



## Antibiotic Sensitivity and ESBL Prevalence among Gram-Negative Bacteria in a Tertiary Care Hospital

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### ABSTRACT

The steady upsurge in the prevalence of ESBL-producing Gram-negative bacteria has become a significant menace to the healthcare systems across the globe, including Pakistan. This study was carried out from November 2023 to May 2024 to determine the prevalence and antibiotic susceptibility of Gram-negative bacteria in clinical samples belonging to the local population of South Punjab, Pakistan as well as to explicate the prevalence of ESBL-producers among the isolates. Various clinical samples were considered including blood cultures, urine, wound swabs, pus, and tracheal secretion. The isolation was followed by Gram staining and biochemical characterization, after which antibiotic susceptibility testing was conducted in accordance with the CLSI guidelines, and ESBLs were detected phenotypically by a double disk synergy test. A total of 111 Gram-negative bacterial isolates were obtained from 238 clinical specimens. These included *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Citrobacter* spp., and *Proteus mirabilis*, in the decreasing order of prevalence. The antibiotic susceptibility profile showed that 76.70% of the isolates were resistant to at least one of the eight selected antibiotics. Amikacin was the most effective antibiotic against most isolates while Colistin was the least effective drug. Overall, 35% of the isolates were positive for the ESBL detection test while 65% were negative. *Escherichia coli* showed the highest prevalence rate for ESBL followed by *Proteus mirabilis*, *Klebsiella* spp., *Citrobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp., respectively. The results emphasized a high prevalence of antibiotic resistance among Gram-negative isolates and a rising ESBL detection rate among the pathogens in clinical settings.

**Keywords:** : *Escherichia coli*; ESBL; Antibiotic resistance; Gram-negative bacteria; Amikacin

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### INTRODUCTION

Antibiotic resistance remains a grave threat to the effectiveness of the treatment of bacterial diseases

and it can be rightfully considered one of the greatest concerns for human health on an international level. Among the worst types of infections are the occasional infections

spontaneously caused by Gram-negative pathogens including extended-spectrum  $\beta$ -lactamase (ESBL) producers (Vasic et al., 2024). ESBL is an enzyme that only a few bacteria produce and confers resistance to several antibiotics. ESBL is especially famous for mediating the hydrolysis of several beta-lactam antibiotics. The mechanism of action of ESBLs leads to deactivation of the antibiotics hence making the bacteria resistant to specific drugs. The emergence of the ESBL genes has been cited as one of the reasons for the increased occurrence of multidrug resistance among emerging Gram-negative bacteria in patients (Muhammad Rizwan et al., 2024). One of the biggest concerns in human health today is extensive rise in  $\beta$ -lactam antibiotic resistance in Enterobacteriaceae. Production of  $\beta$ -lactam enzyme is used to degrade or hydrolyze  $\beta$ -lactam, one of the main mechanism of resistance (Russo et al., 2021).

Beta-lactam antibiotics are considered one of the most frequently recommended drugs worldwide. However, concerns with the utilization of this group of drugs include the fact that patients using these drugs are likely to develop allergic reactions ranging from simple erythematous maculopapular rash to anaphylactic type of shock. Even more severe is the realization that pathogenic bacteria can develop an exceptionally rich and complex resistance to this class of drugs by producing  $\beta$ -lactamases (Turner et al., 2022).  $\beta$ -lactamases are plasmid-inserted genes primarily found in Gram-negative rod-shaped bacteria. They produce enzymes that cause resistance to many classes of antibiotics like penicillins (ampicillin), broad-spectrum cephalosporins (cefotaxime and ceftazidime) as well as monobactams (aztreonam) (Muhammad Rizwan et al., 2024).

In the beginning of 1980s in Europe, the first ESBL infection was reported. The strains of *Escherichia coli* and *Klebsiella* spp. that harbored ESBL of *Streptococcus pneumoniae* were first shown to produce these enzymes. ESBLs are pertinent in the health care setting. Diagnosis of ESBL is a considerable concern in the health sector due to its

devastating impact arising from the predisposition of bacteria towards multiple antibiotic resistance. It is becoming common in bacterial infections with these etiological agents (Bradford, 2001).

