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# A STUDY ON EXTENDED-SPECTRUM β-LACTAMASE GENES FROM CLINICALLY ISOLATED GRAM-NEGATIVE BACTERIA IN BAHAWALPUR

**Muhammad Rizwan[1], Waqas Ashraf[2], Muhammad Moazam Jalees[2], Rafia Anwar[3], Hafiz Muhammad Siddiq[3], Bushra Nizam[4], Firasat Hussain[5], Saleha Zafar[6], Noman Ahmed[7], Muhammad Hanzala Imran[8], Safdar Ali[9*]**

[1]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan
[2,2,5]Assistant Professor, Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan
[3,3]Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University, Faisalabad, Pakistan
[4]Insititute of Biochemistry, Biotechnology and Bioinformatics (IBBB), The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[6]Consultant Pathologist, Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, Pakistan
[7]QC Manager, Microbiology Lab, Modern Gelatin, Kasur, Pakistan
[8]Department of Medical Laboratory Technology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[9*]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

***Corresponding author:** Safdar Ali
[*]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, Email: safdarali5065012@gmail.com

**Abstract**

The extended-spectrum β-lactamase (ESBL) genes have become more common, increasing the prevalence of multidrug-resistant gram-negative bacteria (GNBs) found in clinical patients. This cross-sectional study aimed to find the antimicrobial susceptibility of clinically isolated GNBs and their correlations with ESBL genes. A total of 140 samples were collected without any age discrimination and proceeded for their growth on culture media, gram staining, and biochemical characterization. Antimicrobial susceptibility testing was done by the Kirby-Baur disk diffusion method and PCR was employed for ESBLs associated genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{TEM}$). Out of 140 samples, the majority (60%) were GNBs, with *K. Pneumoniae* being the most prevalent (38.1%), followed by *E. coli* (29.8%), *P. aeruginosa* (15.5%), *Proteus spp* (10.7%), and *Citrobacter spp* (6%). These GNBs were resistant to various antibiotics, including cefixime (70.2%), amoxicillin (50%), cefoperazone (21.4%), imipenem (17.9%), ceftaroline (89%), gentamicin (72.6%), tobramycin (64.3%), amikacin (52.4%). Individual ESBL gene frequencies were *bla*$_{CTX-M}$ (40.5%), *bla*$_{TEM}$ (27%), *bla*$_{OXA}$ (21.6%), and *bla*$_{SHV}$ (10.8%). Species-wise, ESBLs-producing genes *bla*$_{CTX-M}$, and *bla*$_{TEM}$ were most frequent in *K. Pneumoniae* (13.5%) and *E. coli* (27%) while *bla*$_{OXA}$ in *P.*

*aeruginosa* (13.5%) and $bla_{SHV}$ in *Proteus spp* (2.7%). In conclusion, $bla_{CTX\text{-}M}$ was the main gene associated with ESBL production in resistance clinical isolates followed by $bla_{TEM}$, $bla_{OXA}$, and $bla_{SHV}$ genes. To avoid resistance isolates, hospitals must implement infection control and antibiotic stewardship plans.

**Keywords**: ESBLs, gram negative bacteria, disk diffusion method, (PCR), CTXM, TEM

## Introduction

β-lactamases are plasmid-mediated enzymes predominantly produced by gram-negative rod-shaped bacteria. These enzymes propagate resistance to numerous antibiotic classes, such as penicillin (ampicillin), broad-spectrum cephalosporins (cefotaxime and ceftazidime), and monobactam (aztreonam) [1, 2]. β-lactamases are enzymes that are required for GNB resistance to β-lactam drugs. They accomplish this by cleaving the β-lactam ring, rendering β-lactam antibiotics inactive [3,4]. GNBs that produce ESBLs are becoming more common in urinary tract infections (UTIs) in both clinical and community settings. This poses a significant treatment difficulty. [5–7]. Carbapenemase, ESBLs, or AmpCs are frequently produced by GNB as β-lactamases. *blaTEM-1* was initially discovered in the 1960s as a plasmid-mediated β-lactamase (bla) gene [8]. After that, in 1983, an ESBL-coding genes group was detected in *Serratia marcescens* and *K. pneumoniae*, resulting from a specific mutation occurring in the $bla_{TEM}$ and $bla_{SHV}$ genes [9]. In the past, over 600 β-lactamase variants exhibited resistance to β-lactamase inhibitors such as sub-β-lactam, tazobactam, and clavulanic acid. These included $bla_{CTX\text{-}M}$, *blaOXA*, $bla_{TEM}$, and $bla_{SHV}$ variants [10, 11]. Based on protein homology, β-lactamases are divided into four distinct types. *TEM*, *CTX-M,* and *SHV* belong to the serine-based β-lactamase group, while AmpC and *OXA* belong to the D group. Metallo-β-lactamases belong to Group B [12].

The genotypes $bla_{TEM}$ and $bla_{SHV}$ were identified as more prevalent in ESBLs-producing GNB [13]. However, the class A ESBLs have been a significant increase in the occurrence of the *blaCTX-M* gene [14]. Its genotype has become the prevailing variant observed on a global scale. The *CTX-M*-derived ESBLs exhibit greater catalytic efficiencies towards cefotaxime compared to ceftazidime [2, 15]. ESBLs, *CTX-M* type strains are typically resistant to aminoglycosides and fluoroquinolones, whereas strains with *TEM* and *SHV* type ESBLs are additionally resistant to trimethoprim/sulfamethoxazole and tetracycline [16,17].Conditions favorable to the development and uncontainable spread of AmpC genes encoding and ESBL enzymes have been created by the intensive use of antibiotics, insufficient compliance with appropriate hygiene protocols in healthcare settings, and lack of consistent surveillance of drug resistance trends [18].

The presence of ESBL-producing bacteria with resistance to routinely used antibiotics poses a concerning challenge in clinical settings for the treatment and management of infections. There exists a notable deficiency in the knowledge and understanding among both the public and healthcare professionals on the incidence of these diseases and the potential ramifications associated with managing and controlling of those infections [19]. The prevalence of ESBL-producing bacteria has increased significantly in current clinical settings due to insufficient laboratory identification, reporting, and prevention efforts [20, 21]. The WHO has expressed deep concern about the increasing prevalence of antibiotic resistance in developing nations, with a particular attention to Pakistan [2]. The WHO has called on these countries to address this issue and find effective solutions. Various research groups have undertaken research projects in various regions of Pakistan [22] such as Multan (2023) [24], and Lahore (2022) [23]. However, not any study was conducted in Bahawalpur, which is in the Pakistani South region of Punjab. Consequently, this study's significance exists due to insufficient epidemiological data and the incidences of ESBL producers, and their susceptibility patterns to antimicrobial agents, within our population. In considering these facts, a study was undertaken to ascertain antibiotic susceptibility profiles of ESBL-producing isolates used to treat infections caused by those isolates. Additionally, an assessment was conducted to determine the frequencies of ESBL genes ($bla_{SHV}$, $bla_{OXA}$, $bla_{TEM}$, *blaCTX-M*) in clinical isolates obtained from Bahawal Victoria Hospital in Bahawalpur, Pakistan.

