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Prevalence of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae and Their Carbapenem Susceptibility Patterns: A Cross-Sectional Study in Kandahar, Afghanistan

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A B S T R A C T

Purpose:

This study aimed to determine the prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae and to evaluate their carbapenem resistance patterns among clinical isolates in Kandahar, Afghanistan.

Method:

A prospective cross-sectional laboratory-based study was conducted from October 2025 to January 2026 at Mirwais Regional Hospital in Kandahar, Afghanistan. Clinical isolates of Enterobacteriaceae obtained during the study period with complete ESBL testing data (n = 80) were included in the final analysis. ESBL production was detected using the phenotypic confirmatory disk diffusion method.

Antimicrobial susceptibility testing, including imipenem and ertapenem, was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Statistical analysis was conducted to assess associations between ESBL production, carbapenem resistance and prior antibiotic exposure, with $p \leq 0.05$ considered significant.

Results:

Among the 80 Enterobacteriaceae isolates analysed, 21 (26.3%; 95% CI: 17.2–36.8%) were identified as ESBL producers. Among ESBL-producing isolates, susceptibility rates were 66.7% (14/21) for imipenem and 62.5% (10/16) for ertapenem. No statistically significant association was observed between ESBL production and carbapenem resistance ($p > 0.05$). Prior antibiotic use was also not significantly associated with ESBL production or imipenem resistance. High rates of resistance were observed to ampicillin, third-generation cephalosporins, tetracycline, and trimethoprim-sulfamethoxazole, indicating widespread multidrug resistance.

Practical Implications:

The findings emphasise the necessity of routine antimicrobial susceptibility testing for isolated Enterobacteriaceae infections, rational antibiotic prescribing, and strengthened antimicrobial stewardship programs to preserve the effectiveness of carbapenems in this region.

Originality/Novelty:

This study provides baseline data on ESBL prevalence and carbapenem resistance patterns in Kandahar, Afghanistan, a region with limited published antimicrobial resistance data, thereby contributing valuable local evidence to guide clinical practice and policy decisions.

Keywords: ESBL; Enterobacteriaceae; carbapenem resistance; imipenem; ertapenem; antimicrobial resistance; Afghanistan

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1. Introduction

Enterobacteriaceae are major causes of hospital-and community-acquired infections worldwide. They are a diverse family of Gram-negative bacteria with shared biochemical traits, including glucose fermentation, oxidase negativity, and nitrate reduction (Mahon & Lehman, 2019). Antimicrobial resistance (AMR) in bacteria, driven by adaptations that reduce the efficacy of treatments, has become a major global public health concern. In 2019, AMR was associated with an estimated 4.95 million deaths worldwide, including 1.27 million deaths directly attributable to AMR (Murray et al., 2022)
Extended-spectrum β-lactamases (ESBLs) mediate resistance to penicillins, cephalosporins, and aztreonam. They are inhibited by β-lactamase inhibitors, and do not hydrolyse cephamycins or carbapenems (Shakil et al., 2012). Globally, ESBL-producing Enterobacteriaceae are highly prevalent, with pooled estimates of 45.6%, while carbapenemase-producing strains are less common (~16%) (Abera et al., 2023). The emergence of ESBL-producing organisms compromises cephalosporin efficacy, poses substantial challenges to infection control efforts, and leads to longer hospital stays, higher healthcare costs, and increased financial burden on families (Kateregga et al., 2015; Pitout, 2013).

Carbapenem-resistant Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacterales are recognised by the WHO as critical priority pathogens (World Health Organization, 2017). Carbapenem-resistant enterobacteria were first reported in 1996, with carbapenemase-producing Klebsiella pneumoniae identified as the causative organism. Over the past decade, their global prevalence has risen, with CRE infections primarily affecting patients in hospitals and long-term care settings (Caliskan-Aydogan & Alocilja, 2023).

The rising incidence of infections caused by ESBL-producing Enterobacteriaceae has increased reliance on carbapenems, contributing to the emergence of carbapenem-resistant Enterobacteriaceae. (Pang et al., 2018).

Regional studies from China, India, Iran, and Pakistan report high rates of ESBL production in E. coli and K. pneumoniae, moderate resistance to aminoglycosides, and emerging carbapenem resistance (Bhagat, 2023; Khademi et al., 2022; Mustafai et al., 2023; Pan et al., 2024). These findings reflect evolving multidrug resistance trends in hospital settings and highlight the urgent need to monitor resistance patterns.