The predominant mechanism put forth by specific studies on resistance to the beta-lactam antibiotics among Gram-negative rods is the ability to produce ESBL, which causes resistance to the third generation of cephalosporins (Umadevi et al., 2011). Nevertheless, they are unable to hydrolyze cephamycins and can be inhibited by clavulanic acid (Shoorashetty et al., 2011). Clavulanic acid can poorly inhibit AmpC enzymes which impart resistance to cephalosporins and  $\alpha$ -methoxy  $\beta$ -lactams such as cefoxitin, cefotetan, and monobactams. These show vulnerability to advanced spectrum cephalosporins like cefepime, and cefepirome (Jacoby, 2009).

Diagnosing the infections caused by these bacteria requires a comprehensive approach that includes obtaining appropriate clinical specimens, performing microbiological cultures, and conducting antimicrobial susceptibility testing to identify the presence of ESBL-producing organisms as well as determine their susceptibility to drugs (Elshamy and Aboshanab, 2020). Diagnosis of infections caused by Gram-negative ESBL-producers involves the culturing of clinical specimens, such as urine, blood, or wound swabs, to isolate and identify the bacteria. Following isolation, antimicrobial susceptibility testing is performed to assess the susceptibility of the bacteria to various antimicrobials, with a focus on third-generation cephalosporins. ESBL production is confirmed using phenotypic methods like the double-disk synergy method or molecular techniques such as PCR to detect specific ESBL genes.

The World Health Organization arranged a new list of effective drugs against ESBL and carbapenemase-producing Enterobacteriaceae as an urgency of research for trying to inflect the rise in mortality or morbidity caused by such organisms. Carbapenems protecting therapeutic options are strongly advocated (Lombardi et al., 2023). For the detection of ESBL formation, most of the reported studies simply used

phenotypic screening, either with or without a confirming test. Factors such as the kind of screening agents and the impact of AmpC and other ESBL-producing bacterial genes affect the specificity as well as the sensitivity of phenotypic screening and confirmatory testing (Ling et al., 2021). The present study aimed to describe the prevalence of ESBL producers and their antibiotic resistance profile in a tertiary-care hospital of Multan, Pakistan.

## MATERIALS AND METHODS

### *Place and duration of study*

This study was conducted at the microbiology laboratory of MMG department in the Women University Multan, Pakistan between November 2023 and May 2024. Clinical samples were taken from indoor/outdoor departments and burn wards of Nishtar Hospital in Multan. Clinical samples included wounds, urine, pus, blood cultures, and tracheal secretion. A total of 248 patients were involved in the study whose ages ranged from 20–60 years and included both males and females. The study was approved by the Research Ethics Committee of the Women University Multan and all the procedures were in compliance with the declaration of Helsinki. The samples were taken after obtaining informed written consent or assent with consent provided by a relative.

### *Isolation & identification of bacteria*

All the collected samples were cultured on MacConkey and blood agar. The inoculated plates were incubated aerobically at 37 °C for 24 hours. Bacteria were identified based on the colony morphology, Gram staining, and biochemical tests including TSI, SIM, indole, and oxidase tests. The CLSI guidelines were applied to the bacterial identification and susceptibility testing processes.

### *Antimicrobial susceptibility testing*

The susceptibility of the study isolates to various antibiotics was carried out using the Kirby-Bauer disc diffusion method (Haq and Sharif, 2024). All bacterial strains were grown on Mueller-Hinton agar

and the plates were incubated at a temperature of 37°C for 24 hours. The diameter of the zone of inhibition was recorded against the selected antibiotics. Zones of inhibition are the clear circles around the disks, which represent areas of no bacterial growth. The zones of inhibition were categorized as sensitive (S), intermediate sensitive (IS), and resistant (R) as reported by the CLSI. The antibiotic discs that were used for antimicrobial testing included: Amikacin, Aztreonam, Imipenem, Piperacillin/Tazobactam, Ceftriaxone, Colistin, Levofloxacin, and Amoxicillin/Clavulanate.