## Methods and Materials

### *Sample Collection, Preservation, and Transportation*

Eighty-four ($n$ = 84) clinical isolates producing ESBLs were isolated from urine, blood, tracheal aspirates, mucus, and sputum. Without regard to age or gender, samples were collected from patients admitted to various wards of Bahawal Victoria Hospital in Bahawalpur, Pakistan, including emergency, urology, the intensive care unit (ICU), medicine, surgery, cardiology, and gynecology. Blood culture vials (BD BACTECTM Plus Aerobic/F, New Jersey, United States) were obtained for blood samples, while urine and sputum samples were collected in sterile containers), mucus trapper for tracheal aspirants using negative pressure through an automated machine, and pus samples were collected around the wound using sterile swabs [25,26]. Within 2 to 4 hours, urine samples were delivered to the laboratory at ice packs, while Blood culture bottles (BD BACTEC TM Plus Aerobic/F, New Jersey, USA) were kept at 20-24$^0$C for 4 - 8 hours. Urine samples were kept in a refrigerator at range of 2-8°C after processing, and blood culture bottles were kept at 20-24$^0$C for one week [26].

This study was designed to be cross-sectional and carried out at the Cholistan University of Veterinary and Animal Sciences in Bahawalpur, Pakistan, between December 2022 and February 2023, with approval from the Microbiology Department's Institutional Review Board (IRB).

### *Inoculation of a Sample and Bacterial Identification*

The other samples (sputum, pus, tracheal aspirants) were inoculated on chocolate, blood, and MacConkey agar (Oxoid, Basingstoke Hampshire, United Kingdom) while urine The urine specimens were inoculated on CLED agar (Cysteine Lactose Electrolyte Deficient Media, Oxoid, Basingstoke Hampshire, United Kingdom) [27]. Blood culture vials were subjected to a 24-hour incubation period at room temperature before the subsequent step of subculturing onto chocolate, blood, and MacConkey agar. Following the inoculation, the plates were incubated at temperature of 37°C for one night duration. After 24 hours, purified growth was subcultured to prepare bacterial glycerol stock for further testing (gram stain, biochemical analysis for bacterial identification, antimicrobial susceptibility testing, DNA extraction).

### *Identification of Bacteria by Gram Staining and Biochemical Test*

Gram staining was used to distinguish between gram-negative and gram-positive rods or cocci using pure bacterial growths [28]. After confirming the presence of Gram-negative bacteria (GNB), a series of biochemical tests, including the citrate utilizing test, triple sugar iron (TSI), urease test, oxidase test, and sulfur, indole, motility (S.I.M) test, was used to classify the type of bacterium as *E. coli, K. pneumonia*, *P. aeruginosa*, *Proteus spp*, and *Citrobacter spp*. [27]. Subsequently, antimicrobial susceptibility testing took place to check the antibiotic susceptibility and resistance of the strains. In this study, Gram-positive bacteria were eliminated.

### *Antimicrobial Susceptibility Testing*

The antibacterial activity was determined using Kirby-Bauer disc diffusion method, and the ZOI (Zone of Inhibition) was measured. 0.5 McFarland inoculum was used to prepare samples for the current study. It included saline and bacterial colonies in test tube. A cotton swab had used to apply the suspension to MHA agar (Mueller-Hinton, Oxoid, Basingstoke, Hampshire, United Kingdom) plates. Antibiotic disks (Oxoid, Basingstoke, Hampshire, United Kingdom) were then put on the agar. According to the Clinical and Laboratory Standards Institute (CLSI) criteria, the ZOI was evaluated and characterized as susceptible (S), intermediate (I), or resistant (R) [29]. The various antibiotics utilized in the study, along with their corresponding abbreviations and dosages, were as follows: amoxicillin/clavulanate (AMC, 30µg), sulbactam/cefoperazone (SCF, 105µg), imipenem (IPM, 10µg), cefixime (CFM, 5µg), gentamicin (CN, 10µg), amikacin (AK, 30µg), tobramycin (TOB, 10µg), ceftaroline (CPT, 30µg), tigecycline (TGC, 15µg), Polymyxin B (PB, 300µg), Colistin (CT, 10µg), fosfomycin (FOS, 50µg), and nitrofurantoin (F, 300µg). These antibiotics were utilized against *E. coli*, *Klebsiella pneumonia*, *Citrobacter spp*, *Proteus species*, and *P. aeruginosa*.

### *Molecular Detection of ESBLs*

In microcentrifuge tubes, 1 to 2 bacterial colonies were dissolved in distilled water and then placed in water bath at 100°C for 20 minutes, to extract DNA using the boiling method. To collect the DNA-containing supernatant and discard the precipitate, centrifuged at 3000 rpm for 5 minutes [30]. At -20°C, DNA was stored in a deep freezer. UV light was used to validate the DNA extraction, and ethidium bromide-containing 1% agarose gel was utilized. PCR-specific primer sequences, as shown in **Table 1**, were used to identify genes (*bla*$_{CTX-M}$, *bla*$_{OXA}$, *bla*$_{SHV}$, *bla*$_{TEM}$). The PCR was performed using 15µL of reaction mixture containing each forward and reverse primer 1µL (5 µM), master mix (Vazyme Biotech co., Nanjing, China) 7.5µL, DNA (125 ng) 2.5µL, and distilled water 3µL. On Thermal Cycler Bio-Rad T100 (Hercules, California, United States), Initial cycle at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 50°C (*bla*$_{CTX-M}$) / 56°C (*bla*$_{OXA}$, *bla*$_{TEM}$, and *bla*$_{SHV}$) for 40 seconds, 72°C for 40 seconds, and a 5-minute extension at 72°C. Gel electrophoresis was used to evaluate the PCR result, 220 volts applied for 40 minutes, and using a 1.2% agarose gel stained with ethidium bromide before visibility under UV light. After that, the gel was visualized under the UV lights as shown in **Figure 1**.