Although ESBL-producing Enterobacteriaceae are increasingly reported worldwide (Mansouri et al., 2019)Information from hospitals in Afghanistan, particularly in Kandahar, remains limited. Afghanistan's efforts to combat antimicrobial resistance are hindered by a weakened health care system, limited laboratory capacity, fragmented and under-resourced AMR surveillance, constrained antimicrobial stewardship programs, and a lack of nationally representative epidemiological data on resistance patterns (H. Ehsan et al., 2025). Although some initiatives have been launched to tackle antimicrobial resistance, data on the true extent of resistance remain limited, and significant gaps persist in the country's infrastructure for monitoring and responding to AMR (Médecins Sans Frontières, 2024). The lack of locally generated data on their prevalence and carbapenem-resistance patterns limits evidence-based antibiotic selection and effective infection-control practices. In a resource-limited healthcare system such as Afghanistan, such data are essential for strengthening antimicrobial stewardship and improving patient outcomes.

This study therefore aimed to determine the prevalence of ESBL-producing Enterobacteriaceae and to evaluate their carbapenem susceptibility patterns among clinical isolates at Mirwais Regional Hospital.

2. Methods and Materials

2.1 Study design and setting

This was an observational, hospital-based, cross-sectional study conducted over three months, from 30/10/2025 to 31/01/2026, at Mirwais Regional Hospital in Kandahar, Afghanistan. Mirwais regional hospital in Kandahar is a 650-bed hospital and one of the largest in southwestern Afghanistan. It treats about 32,000 outpatients and 6,000 inpatients monthly, performing around 1,200 major and 800 minor surgeries.

2.2 Study population

Clinical specimens were collected aseptically from patients of all ages suspected of infectious diseases within the laboratory and processed within 1 hour of collection. All Enterobacteriaceae isolates from the same patient were included only if they were obtained from different specimen types or at different time points. Specimens yielding non-Enterobacteriaceae bacteria or those that were contaminated were excluded.

2.3 Sample size Determination

The required sample size for estimating the prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae was calculated using the single population proportion formula:

$$n= z2\ P(1–P)/d2$$

Where n is the required sample size, Z is the Z-score corresponding to the 95% confidence level (1.96), and P is the estimated prevalence of ESBL-producing Enterobacteriaceae (30%) based on previous studies (B. Ehsan et al., 2023), and d is the margin of error (5%). Based on this formula, the minimum required sample size was approximately 323 isolates. Although the calculated minimum sample size was 323 isolates, the study included all eligible Enterobacteriaceae isolates obtained during the predefined study period, resulting in a final sample of 80 isolates.

2.4 Bacterial isolation and identification

Blood samples were inoculated into tryptic soy broth (TSB) and incubated at 37 °C for 24 hours, followed by subculture onto blood agar, chocolate agar and MacConkey agar. Cerebrospinal fluid, pleural fluid, and peritoneal fluid specimens were inoculated into brain heart infusion (BHI) broth and incubated under identical conditions before subculture. Other clinical specimens were directly cultured onto the aforementioned media and incubated at 37 °C for 24 hours.

Bacterial isolates were identified using standard microbiological techniques, including Gram staining, catalase, oxidase, indole production, citrate utilisation, methyl red, triple sugar iron (TSI) and motility tests. All culture media and reagents were obtained from Becton, Dickinson and Company. (BD BBL ™, USA).

2.5 Antimicrobial susceptibility test

The Modified Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. Mueller Hinton agar (BD BBL, USA) was prepared according to the manufacturer's instructions, aseptically poured into sterile 90 mm Petri dishes, and left at room temperature until complete solidification. Inoculation of agar was carried out using a sterile cotton swab dipped into a bacterial suspension standardised to 0.5 McFarland turbidity. Excess fluid was removed by gently pressing the swab against the side of the tube. The surface of each plate was streaked in three different directions, with the plate rotated approximately 60° between streaking to ensure uniform bacterial distribution. Antibiotics discs of Gentamicin (GM) 10 µg, Amikacin (AN) 30 µg, Nitrofurantoin (FM) 100 µg, Amoxicillin/Clavulanic acid (AMC) 20/10 µg, Trimethoprim- sulfamethoxazole(SXT,1.25/23.75), Imipenem (IMP) 10 µg, Ertapenem (ETP) 10 µg, Ciprofloxacin (CIP) 5 µg, Cefotaxime (CTX) 30 µg, Ceftriaxone (CRO) 30 µg, Ceftazidime (CAZ) 30 µg, Nalidixic acid (NA) 30 µg, Tetracycline (TE) 30 µg, Levofloxacin (LEV) 5 µg, Ceftazidime/Avibactam (CZA) 10/4 µg, Ampicillin (AM) 10 µg, (Mast group, Merseyside, UK or BD BBL Sensi-Disc, USA). Multidrug resistance (MDR) was defined as resistance to ≥3 classes of antimicrobial agents (Magiorakos et al., 2012).