### *ESBL detection test*

One of the conventional methods that are applied routinely for the detection of ESBLs is the double disc diffusion method (Aliero et al., 2024). It involves applying disks containing cephalosporins like ceftazidime or ceftriaxone as a preliminary indicator to evaluate resistance or reduced susceptibility. In the next step, a disk containing the molecules belonging to the group of beta-lactamase inhibitors like clavulanic acid is applied together with a cephalosporin to distinguish between resistance due to ESBL and other causes. The isolates were cultured on the agar plates containing disks and incubated at 37°C for 18-24 hours. If the isolates had a zone of inhibition  $\geq 5$  mm for the combined discs than the cephalosporin alone, these were confirmed as ESBL producers.

### *Statistical analysis*

Mean and standard deviation were calculated in Microsoft Excel. Chi-squared test was used to statistically analyze the results for the ESBL detection test using SPSS 29.0. A *p*-value less than 0.05 was considered significant.

## RESULTS

### *Age and gender distribution*

A total of 111 isolates of Gram-negative bacteria were obtained from clinical specimens belonging to 248 patients. Out of these 111 isolates, 55 (49.5%) belonged to the male participants and 56 (50.5%) were isolated from clinical samples belonging to females. In terms of age, the study participants were 20-60 years old. 32 (28.8%) samples were from

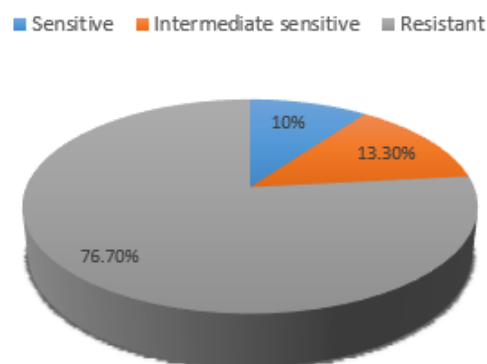
patients who were 20-30 years old, 32 (28.8%) were from patients who were 30-50 years old, and 47 (42.3%) were from patients who were 50-60 years of age.

### Identification of isolates

Following the selection of Gram-negative isolates based on the results of Gram staining, the isolates were identified on the basis of biochemical tests following the scheme of Bergey's manual of systematic bacteriology. *Escherichia coli* (N=34, 30.6%) was the most commonly isolated pathogen followed by *Klebsiella* spp. (N=25, 22.5%), *Acinetobacter* spp. (N=21, 18.9%), *Pseudomonas aeruginosa* (N=15, 13.5%), *Citrobacter* spp. (N=9, 8.1%), and *Proteus mirabilis* (N=7, 6.3%).

### Antibiotic sensitivity of isolates

Overall results for the sensitivity of isolates to the selected antibiotics revealed that a vast majority of the isolates were absolutely resistant to the tested drugs and only a few were completely susceptible to these (Figure 1).



**Figure 1:** Overall prevalence of antibiotic resistance among the isolated pathogens

The average results for antibiotic sensitivity testing showed that Amikacin was the most effective drug against all bacteria as none of the isolates exhibited resistance (Table 1). On the other hand, Colistin was the least effective of all tested drugs as all the

**Table 1:** Zones of inhibition (mm) of bacterial isolates against selected antibiotics according to the CLSI guidelines