**Table 1:** Primers used for the PCR-based molecular identification of ESBLs.

| Target Genes | Primer Sequence (5' to 3') | Amplicon Size, bp | Annealing Temperature, °C | References |
|---|---|---|---|---|
| ***bla*$_{TEM}$** | F: TCAACATTTCCGTGTCG<br>R: CTGACAGTTACCAATGCTTA | 860 | 56 | [31] |
| ***bla*$_{CTX-M}$** | F: ATATCTCTACTGTTGCATCTCC<br>R: TGGGTRAARTARGTSACCAGA | 593 | 50 | [32] |
| ***bla*$_{SHV}$** | F: AGGATTGACTGCCTTTTTG<br>R: ATTTGCTGATTTCGCTCG | 392 | 56 | [33] |
| ***bla*$_{OXA}$** | F: ATATCTCTACTGTTGCATCTCC<br>R: AAACCCTTCAAACCATCC | 619 | 56 | [33] |

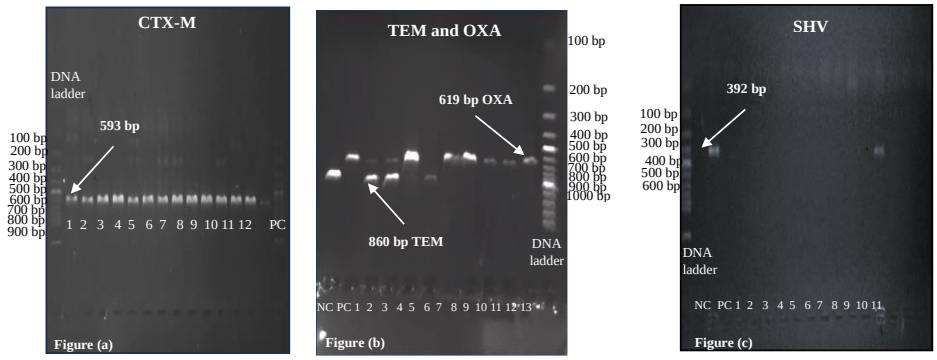


**Figure 1:** The gel electrophoresis visualization of DNA bands. **(a)** Lane PC represents a positive control; Lanes 1 to 12 demonstrate a 593bp band related to the gene *bla*$_{CTX-M}$. **(b)** Lane PC and NC represent positive and negative control, respectively. Lanes 2, 3, and 7 demonstrate 860 bp bands, which are associated with the *bla*$_{TEM}$ gene. Lanes 1, -5, -8,10, and 13 demonstrate 619 bp bands, which are associated with the *bla*$_{OXA}$ gene. Lanes 2 and 3 demonstrate 619 bp and 860 bp bands, which correspond to the *bla*$_{OXA}$ and *bla*$_{TEM}$ genes, respectively. **(c)** Lane PC and NC represent positive and negative control, respectively. Lane11 demonstrates bands of 392 bp, which are associated with the *bla*$_{SHV}$ gene, whereas Lanes -1 to -10 demonstrate no bands. utilized for molecular PCR identification of ESBLs.

### *Quality Control*

The accuracy, precision, and reproducibility of the growth-supporting prepared media characteristics (CLED agar, Chocolate agar, Blood agar, MacConkey agar, and MHA agar), gram staining,

biochemical tests (oxidase, S.I.M, urease, citrate, and TSI), and gram staining were evaluated using different ATCC strains (Manassas, Virginia, near Washington DC, USA) [27]. Antibiotics were administered to ATCC isolates to corroborate the accuracy of susceptibility testing, and the results were interpreted according to CLSI recommendations [34].

### *Statistical Analysis*

The statistical software SPSS-27 (IBM SPSS Statistics for Macintosh, Version 27.0, USA) and Microsoft Excel 365 were used to compare the frequencies of *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{TEM}$, and *bla*$_{OXA}$ genes in gram-negative bacteria isolated clinically in Bahawalpur. Statistical analysis was performed on the data presented in this study using the chi-square test. A *p*-value less than or equal to 0.05 indicated that the data were statistically significant.

## Results

### *Socio-Demographic Data of Isolated GNBs*

84 clinical isolates producing ESBL were isolated. By sample type, 33.3% (*n* = 28) urine, 25% (*n* = 21) sputum, 15.5% (*n* = 13) blood, 14.3% (*n* = 12) tracheal-aspirant and 11.9% (*n* = 10) pus were collected. Among the 84 samples analyzed, it was observed that males were 63.1% (*n* = 53), while females were 36.9% (*n* = 31) The frequency of ESBL was more abundant in an intensive care unit (ICU) at 40.5% (*n* = 34) followed by 23.8% (*n* = 20) from the urology department, 15.5% (*n* = 13) from the medical ward, 8.3% (*n* = 07) from gynecology, 6% (*n* = 05) from surgical wards, 3.6% (*n* = 03) from cardiology, and 2.4% (*n* = 02) from the emergency department. The findings of this study indicate that *Klebsiella pneumoniae* was the most identified gram-negative producing ESBLs accounting for 38.1% of the total isolates (*n* = 32/84). *Escherichia coli* followed as the second most prevalent ESBL-producing organism, representing 29.8% of the isolates (*n* = 25/84). *Pseudomonas aeruginosa*, *Proteus spp*, and *Citrobacter spp* were also identified as ESBL producers, with proportions of 15.5% (*n* = 13/84), 10.7% (*n* = 9/84), and 6% (*n* = 5/84), respectively **(Table 2).**