The plates were incubated aerobically at 37 °C for 16-18 hours. After incubation, the diameters of the inhibition zones were measured in millimetres and interpreted as sensitive, intermediate, or resistant according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2025) for Enterobacteriaceae.

Due to the specific use of certain antibiotics, not all isolates were tested against every agent, resulting in variable N per antibiotic. Nalidixic acid was tested only on urinary isolates, and because the number of urine samples in this study was limited, N for this agent was low. Cefoxitin was tested only selectively, as it is not routinely used against Enterobacteriaceae but is primarily used against Gram-positive bacteria.

2.6 Phenotypic detection of ESBLs

All confirmed Enterobacteriaceae that showed resistance to third-generation cephalosporins were screened for ESBL production. According to CLSI guidelines, the recommended confirmatory method is the cephalosporin-clavulanic acid combination disk test. However, because combination disks were unavailable in our laboratory, ESBL production was confirmed using the Double Disk Synergy Test (DDST), a widely accepted phenotypic alternative (Drieux et al., 2008).

A standardised bacterial suspension equivalent to 0.5 McFarland turbidity was inoculated onto Mueller–Hinton agar plates. An amoxicillin/clavulanic acid (20/10 µg) disk was placed at the centre of the plate, and ceftriaxone (30 µg), ceftazidime (30 µg), and cefotaxime (30 µg) disks were positioned 30 mm away on three sides. Plates were incubated aerobically at 37°C for 16–18 hours. Enhancement of the inhibition zone of any cephalosporin disk toward the amoxicillin/clavulanic acid disk was interpreted as confirmation of ESBL production.

2.7 Quality control and limitations

Due to the unavailability of certified ESBL-positive and ESBL-negative control strains (e.g., Klebsiella pneumoniae ATCC700603), external quality control for ESBL detection could not be performed. However, general bacterial reference strains provided by Snow Pharma were used for internal quality control, including Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, and Pseudomonas aeruginosa ATCC 9027, to ensure disc performance. The lack of certified ESBL control strains is addressed in the study's limitations section and may affect the reliability of ESBL detection.

2.8 Statistical analysis

Data were entered, cleaned, and coded using Microsoft Excel. Statistical analyses were performed using IBM SPSS Statistics, version 27. Descriptive statistics, including frequencies, percentages, and cross–tabulations, were calculated. The Chi-square test was used when expected cell counts were ≥5; otherwise, Fisher's exact test was applied to assess associations. A p-value of ≤0.05 was considered statistically significant.

2.9 Ethical consideration

Official permission to conduct the study was obtained from Mirwais Regional Hospital, the Kandahar Provincial Public Health Directorate, and the Research Committee of Kandahar University. The study utilised routine clinical specimens collected for diagnostic purposes, and no additional procedures were performed on patients for research purposes. Patients' confidentiality was strictly maintained, and no personal identifiers were recorded. All data were handled anonymously and used solely for research purposes.

3. Results

3.1 Demographic and specimen distribution

A total of 496 clinical samples were processed during the study period. Of these, 242 (48.8%) showed bacterial growth, while 253 (51.0%) showed no growth, and 1 sample (0.2%) had missing data. Among the study participants (n=496), 275 (55.4%) were male and 221 (44.6%) were female. The ages of participants ranged from 15 days to 85 years, with a mean age of 27.2± 20.4 years. Ear pus was the most frequently processed specimen (101/496, 20.4%), followed by wound pus (68/496, 13.7%) and pleural fluid (41/496, 8.3%).

3.2 Bacterial isolates

Among the 242 culture-positive isolates, the predominant isolate was Staphylococcus aureus (n = 74, 30.6%), followed by Pseudomonas aeruginosa (n = 41, 16.9%), Escherichia coli (n = 27, 11.2%) and Klebsiella pneumoniae (n = 25, 10.3%). Other isolates included Proteus spp. (n = 24, 9.9%), Acinetobacter baumannii (n = 18, 7.4%), coagulase- negative staphylococci (n = 21, 8.7%), Enterobacter spp. (n = 5, 2.1%), Providencia spp. (n = 2, 0.8%), Streptococcus pyogenes (n =4, 1.7%), and Citrobacter spp. (n = 1, 0.4%) (Table 1).