No.	Isolates	Amikacin	Aztreonam	Imipenem	Piperacillin/ Tazobactam	Ceftriaxone	Colistin	Levofloxacin	Amoxicillin/ Clavulanic acid
1	<i>Escherichia coli</i> (n=34)	17.36±4.14	17.82±3.45	19.27±5.56	17.54±4.01	20.36±1.56	6.24±2.51	16.00± 3.68	17.42 ±3.84
		S	IS	R	IS	IS	R	IS	IS
2	<i>Klebsiella</i> spp. (n=25)	17.40±4.04	16.48±3.84	19.48±5.18	18.68±3.47	21.68±7.12	6.64±1.05	15.96± 4.09	17.36 ±3.69
		S	IS	R	IS	IS	R	R	IS
3	<i>Acinetobacter</i> spp. (n=21)	16.33±4.72	17.14±4.22	20.42±7.01	17.81±4.30	19.14±7.24	5.67±3.89	15.00± 4.10	16.90 ± 4.47
		IS	R	IS	R	R	R	R	IS
4	<i>Pseudomonas aeruginosa</i> (n=15)	15.53±3.83	15.53±3.66	15.67±2.50	14.80±3.00	19.27±5.73	4.73±1.89	15.20± 4.07	14.73±3.28
		IS	R	R	R	R	R	R	IS
5	<i>Citrobacter</i> spp. (n=9)	18 ± 3.71	14.22±3.28	15.11±1.83	14.44±2.40	19.22±7.74	4.77±1.11	16.89± 3.98	13.66 ±2.83
		S	R	R	R	R	R	IS	R
6	<i>Proteus mirabilis</i> (n=7)	17.57±2.93	14.00±3.55	20.86±9.93	18.86±2.85	20.43±5.30	5.43±2.08	17.57± 4.08	15.71 ±3.90
		S	R	IS	IS	IS	R	IS	IS

Results are shown as Mean±SD; S=Sensitive; IS=Intermediate sensitive; R=Resistant

*Escherichia coli* isolates manifested resistance to Imipenem and Colistin, but susceptibility to Amikacin. These showed intermediate sensitivity to the remaining antibiotics. For *Klebsiella* spp., the most effective drug was Amikacin, followed by Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, and Amoxicillin/Clavulanate. However, these were completely resistant to Imipenem and Colistin. *Acinetobacter* spp. were not sensitive to any of the selected antibiotics and were intermediate sensitive to Amikacin, Imipenem, and Amoxicillin/Clavulanate. These showed resistance to the other antibiotic drugs. *Pseudomonas aeruginosa* demonstrated resistance to all of the selected drugs, except two. Amikacin and Amoxicillin/Clavulanate were the most effective drugs against *Pseudomonas aeruginosa*, although the isolates were intermediate sensitive to these drugs. *Citrobacter* spp. exhibited sensitivity to Amikacin and intermediate sensitivity to Levofloxacin. Against all the other drugs, these were completely resistant. As far as *Proteus mirabilis* is concerned, Amikacin was the most effective drug whereas Aztreonam and Colistin were the least effective drugs. These isolates were intermediate sensitive to the remaining antibiotics.

#### ESBL detection

ESBL detection was done by the double disk diffusion test to identify the production of ESBL by Gram-negative bacteria. Amoxicillin/Clavulanate and Ceftazidime were used for the identification of ESBL-producing bacterial strains. Co-amoxiclavulanate contains clavulanic acid, which inhibits the breakdown of Amoxicillin in the disc by beta-lactamase-producing organisms in combination with the third generation cephalosporin and/or Aztreonam and leads to increased zone size of these antimicrobials or the elliptical zone between the tested antibiotic and discs containing Co-amoxiclavulanate. Out of 111 Gram-negative isolates, 39 (35.1%) showed positive result for ESBL production while 72 (64.9%) tested negative (Figure 2). Among all, *Escherichia coli* isolates showed the highest prevalence of ESBL as only around one-third tested negative for ESBL (Table 2).



**Figure 2:** Positive and negative test results for ESBL detection

The chi-squared test was used to document the statistically significant difference between the two groups with degrees of freedom of 5 and a  $p$  value of  $<0.05$ . On the contrary, *Acinetobacter* spp. and *Pseudomonas aeruginosa* manifested the lowest prevalence of ESBL with 86-90% of the isolates testing negative for ESBL. For *Klebsiella* spp. and *Citrobacter* spp., only around one-quarter of the isolates showed ESBL positive result whereas the majority were negative for ESBL test. As far as *Proteus mirabilis* is concerned, more than half of the isolates did not produce ESBL but slightly less than half did.