**Table 2:** Sociodemographic data on clinically isolated bacteria.

| | **Age** | **10-19** | **20-29** | **30-39** | **40-49** | **50-59** | **≥60** |
|---|---|---|---|---|---|---|---|
| | *n* = 84 | 01 | 11 | 35 | 22 | 07 | 08 |
| | Percentage (%) | 1.2% | 13.1% | 41.7% | 26.2% | 8.3% | 9.5% |
| **Gender** | Male (*n* = 53) | 01 | 05 | 22 | 14 | 04 | 07 |
| | 63.1% | 1.2% | 6.0% | 26.2% | 16.7% | 4.8% | 8.3% |
| | Female (*n* = 31) | 0 | 06 | 13 | 08 | 03 | 01 |
| | 36.9% | 0.0% | 7.1% | 15.5% | 9.5% | 3.6% | 1.2% |
| **Wards** | ICU (*n* = 34) | 01 | 3 | 14 | 05 | 03 | 08 |
| | 40.5% | 1.2% | 3.6% | 16.7% | 6.0% | 3.6% | 9.5% |
| | Urology (*n* = 20) | 0 | 04 | 06 | 09 | 01 | 0 |
| | 23.8% | 0.0% | 4.8% | 7.1% | 10.7% | 1.2% | 0.0% |
| | Medical (*n* = 13) | 0 | 01 | 08 | 03 | 01 | 0 |
| | 15.5% | 0.0% | 1.2% | 9.5% | 3.6% | 1.2% | 0.0% |
| | Gynecology (*n* = 07) | 0 | 0 | 03 | 04 | 0 | 0 |
| | 8.3% | 0.0% | 0.0% | 3.6% | 4.8% | 0.0% | 0.0% |
| | Surgical (*n* = 05) | 0 | 01 | 03 | 0 | 01 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6.0% | 0.0% | 1.2% | 3.6% | 0.0% | 1.2% | 0.0% |
| | Cardiology (*n* = 03) | 0 | 01 | 01 | 01 | 0 | 0 |
| | 3.6% | 0.0% | 1.2% | 1.2% | 1.2% | 0.0% | 0.0% |
| | Emergency (*n* = 02) | 0 | 01 | 0 | 0 | 01 | 0 |
| | 2.4% | 0.0% | 1.2% | 0.0% | 0.0% | 1.2% | 0.0% |
| **Specimens** | Urine (*n* = 28) | 0 | 05 | 07 | 13 | 03 | 0 |
| | 33.3% | 0.0% | 6.0% | 8.3% | 15.5% | 3.6% | 0.0% |
| | Sputum (*n* = 21) | 0 | 0 | 17 | 0 | 0 | 04 |
| | 25% | 0.0% | 0.0% | 20.2% | 0.0% | 0.0% | 4.8% |
| | Blood (*n* = 13) | 01 | 02 | 05 | 05 | 0 | 0 |
| | 15.5% | 1.2% | 2.4% | 6.0% | 6.0% | 0.0% | 0.0% |
| | TA (*n* = 12) | 0 | 02 | 01 | 04 | 03 | 02 |
| | 14.3% | 0.0% | 2.4% | 1.2% | 4.8% | 3.6% | 2.4% |
| | Pus (*n* = 10) | 0 | 02 | 05 | 0 | 01 | 02 |
| | 11.9% | 0.0% | 2.4% | 6.0% | 0.0% | 1.2% | 2.4% |
| **Bacteria Isolates** | *K. pneumonia* (*n* = 32) | 0 | 03 | 14 | 08 | 0 | 07 |
| | 38.1% | 0.0% | 3.6% | 16.7% | 9.5% | 0.0% | 8.3% |
| | *E. coli* (*n* = 25) | 0 | 03 | 10 | 06 | 05 | 01 |
| | 29.8% | 0.0% | 3.6% | 11.9% | 7.1% | 6.0% | 1.2% |
| | *P. aeruginosa* (*n* = 13) | 01 | 01 | 03 | 06 | 02 | 0 |
| | 15.5% | 1.2% | 1.2% | 3.6% | 7.1% | 2.4% | 0.0% |
| | *Proteus spp* (*n* = 09) | 0 | 02 | 07 | 0 | 0 | 0 |
| | 10.7% | 0.0% | 2.4% | 8.3% | 0.0% | 0.0% | 0.0% |
| | *Citrobacter spp* (*n* = 05) | 0 | 02 | 01 | 02 | 0 | 0 |
| | 6.0% | 0.0% | 2.4% | 1.2% | 2.4% | 0.0% | 0.0% |

*ICU = Intensive Care Unit, Tracheal aspirant = TA

***Antibiotic susceptibility of ESBL-producing Gram-negative bacteria (GNBs).***

The effectiveness of various antibiotics against ESBL-producing GNB was evaluated such as *K. pneumonia, E. coli, P. aeruginosa, Citrobacter spp,* and *Proteus spp*. Ceftaroline (CPT) exhibited the highest level of resistance, followed by gentamicin (CN) >cefixime (CFM) > tobramycin (TOB) > amikacin (AK) > amoxicillin/clavulanate (AMC) > cefoperazone (SCF) > imipenem (IPM). GNBs were most sensitive to the following agents, in descending order: IPM > SCF > AMC > AK > TOB > CFM > CN > CPT. The producers of ESBL had the lowest MICs for IPM, SCF, and AMC. Consequently, these agents could be utilized as prospective treatment options. In contrast, AK, TOB, CFM, and CN exhibited elevated resistance rates to GNRs and as a result, it should not be utilized for curl **(Table 3)**.

***K. pneumonia Susceptibility to Various Antibiotics***

*K. pneumonia* 38.1%, *n* = 32/84 was the most common type of bacteria identified within ESBL-producing clinical isolates studied, and their antibiotic susceptibility was as follows: IPM >SCF >AMC >AK >TOB > CFM >CN > CPT while resistance rate against of *K. pneumonia* was CPT > CN > CFM >AK >TOB > AMC > SCF > IPM. IPM, SCF, and AMC were the most effective antibiotics against *K. pneumonia*, whereas AK, TOB, CFM, and CN were not, as shown in **Table 3**.

***E. coli Susceptibility to Various Antibiotics***

*E. coli* (29.8%, *n* = 25/84) was the second most frequent bacteria discovered in the tested ESBL-producing bacterium after *K. pneumonia*. Antibiotic efficacy rates against *E. coli* were as follows: IPM > AK > SCF > TOB> CN >AMC > CPT > CFM. IPM, AK, and SCF most effective drugs against *E. coli* **(Table 3)**.

***P. aeruginosa Susceptibility to Various Antibiotics***

*P. aeruginosa* (15.5%, *n* = 13) was the third most identified ESBL-producing GNB after *K. pneumonia* and *E. coli.* Antibiotic efficacy rates against *P. aeruginosa* were as follows: IPM > AK > SCF > TOB> CN >AMC > CPT > CFM. IPM, AK, and SCF most effective drugs against *P. aeruginosa* **(Table 3)**.

***Susceptibility of Proteus spp to Various Antibiotics***

*Proteus spp* (10.7%, *n* = 09/84) was the fourth most identified ESBL-producing GNB after *K. pneumonia*, *E. coli*, and *P. aeruginosa*. Antibiotic efficacy rates against *Proteus spp* were as follows: IPM > SCF > AMC> CFM> TOB> CN > CPT > AK. IPM, AMC, and SCF most effective drugs against *Proteus spp* **(Table 3)**.

