics are among the safest and most commonly given antibiotics in the world, their effectiveness has been significantly reduced by the advent and global spread of beta-lactam antibiotic resistance in bacteria. Extended-spectrum beta-lactamase production in Enterobacteriaceae is the most prevalent resistance mechanism, with strains being increasingly isolated from throughout the globe (17).

According to a study from Ziauddin University in Karachi, Pakistan, 84.16% of reported cases of Klebsiella pneumonia produce ESBLs. This prevalence was higher than our current study (29.6%) (18). A study showed that the prevalence of ESBL-producing clinical isolates was 8.6%, this result is lower as compared to this study (19). In one study, the prevalence was 14.93%. It was carried out at The Children's Hospital and Institute of Child Health in Lahore in the Microbiology department. This result is low as compared to our study (20). According to a study conducted in three main hospitals of N'Djamena, the capital city of Chad, the prevalence of ESBL-producing clinical isolates was 47.72% (21).

Our results showed that the majority of the clinical isolates were obtained from males (50.8%) than females (49.2%). In another study, 63.6% of the ESBL-producing isolates were reported from males than females (36.4%) (90). in a previous study, the majority of samples were from females (52.5%) than males (47.5%). Another study reported majority of samples from males (50.85) than females (49.2%) (22). In our study, the maximum number of ESBL-producing isolates was obtained from the age group 61-70 years (18%) and the least infected group was 81-90 years (3.3%). In another study, the age group of 11-20 (0.05%) showed the least infection with ESBL and the maximum infected group was 21- 30 (19.7%) (23). our study reported that E. coli was the maximum public ESBL-producing Pathogen (85.2%) followed by Klebsiella (11.5%). Similarly, E. coli was the most common ESBL-producing pathogen (63.8%) followed by K. pneumoniae (21.2%) in another study. One study conducted in Mashhad, Iran showed that E. coli was the leading ESBL-producing isolate, among 336 ESBL-producing isolates, 281 isolates were E. coli (83.6%) and 55 isolates were Klebsiella species (16.4%) (24). Urine was the most common specimen from which ESBL-positive strains were isolated (44.3%). The study at the Department of Microbiology, Manmohan Memorial Medical College and Teaching Hospital (MMTH) in Kathmandu, Nepal also showed that the ESBL-producing Enterobacteriaceae were greatest often isolated from urine samples (51.6%) (25). in our study, the frequency of urine isolates was 27 followed by pus (13), blood (5), HVS (4), sputum (2), and body fluid (1) isolates. In another study conducted in five hospitals in China, the frequency of urine isolate was 36 followed by blood (21), sputum (20), pus (18), body fluid (12), and HVS (2) isolates (26). Similarly, another study reported ESBLs from the clinical samples: urine (344) followed by pus (109), blood (15), tip (10), sputum (12), and body fluids (10) (27). The majority of ESBL-positive samples were received from the Medicine department (28) followed by the medical ICU (9), ER (13), Surgery (6), and pediatric ICU (5). In another study, most of the samples were received from the Surgery ward (110) followed by ICU (63), Pulmonology (27), Neurosurgery (22), Thoracic Surgery (21), Oncology (11), Orthopedic Surgery (9), Cardiology (9), Gastroenterology (8), Endocrinology (7) and Neurology (2) (28). In our study, few samples were received from two different ICUs, 14.8% from the Medical ICU and 8.2% from the Pediatric ICU. In another study conducted in the ICU at Hamad Medical Corporation, Qatar, most of the samples collected were from different ICUs, 29.4% from Medical, 28.5% from Surgical, 16.5% from trauma, 15.6% from Pediatric, and 10% from Neonatal.

The present study found that all ESBL strains tested were 98.4% sensitive to imipenem and 18% sensitive to cefepime. The study found slightly lower sensitivity than earlier research performed at the School of Medical Sciences & Research, Greater Noida, India, which indicated that 100% ESBL strains were sensitive to imipenem and 35.5% sensitive to cefepime (29). In our study, ESBL stains were 88.5% sensitive to amikacin, 52.5% sensitive to gentamicin, 75.4% sensitive to piperacillin-tazobactam, 13.1% sensitive to ciprofloxacin, 9.8% sensitive to levofloxacin and for urine isolates 92.3% sensitive to nitrofurantoin. In

another study conducted at Department of Microbiology, Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, ESBL strains were 88.4% sensitive to amikacin, 63% sensitive to gentamicin, 87% sensitive to piperacillin-tazobactam, 26.8% sensitive to levofloxacin, 17.4% sensitive to ciprofloxacin and for urine samples 83.6% sensitive to nitrofurantion (30). In our study, all ESBL-producing Enterobacteriaceae were the highest resistant to levofloxacin 90.2%, ciprofloxacin 86.9%, cefepime 82%, trimethoprim-sulfamethoxazole 78.7% and minocycline 47.5%, and least resistant to imipenem 1.6%, meropenem 1.6%, fosfomycin 1.6% and nitrofurantion 1.6% All Enterobacteriaceae showed the highest resistance to amoxicillin (89.1%), sulfamethoxazole-trimethoprim (83.6%), and cefotaxime (85.5%), and least resistance to nitrofurantoin (36.9%), imipenem (12.2%) and meropenem (14.6%) in another study (31).

# CONCLUSION

The extended-spectrum beta-lactamase (ESBL) positivity among the members of the Enterobacteriaceae family is high (29.6%) in our study. Among them, Escherichia coli was the most common ESBL-producing isolate (85.2%) followed by Klebsiella pneumoniae (11.5%). This is alarming as these isolates show cross-resistance to a variety of antibiotics. The ESBL-producing isolates showed the best sensitivity to imipenem (98.4%) and meropenem (98.4%) followed by amikacin (88.5%) cefoperazone-sulbactam (82%), piperacillin-tazobactam (75.4%). Nitrofurantoin (96.3%) and fosfomycin (96.3%) are the best alternatives in case of urinary tract infections caused by ESBL-producing Enterobacteriaceae. Tigecycline showed 100% sensitivity in pus/wound swabs and can be used in wound infections. There was no significant association between ESBL positivity and patient age, gender, clinical department, clinical samples, and members of the Enterobacteriaceae family (p-value > 0.05)

The results of this study can be utilized to direct the selection of an appropriate empirical treatment regime for suspected infection with members of the Enterobacteriaceae family. The definitive therapy should be initiated only after culture and antibiotic susceptibility testing. In addition, antibiotics should undoubtedly be prescribed with caution. This would help in combating antibiotic resistance among bacteria and implementing of antibiotic stewardship program.

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## Conflict of Interest:

Authors have no conflict of interest.

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