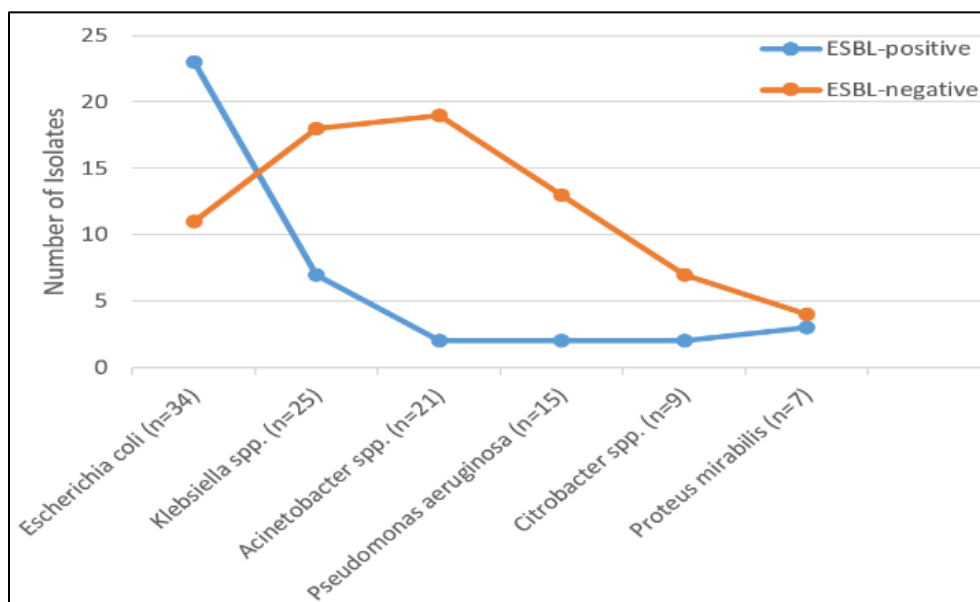
**Table 2:** Results for the detection and distribution of ESBL-producing pathogens

Sr. No.	Bacterial Strains	ESBL Positive (%)	ESBL Negative (%)
1	<i>Escherichia coli</i> (n=34)	67.6	32.3
2	<i>Pseudomonas aeruginosa</i> (n=15)	13.3	86.6
3	<i>Klebsiella</i> spp. (n=25)	28	72
4	<i>Acinetobacter</i> spp. (n=21)	9.5	90.4
5	<i>Proteus mirabilis</i>	42.85	57.1

	(n=7)		
6	<i>Citrobacter</i> spp. (n=9)	22.2	77.7
7	Total (n=111)	35.14	64.86

Figure 3 shows the results of the ESBL detection test in terms of the number of isolates. A  $p$ -value of 0.05 for the chi-squared test was calculated with degrees of freedom of 5 which indicated that there were no statistically significant differences between the ESBL-positive and ESBL-negative groups.

It was observed that 23 of the 34 *Escherichia coli* isolates were positive for ESBL production. 7 of the 25 isolates of *Klebsiella* spp. showed positive results for the ESBL detection test. For *Acinetobacter* spp., only 2 out of 21 isolates were found to be possible ESBL producers. In the case of *Pseudomonas aeruginosa*, 2 out of 15 isolates showed positive test results for ESBL production. Furthermore, 2 of the 9 *Citrobacter* spp. isolates were among probable ESBL producers. Finally, 3 of the 7 *Proteus mirabilis* isolates revealed ESBL-positive results.



**Figure 3:** Prevalence of ESBL producers among Gram-negative isolates

## DISCUSSION

The spread of *Enterobacteriaceae* carrying plasmid-mediated ESBLs has made resistance to  $\beta$ -lactams a worldwide concern. ESBLs can hydrolyze  $\beta$ -lactams such as Cephalosporins, Cephamycin, and Aztreonam. Nevertheless, they remain sensitive to Fosfomycin, Nitrofurantoin, and Carbapenems, and are likely to be inhibited by  $\beta$ -lactamase inhibitors such as Clavulanic acid, Sulbactam, and Tazobactam (Tseng et al., 2023). Categorizing the specific ESBL genes within the bacteria that produce ESBL and identifying the level of antimicrobial resistance can offer practical data relative to the risk factors and epidemiology of the ESBL-producing organisms and their infections (Ghaderi et al., 2020). In this