***Susceptibility of Citrobacter spp to Various Antibiotics***

*Citrobacter spp* (6%, n= 05/84) was the least identified ESBL-producing GNB as compared to *K. pneumonia*, *E. coli*, *P. aeruginosa,* and *Proteus spp.* Antibiotic efficacy rates against *Citrobacter spp* were as follows: IPM > SCF > AK > CN > CPT >TOB >AMC> CFM. IPM and SCF were the most effective drugs against *Citrobacter spp* **(Table 3).**

**Table 3:** Antimicrobial susceptibility testing against *K. pneumonia, E. coli, P. aeruginosa*, *Citrobacter spp*, and *Proteus spp*. that produce ESBLs.

| Antibiotics | *Citrobacter spp* *n* = 05 | | | *E. coli* *n* = 25 | | | *K. pneumonia* *n* = 32 | | | *P. aeruginosa* *n* = 13 | | | *Proteus spp* *n* = 09 | | | Total *n* = 84 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | I | S | R | I | S | R | I | S | R | I | S | R | I | S | R | I | S |
| AMC | 05 6.0 % | 0 0.0 % | 0 0.0 % | 18 21.4 % | 05 6.0 % | 02 2.4 % | 08 9.5 % | 0 0.0 % | 24 28.6 % | 10 11.9 % | 0 0.0 % | 03 3.6 % | 01 1.2 % | 01 1.2 % | 07 8.3 % | 42 50.0 % | 06 7.1 % | 36 42.9 % |
| SCF | 01 1.2 % | 03 3.6 % | 01 1.2 % | 05 6.0 % | 0 0.0 % | 20 23.8 % | 04 4.8 % | 01 1.2 % | 27 32.1 % | 07 8.3% | 0 0.0 % | 06 7.1 % | 01 1.2 % | 0 0.0 % | 08 9.5 % | 18 21.4 % | 04 4.8 % | 62 73.8 % |
| CFM | 05 6.0 % | 0 0.0 % | 0 0.0 % | 24 28.6 % | 0 0.0 % | 01 1.2 % | 14 16.7 % | 0 0.0 % | 18 21.4 % | 12 14.3 % | 0 0.0 % | 01 1.2 % | 04 4.8 % | 0 0.0 % | 05 6.0 % | 59 70.2 % | 0 0.0 % | 25 29.8 % |
| IPM | 0 0.0 % | 0 0.0 % | 05 6.0 % | 04 4.8 % | 04 4.8 % | 17 20.2 % | 04 4.8 % | 0 0.0 % | 28 33.3 % | 06 7.1% | 0 0.0 % | 07 8.3 % | 01 1.2 % | 0 0.0 % | 08 9.5 % | 15 17.9 % | 04 4.8 % | 65 77.4 % |
| AK | 03 3.6 % | 01 1.2 % | 01 1.2 % | 04 4.8 % | 04 4.8 % | 17 20.2 % | 21 25% | 01 1.2 % | 10 11.9 % | 07 8.3% | 02 2.4 % | 04 4.8 % | 09 10.7 % | 0 0.0 % | 0 0.0 % | 44 52.4 % | 08 9.5 % | 32 38.1 % |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CT** | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 07 63.6 % | 02 18.2 % | 02 18.2 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 07 63.6 % | 02 18.2 % | 02 18.2 % |
| **CPT** | 04 4.9 % | 0 0.0 % | 01 1.2 % | 20 24.4 % | 02 2.4 % | 02 2.4 % | 31 37.8 % | 0 0.0 % | 01 1.2 % | 10 12.2 % | 01 1.2 % | 01 1.2 % | 08 9.8 % | 01 1.2 % | 0 0.0 % | 73 89.0 % | 04 4.9 % | 05 6.1 % |
| **FOS** | 0 0.0 % | 0 0.0 % | 0 0.0 % | 02 4.7 % | 02 4.7 % | 08 18.6 % | 01 2.3 % | 06 14% | 15 34.9 % | 0 0.0% | 0 0.0 % | 01 2.3 % | 01 2.3 % | 0 0.0 % | 07 16.3 % | 04 9.3 % | 08 18.6 % | 31 72.1 % |
| **CN** | 03 3.6 % | 0 0.0 % | 02 2.4 % | 14 16.7 % | 03 3.6 % | 08 9.5 % | 27 32.1 % | 01 1.2 % | 04 4.8 % | 10 11.9 % | 02 2.4 % | 01 1.2 % | 07 8.3 % | 0 0.0 % | 02 2.4 % | 61 72.6 % | 06 7.1 % | 17 20.2 % |
| **F** | 0 0.0 % | 0 0.0 % | 0 0.0 % | 04 8.3 % | 0 0.0 % | 12 25.0 % | 19 39.6 % | 02 4.2 % | 03 6.3 % | 0 0.0% | 0 0.0 % | 0 0.0 % | 08 16.7 % | 0 0.0 % | 0 0.0 % | 31 64.4 % | 02 4.2 % | 15 31.3 % |
| **PB** | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 05 45.5 % | 03 27.3 % | 03 27.3 % | 0 0.0 % | 0 0.0 % | 0 0.0 % | 05 45.5 % | 03 27.3 % | 03 27.3 % |
| **TGC** | 0 0.0 % | 0 0.0 % | 0 0.0 % | 04 8.2 % | 0 0.0 % | 12 24.5 % | 6 12.2 % | 09 18.4 % | 09 18.4 % | 01 2.0% | 0 0.0 % | 0 0.0 % | 0 0.0 % | 01 2.0 % | 07 14.3 % | 11 22.4 % | 10 20.4 % | 28 57.1 % |
| **TOB** | 04 4.8 % | 0 0.0 % | 01 1.2 % | 12 14.3 % | 06 7.1 % | 07 8.3 % | 21 25% | 06 7.1 % | 05 6.0 % | 10 11.9 % | 01 1.2 % | 02 2.4 % | 07 8.3 % | 01 1.2 % | 01 1.2 % | 54 64.3 % | 14 16.7 % | 16 19% |

*R=Resistant, S=Susceptible, I=Intermediate, SCF=Cefoperazone, IPM=Imipenem, AMC= Amoxicillin/clavulanate, CFM=Cefixime, CN=Gentamicin, AK=Amikacin, TOB=Tobramycin, CPT=Ceftaroline, TGC=Tigecycline, PB =Polymyxin B, CT=Colistin, FOS= Fosfomycin, F=Nitrofurantoin.

***bla$_{CTX-M}$, bla$_{OXA}$, bla$_{TEM}$, and bla$_{SHV}$ prevalence among GNBs***

Out of 84 isolates, 37 were multi drugs resistance (MDR) ESBL-producing GNBs. *E. coli* was 32.4 % (*n* = 12/37), followed by *P. aeruginosa* was 29.7% (*n* = 11/37), *K. pneumonia* was 21.6% (*n* = 08/37), *Citrobacter spp* was 10.8% (*n* = 04/37) and *Proteus spp* was 5.4% (*n* = 02/37). In our study, the ESBL gene was detected in MDR-ESBL-producing GNBs. Among MDR clinical isolates, *bla$_{CTX-M}$* (40.5%, *n* = 15/37) was the very common coding of the gene for ESBL production, followed by *bla$_{TEM}$* (27%, *n* = 10/37), *bla$_{OXA}$* (21.6%, *n* = 08/37) and *bla$_{SHV}$* (10.8%, *n* = 04/37). As shown in Table 3, the most significant gene among *E. coli*, *K. pneumonia*, and *P. aeruginosa* was *bla$_{CTX-M}$*, followed by *bla$_{TEM}$* and *bla$_{OXA}$* in *E. coli*, *K. pneumonia*, and *P. aeruginosa*, and *bla$_{SHV}$* in *E. coli*, *K. pneumonia*, and *Proteus spp* **(Table 4)**.

**Table 4:** ESBL coding gene distribution within GNB bacteria isolated from clinical specimens.

| Genes | *bla$_{CTX-M}$* | | *bla$_{TEM}$* | | *bla$_{OXA}$* | | *bla$_{SHV}$* | |
|---|---|---|---|---|---|---|---|---|
| | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg |
| E. coli (%) ( n = 12) | 10 27% | 02 5.4% | 04 10.8% | 08 21.6% | 01 2.7% | 11 29.7% | 02 5.4% | 10 27.0% |
| K. pneumonia (%) (n =8) | 05 13.5% | 03 8.1% | 05 13.5% | 03 8.1% | 02 5.4% | 06 16.8% | 01 2.7% | 07 18.9% |
| P. aeruginosa (%) (n =11) | 0 0.0% | 11 29.7% | 01 2.7% | 10 27% | 05 13.5% | 06 16.8% | 0 0.0% | 11 29.7% |
| Proteus spp (%) (n =2) | 0 0.0% | 02 5.4% | 0 0.0% | 02 5.4% | 0 0.0% | 02 5.4% | 01 2.7% | 01 2.7% |
| Citrobacter spp (%)(n= 4) | 0 0.0% | 04 10.8% | 0 0.0% | 04 10.8% | 0 0.0% | 04 10.8% | 0 0.0% | 04 10.8% |

| | | | | |
|---|---|---|---|---|
| *p-value* | 0.00 | 0.04 | 0.16 | 0.26 |

* Genetic prevalence of ESBLs (*blaCTX-M*, $bla_{OXA}$, $bla_{TEM}$, and $bla_{SHV}$) in *K. pneumonia, E. coli, P. aeruginosa, Citrobacter spp,* and *Proteus spp*.

***Antibiotic Resistance and ESBL Gene Correlation***

Most of the clinical isolates (8.1%, $n$ = 3/37) had the *blaCTX-M* + $bla_{TEM}$ gene combination. Following that, 5.4% ($n$ = 2/37) of isolates showed combinations of *blaCTX-M* + $bla_{TEM}$ + $bla_{OXA}$, as well as $bla_{TEM}$ + $bla_{OXA}$. Additionally, 2.7% ($n$ = 1/37) of cases had $bla_{CTX-M}$ + $bla_{SHV}$ and $bla_{CTX-M}$ + $bla_{TEM}$ + $bla_{SHV}$ genes. β-lactam antibiotics, AMC, CFM, and IPMs were all resistant to the ESBL isolates. TOB, AK, CN, CPT, PB, CT, F, and FOS, in addition to non-β-lactam antibiotics, were ineffective against bacteria with resistance genes, such as *blaTEM*, $bla_{TEM}$ *blaSHV*, *blaCTX-M*, and $bla_{OXA}$, *blaCTX-M* + $bla_{TEM}$ + $bla_{OXA}$, *blaCTX-M* + $bla_{TEM}$, $bla_{TEM}$ + $bla_{OXA}$, *blaCTX-M* + $bla_{TEM}$ + $bla_{SHV}$ and $bla_{CTX-M}$ + $bla_{SHV}$. In this study, no clinical isolates contained the $bla_{OXA}$ + $bla_{SHV}$, $bla_{CTX-M}$ + $bla_{OXA}$, $bla_{TEM}$ + $bla_{SHV}$ + $bla_{OXA}$, or $bla_{TEM}$ + $bla_{SHV}$ genes. **(Table 5).**

**Table 5:** Clinical isolates exhibit antibiotic resistance and the presence of ESBL-coding genes.

| Genes | Total (*n*) % | Antibiotics |
|---|---|---|
| $bla_{CTX-M}$ | (08)21.6% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT |
| $bla_{CTX-M}$ + $bla_{TEM}$ | (03)8.1% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT |
| $bla_{CTX-M}$ + $bla_{TEM}$ + $bla_{OXA}$ | (02)5.4% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT, F, FOS, TGC |
| $bla_{CTX-M}$ + $bla_{TEM}$ + $bla_{SHV}$ | (1)2.7% | AMC, CFM, IMP, TOB, AK, CN, CPT |
| $bla_{CTX-M}$ + $bla_{SHV}$ | (1)2.7% | AMC, CFM, IMP, TOB, AK, CN, CPT, F |
| $bla_{CTX-M}$ + $bla_{OXA}$ | (0)0.0% | NIL |
| $bla_{CTX-M}$ + $bla_{TEM}$ + $bla_{SHV}$ + $bla_{OXA}$ | (0)0.0% | NIL |
| $bla_{TEM}$ | (02)5.4% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT |
| $bla_{TEM}$ + $bla_{OXA}$ | (02)5.4% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT, FOS |
| $bla_{TEM}$ + $bla_{SHV}$ | (0)0.0% | NIL |
| $bla_{TEM}$ + $bla_{SHV}$ + $bla_{OXA}$ | (0)0.0% | NIL |
| $bla_{SHV}$ | (02)5.4% | AMC, CFM, IMP, AK, CPT |
| $bla_{SHV}$ + $bla_{OXA}$ | (0)0.0% | NIL |
| $bla_{OXA}$ | (04)10.8% | AMC, CFM, SCF, IMP, TOB, AK, CN, CPT, PB, CT, TGC |

* AMC=Amoxicillin/clavulanate, CFM=Cefixime, CN=Gentamicin, AK=Amikacin, TOB=Tobramycin, CPT=Ceftaroline, TGC=Tigecycline, PB =Polymyxin B, CT=Colistin, FOS= Fosfomycin, F=Nitrofurantoin.