investigation, the prevalence of ESBL in Gram-negative bacteria was determined in a tertiary-care hospital. Among these, less than half of the patients were 50-60 years old, while slightly more than half were 20-50 years of age. Males and females were equally distributed among the study population. In another study in a Greek population, 267 Gram-negative bacteria were identified in clinical specimens from 235 patients. In terms of gender, 60% of the patients were male while 40% were female in their population. Furthermore, they reported an average age of 47 years and a range of 1-94 years for study subjects (Maraki et al., 2020).

In this study, *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*,

*Citrobacter* spp., and *Proteus mirabilis* were isolated from the clinical specimens in descending order of prevalence. In another study, which was conducted in a different city in Pakistan, the most prevalent Gram-negative pathogen in clinical isolates was *Escherichia coli*, followed by *Klebsiella* spp., *Proteus mirabilis*, and *Pseudomonas aeruginosa*, respectively, which is similar to our observations (Saleem et al., 2022). More than three-quarters of the pathogens isolated in the present study were resistant to the selected antibiotics. A systematic analysis was recently conducted to estimate the burden of antibiotic resistance in Pakistan which revealed an alarmingly high prevalence of resistance among the clinical samples collected in different provinces of Pakistan (Bilal et al., 2021). This highlights the variability in antibiotic effectiveness across different bacterial pathogens emphasizing the need for targeted antibiotic treatment based on specific bacterial susceptibility profiles.

In our study, *Escherichia coli* showed sensitivity to Amikacin but resistance to Imipenem and Colistin, in general. A previous study reported a high level of resistance to Carbapenem and Colistin among *Escherichia coli* isolated from clinical samples in Nepal (Karki et al., 2021). *Klebsiella* spp. were resistant to Imipenem, Colistin, and Levofloxacin but sensitive to Amikacin only. A previous study in a Chinese population focused on the isolation of Colistin-resistant Gram-negative bacteria from clinical specimens and found that, in addition to Colistin, these isolates were highly resistant to other drugs including Imipenem and Levofloxacin. They also reported Amikacin as the most effective drug against these MDR pathogens, similar to our findings (Chen et al., 2021). *Acinetobacter* spp. were mostly MDR and showed a very high level of resistance to Aztreonam, Ceftriaxone, Colistin, Levofloxacin, and Piperacillin/Tazobactam but a lower level of resistance to Amikacin, Imipenem, and Amoxicillin/Clavulanate. Nonetheless, a study in Thailand reported the non-susceptibility of *Acinetobacter baumannii* to several antibacterial agents including Amikacin, Ceftriaxone, Colistin, Imipenem, Levofloxacin, and Piperacillin/

Tazobactam. The only effective drugs against their isolates were Colistin and Tigecycline (Chukamnerd et al., 2024).

*Pseudomonas aeruginosa* showed intermediate susceptibility to Amikacin and Amoxicillin/Clavulanate, while these were resistant to Aztreonam, Ceftriaxone, Colistin, Imipenem, Levofloxacin, and Piperacillin/Tazobactam. A retrospective study in a Chinese population documented that *Pseudomonas aeruginosa* isolates showed the highest resistance to Imipenem followed by Aztreonam, Levofloxacin, and Piperacillin/Tazobactam. The lowest resistance rate was against Amikacin which was 1.8% (Lyu et al., 2023). Isolates of *Citrobacter* spp. in our study were resistant to all antibiotics except Amikacin. In a meta-analysis, *Citrobacter* spp. was found to show the highest rate of resistance to Amikacin, followed by Piperacillin/Tazobactam and Imipenem, respectively. This emphasizes its emergence as an MDR pathogen in hospitals rapidly acquiring resistance to antimicrobial drugs and playing an active role in nosocomial outbreaks (Fonton et al., 2024). In our study, *Proteus mirabilis* isolates were resistant to Aztreonam and Colistin but sensitive to Amikacin. Previously, clinical isolates of *Proteus mirabilis* belonging to an Egyptian population showed the highest rate of resistance to Ceftriaxone followed by Levofloxacin, Amoxicillin/Clavulanate, and Aztreonam. However, these were still susceptible to Piperacillin/Tazobactam, Imipenem, and Amikacin which is in line with our observations (Elhoshi et al., 2023).