**Discussion**

Globally, the emergence of ESBL-producing Enterobacteriaceae has become a major concern. These multidrug-resistant organisms produce infections with a high mortality rate and limited treatment options [35, 36]. Numerous GNBs generate ESBL enzymes capable of hydrolyzing cephalosporins and penicillin, which are inhibited by clavulanic acid [37]. Multiple non-β-lactam antibiotics,

including cotrimoxazole, nalidixic acid, norfloxacin, ciprofloxacin, and amikacin, are ineffective against ESBL-producing GNRs. Resistance genes are frequently encoded by the identical plasmids required for ESBL production. [38]. *K. pneumoniae* had the highest percentage of ESBL (38.1%, *n* = 32/84) among GNBs in our study. *E. coli* was 29.8% (25/84) and *P. aeruginosa* was 15.5% (13/84). The study found 10.7% *Proteus spp*. and 6% *Citrobacter spp*. In 2020, Bilal *et al*. found 11.7% of *K. pneumoniae* isolates produced ESBLs [39]. In 2017, Shakya *et al*. found 17.64% of *K. pneumoniae* isolates in Lalitpur, Nepal, produced ESBLs [40]. Ahmed et al. found that 24.5% of *K. pneumoniae* isolates in Pakistan were ESBL-positive [37], a higher prevalence than in the current study. In 2016, Batool *et al*. found 34% of 97 *K. pneumoniae* isolates in Pakistan were ESBL-positive [41]. Ejaz *et al*. found that 71.75% of *K. pneumoniae* isolates produced ESBL [42]. In India, Mathai *et al*. (2015) found that *Klebsiella spp*, (15.6%), *Pseudomonas spp*, (12.8%), and *E. coli* were the most common GNBs [43]. Different ESBL-producing isolate phenotypic identification methods and geographical factors may explain prevalence differences. These studies demonstrate that *Klebsiella spp* or *K. pneumoniae* are common ESBL-producing gram-negative bacteria.

The predominant bacteria responsible to produce ESBLs were identified as *E. coli* in a study conducted by Idrees *et al*., (2022b) in Multan, Pakistan. Out of the 272 isolates examined, *E. coli* accounted for 64% (*n* = 174) of the total isolates. *Klebsiella spp* accounted for 27.2% (*n* = 74) of the isolates, while Acinetobacter species comprised 2.2% (n = 18) [24]. A total of six bacteria, accounting for 2.2% of the overall population, were identified. It has the capability to effectively impede the activity of said enzymes. Similarly, the study conducted in India, in 2022, *E. coli* was found to be the most prevalent pathogen, accounting for 70.8% of the isolates. This was followed by *K. pneumoniae*, which constituted 22.0% and 7.2% of the other isolates [44]. According to these studies, the prevalence of *K. pneumoniae* was comparatively low than *E. coli* while according to the current study, *K. pneumoniae* had a high prevalence as comparative *E. coli.* Another study by Haider *et al*. (2022) in Lahore, Pakistan, assessed that, *P. aeruginosa* was the most common isolate, accounting for 41.4% (n=43/104) [23], while the current study showed a low prevalence of *P. aeruginosa*. Various studies have confirmed the incidence of *E. coli*, *Klebsiella spp*, and *P. aeruginosa* in Pakistan, supporting our findings [44, 45].

Our study results demonstrated, significant incidences of antibiotic resistance against β-lactam antibiotics, particularly cephalosporins (cefixime (CFM) 70.2%, cefoperazone (SCF) 26.2%), penicillin (amoxicillin/clavulanate (AMC) 50%), and carbapenem (Imipenem (IMP) 22.7%). The results of our research have been confirmed by various studies conducted in Argentina [46], Pakistan [23, 24, 42, 48], Algeria [45], Turkey [47], and the United States [49]. The high prevalence of antibiotic resistance is due to the extensive and used of modern antibiotics was uncontrolled, particularly in the treatment of post-operative and ICUs patients.

Our study results indicated, cefoperazone, imipenem, gentamicin, amikacin, nitrofurantoin, and fosfomycin demonstrated the highest efficacy in reducing ESBL-producing isolates. Hence, it is probable that these antibiotics can be employed for the purpose of treating bacterial infections. In contrast, the remaining antibiotics displayed a lack of efficacy, rendering them unsuitable as viable treatment options. Several studies have determined that, gentamicin, amikacin, nitrofurantoin, imipenem, and fosfomycin are the most effective antibiotics for treating ESBL producer's infections [8, 24, 42, 44, 47, 48, 50].

Hence, it is imperative to consistently carry out similar research in developing nations to evaluate the efficacy of widely utilized medication in combating infections. In the present study, ESBL-coding genes prevalence, namely *blaCTX-M, bla$_{TEM}$, bla$_{OXA}$*, and *bla$_{SHV}$*, was studied. Notably, the prevalence rates of these genes were found to be 40.5% for *blaCTX-M*, 27% for *bla$_{TEM}$*, 21.6% for *bla$_{OXA}$*, and 10.8% for *bla$_{SHV}$* among GNRs. These findings demonstrate a remarkable resemblance to the prevalence rates reported in various studies conducted in neighbouring countries as well as globally [51–55]. The results found the prevalence of *blaCTX-M, bla$_{TEM}$, bla$_{OXA}$*, and *bla$_{SHV}$* genes among the *E. coli* was found to be 27%, 10.8%, 2.7%, and 5.4% respectively. In the case of *K. pneumonia*, the observed prevalence rates for *blaCTX-M*, *bla$_{OXA}$*, and *bla$_{TEM}$* and *bla$_{SHV}$* were found to be 13.5%, 5.4%, and 2.7% respectively. In the case of *P. aeruginosa*, it was observed that the genes *blaCTX-M*