In addition to being crucial for management and control, screening for ESBL-producing Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, is also crucial for containment. Among the tests that are used for ESBL identification, one of the most popular is the double disk diffusion test which is more economical and feasible than the other tests, yet it has been established to be slightly less accurate than genotyping and other approaches (Bhat et al., 2020). We found that 35% of the isolates were likely ESBL-producers. Another study in a similar

population described the positivity rate for ESBL ranging from 5% to 45% in samples originating from different sections of a hospital (Idrees et al., 2022). Owing to the ability of ESBLs to enable resistance against the majority of the antimicrobial classes, there has been a rising ESBL prevalence among bacteria in the present world (Gharavi et al., 2021). Besides, other reports have revealed that the prevalence of ESBL producers in Enterobacteriaceae is on the rise in the community settings, globally (Bezabih et al., 2021).

Among the ESBL-producers, we found the highest positivity rate for *Escherichia coli*, followed by *Proteus mirabilis*, *Klebsiella* spp., *Citrobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Owusu et al. (2023) in 2023 reported that among Gram-negative isolates from clinical specimens, *Escherichia coli* was the most prevalent, followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp., respectively. Another research also disclosed that *Escherichia coli* was the most predominant ESBL producer followed by *Klebsiella pneumoniae*. Nearly 68% of the *Escherichia coli* isolates were found to be ESBL-positive in the present study. A recent research focused on genotyping of MDR *Escherichia coli* and confirmed the presence of one or more ESBL-related genes in 29 of the 35 isolates (Ibrahim et al., 2023). It has been estimated that the proportion of ESBL-producing isolates of *Escherichia coli* has been rising in recent years with relative average annual increments of 7% (Kaye et al., 2024).

*Klebsiella* spp., in our study, showed an ESBL-positivity rate of 28%. A retrospective study in a hospital determined that 37% of the *Klebsiella pneumoniae* isolates gave positive results for ESBL detection by phenotypic method (Ljubović et al., 2023). Approximately 10% of the *Acinetobacter* spp. included in the present study were positive for the ESBL detection test. A similar study in an Ethiopian population found that 11.5% of the *Acinetobacter baumannii* isolated from clinical samples were positive for ESBL (Gashaw et al., 2024). Herein, around 13% of the *Pseudomonas aeruginosa* isolates

tested positive for ESBL. Previously, 29.58% of *Pseudomonas aeruginosa* isolated from the clinic as well as the environment tested positive in the phenotypic detection test for ESBL (Alkhulaifi and Mohammed, 2023). Among *Citrobacter* spp., around 22% of the isolates were likely to possess ESBL in our study. In a Palestinian population, the prevalence of ESBL among *Citrobacter* spp. isolated in a clinical setting was 38.4% (El Aila et al., 2023). *Proteus mirabilis* showed a very high prevalence of ESBL and nearly 43% of the isolates gave positive results, although the number of isolates was small. Similarly, ESBLs were detected in 57.6% of *Proteus mirabilis* clinical isolates collected from an Egyptian population (ElTaweel et al., 2024).

### Conclusion

The present research showed the diversity of ESBL producers among various clinical pathogens, which play an important role in the dissemination of diseases in hospital settings. An increase in the frequency of nosocomial infections casts an adverse impact on healthcare. The identification of significant drug resistance patterns among the pathogenic Gram-negative bacteria and the high prevalence of ESBL producers in the hospital environment raise concerns about public health. Furthermore, it highlights the necessity of implementing infection control and surveillance programs as well as administering adequate antimicrobial treatment procedures in hospitals.

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The authors declare that they have no acknowledgments to disclose.

### Ethical Statement

Not Applicable

### Conflict of Interest

Authors declare no conflict of interests.

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