and *bla$_{SHV}$* were not detected. However, the prevalence of the genes *bla$_{TEM}$* and *bla$_{OXA}$* was found to be 2.7% and 13.5% respectively. Further on to *Proteus spp*, only the gene *bla$_{SHV}$* was identified, with a frequency of 2.7%. The study was conducted by Idress *et al*., (2022), *blaCTX-M, bla$_{TEM}$, bla$_{OXA}$*, and *bla$_{SHV}$* genes prevalence was evaluated in isolates of *Acinetobacter spp*, *Klebsiella spp*, *E. coli*, and other related gram-negative rods. The observed prevalence varied between 66.7% to 92.0% for *blaCTX-M*, 60.3% to 72.2% for *bla$_{TEM}$*, 16.7% to 40.8% for *bla$_{OXA}$*, and 14.4% to 22.2% for *bla$_{SHV}$*, respectively [24]. However, the study conducted by Haider *et al*., (2022) in Lahore, showed the prevalence of *bla$_{TEM}$* positive isolates was found to be most prevalent among ESBLs producers, accounting for 81.8% of the cases. This was followed by 27.3% of isolates carrying *bla$_{OXA}$ 1* and *bla$_{SHV}$* genes, respectively among the GNBs [23]. According to the results of the study conducted in Iraq and neighboring [56, 57], it has been observed that the *blaCTX-M* gene showed a prominent presence in both *E. coli* and *K. pneumonia*. Presently, it has been observed that *CTX-M* enzymes are now recognized as frequent ESBL type in *E. coli*, above the previously dominant *SHV* and *TEM* enzymes. However, reports from studies conducted in India and Turkey have indicated that *TEM* enzymes showed the highest prevalence rate [58].

The most frequent ESBL gene combination observed in this study was *bla$_{CTX-M}$* + *bla$_{TEM}$*, accounting for 13.5% of the cases. This was followed by *blaCTX-M* + *bla$_{OXA}$* + *bla$_{TEM}$* (5.4%), *bla$_{OXA}$* + *bla$_{TEM}$* (5.4%), *blaCTX-M* + *bla$_{OXA}$*, *blaCTX-M* + *bla$_{TEM}$* + *bla$_{SHV}$*, *blaCTX-M* + *bla$_{SHV}$* (2.7%), and *blaCTX-M* + *bla$_{OXA}$* + *bla$_{SHV}$* (0%).According to research results of a previous study occurred in Nigeria, it was observed that the combination of *bla$_{SHV}$* + *bla$_{TEM}$* + *blaCTX-M* (70%) was the most prevalent, while the combination of *bla$_{TEM}$* + *blaCTX-M* (15%) was followed similarly[57]. However, our own findings contradict these results. Additional examples are provided in this study. The identification of multiple genes (*bla$_{SHV}$, bla$_{TEM}$, blaCTX-M*) within the genomes of certain isolates indicates the possible existence of resistance plasmids [52]. In Iraq (2016), in a study conducted by Poles *et al*., six distinct genotype patterns were identified. The genotype labeled as *blaCTX-M* exhibited the highest prevalence rate, accounting for 40% of the observed genotypes. Following closely behind was the genotype combination of *bla$_{TEM}$* and *blaCTX-M*, which constituted 30.9% of the genotypes identified. The frequencies of the genotypes (*bla$_{TEM}$, bla$_{TEM}$* + *bla$_{SHV}$, bla$_{SHV}$* + *blaCTX-M, and bla$_{TEM}$* + *bla$_{SHV}$* + *blaCTX-M*) were 7.3%, 5.5%, 5.4%, and 10.9%, respectively [51]. In a recent study conducted by Idress *et al*., in the year 2022, the study findings revealed that the combination of *blaCTX-M* and *bla$_{TEM}$* genes was the most prevalent, accounting for approximately 30.5% of the observed cases. This was followed by the combination of *blaCTX-M, bla$_{OXA}$*, and *bla$_{TEM}$* genes, which accounted for approximately 14.0% of the cases. Additionally, the combination of *blaCTX-M* and *bla$_{OXA}$* genes was observed in approximately 13.6% of the cases. Other combinations, such as *blaCTX-M, bla$_{TEM}$*, and *bla$_{SHV}$* (7.0%), *blaCTX-M* and *bla$_{SHV}$*, *bla$_{OXA}$, blaCTX-M*, and *bla$_{SHV}$* (2.2%), as well as *bla$_{OXA}$* and *blaTEM* (1.8%), were also identified, even with lower frequencies [24]. These gene patterns are comparatively higher than our study.

The presence of multiple ESBL genes in numerous ESBL-producing strains can give complicated antimicrobial resistance patterns, leading to the development of co-resistance against antibiotic groups other than β-lactam antibiotics. In our study, the *bla$_{TEM}$* gene, which encodes for an ESBL, consistently co-occurred with the *blaCTX-M* gene. Results of this study based on, coexistence of *bla$_{TEM}$* + *blaCTX-M* + *bla$_{OXA}$* genes has been associated with resistance development against penicillin, cephalosporins, and aminoglycosides. As penicillin-resistant strains proliferate, cephalosporins and aminoglycosides, which have been widely prescribed to treat infections caused by ESBL-producing bacteria, are progressively losing their efficacy. In both community and clinical settings, the current situation is cause for concern and graveness. This highlights the importance of using rational antibiotic treatment to limit spread of those strains within healthcare facilities, as well as increasing our clinical manifestations of ESBL type knowledge. Gene sequencing has potential to provide significant insights into the phylogenetic history of ESBL-associated genes, which is crucial for preventing their further spread [22, 59].

## Conclusion

In conclusion, *blaCTX-M* emerged as the predominant gene responsible for encoding ESBL production in clinical isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Proteus spp*. This was followed by the presence of $bla_{OXA}$, $bla_{TEM}$, and $bla_{SHV}$ genes. 37/84 isolates of GNRs were analyzed to identify the various genetic combinations of ESBL-coding genes, namely *blaCTX-M,* $bla_{TEM}$, $bla_{OXA}$, and $bla_{SHV}$. It was observed that all possible combinations of these genes were present, except for the combination of *blaCTX-M*, $bla_{OXA}$, and $bla_{SHV}$. The ESBLs production makes β-lactam antibiotics (such as cephalosporins and penicillins) ineffective, thereby contributing to the increase in bacterial resistance. Additionally, antibiotics that are not β-lactams (such as aminoglycosides) become ineffective. According to the results of our study, tigecycline, imipenem, cefoperazone, and fosfomycin have the potential to treat infections caused by ESBLproducing bacteria. It is recommended that clinical microbiology laboratories consistently employ ESBL-identification tools in order to monitor multidrug-resistant isolates. Additionally, the use of antibiograms can provide valuable guidance to physicians and clinical staff when making decisions regarding empirical therapy for infections. The implementation of infection prevention and control programs, as well as antibiotic stewardship programs, is crucial in hospital settings in order to effectively mitigate the transmission of resistant isolates.

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