Table 1. Frequency and distribution of bacterial isolates from positive clinical samples (n=242)

Isolated bacteria	N	%
Staphylococcus aureus	74	30.6
Pseudomonas aeruginosa	41	16.9
Escherichia coli	27	11.2
Klebsiella pneumoniae	25	10.3

Proteus spp.	24	9.9
Acinetobacter baumannii	18	7.4
Coagulase-negative staphylococci	21	8.7
Enterobacter spp.	5	2.1
Providencia spp.	2	0.8
Streptococcus pyogenes	4	1.7
Citrobacter spp.	1	0.4

Note: Percentages were calculated from total isolates (n=242)

3.3 Enterobacteriaceae and ESBL Production

Of the 242 culture-positive isolates, 84 (34.7%) belonged to the family Enterobacteriaceae, while 158 (65.3%) were non-Enterobacteriaceae. ESBL production status was available for 80 (95.2%) of the 84 Enterobacteriaceae isolates; four isolates had missing data and were excluded from this analysis. Among the 80 Enterobacteriaceae isolates with available data, 21 (26.3%; 95% CI: 17.2-36.8%) were ESBL positive, while 59 (73.8%) were ESBL negative.

Urine and sputum specimens showed the highest proportion of Enterobacteriaceae (54.5% each), followed by wound pus (38.2%) and pleural fluid (34.1%). Specimen type information was available for 239 culture- positive isolates, including 82 Enterobacteriaceae isolates; two isolates had missing specimen data and were excluded from this analysis. No statistically significant association was observed between specimen type and Enterobacteriaceae isolation (p = 0.207). (**Table *2***).

Table 2. Distribution of Enterobacteriaceae according to specimen type among isolates with available specimen data (n=239)

Specimen type	Total (n)	Enterobacteriaceae(n)	%
Pleural fluid	41	14	34.1
Blood	4	0	0.0
Urine	11	6	54.5
Ear pus	101	29	28.7
Wound pus	68	26	38.2
Sputum	11	6	54.5
Other	3	1	–
Total	239	82	34.3

3.4 Association Between ESBL Production and Carbapenem Resistance

The relationship between ESBL production and carbapenem resistance was assessed using the chi-square test. No statistically significant association was observed between ESBL production and imipenem resistance (p = 0.354) or ertapenem resistance (p = 0.400). These findings indicate that ESBL production was not significantly associated with carbapenem resistance among Enterobacteriaceae isolates in this study.

The susceptibility of ESBL-producing Enterobacteriaceae isolates to carbapenems is summarised in Table 3. Among ESBL-producing isolates, 66.7% were sensitive to imipenem, and 62.5% were sensitive to ertapenem, indicating moderate susceptibility to carbapenem (Table 3).

Table 3. Carbapenem susceptibility of ESBL-producing Enterobacteriaceae isolates (n = 21)

ESBL production	Antibiotic	N	Sensitive n (%)	Resistant n (%)	Intermediate n (%)
Positive	Imipenem	21	14 (66.7)	7 (33.3)	0 (0.0)
Positive	Ertapenem	16	10 (62.5)	6 (37.5)	0 (0.0)

Note: percentages were calculated based on the number of ESBL-positive isolates tested for each antibiotic

3.5 Antibiotic susceptibility profile

Enterobacteriaceae isolates demonstrated high rates of resistance to commonly used antibiotics, particularly ampicillin (98.6%), cefazolin (98.4%), amoxicillin/clavulanic acid (94.8%), tetracycline

(94.6%), and trimethoprim-sulfamethoxazole (94.4%). High resistance was also observed for ceftazidime (94.7%) and ceftriaxone (91.4%).

Carbapenems showed relatively higher activity, with sensitivities of 51.2% and 53.4% to imipenem and ertapenem, respectively. Amikacin also demonstrated moderate activity (51.2% sensitivity). Overall, the isolates exhibited a high level of multidrug resistance (Table 4).

Table 4. Antimicrobial susceptibility pattern of Enterobacteriaceae isolates (n = 84)

Antibiotic	N	Sensitive n (%)	Resistant n (%)	Intermediate n (%)
Ceftriaxone	81	6 (7.4)	74 (91.4)	1 (1.2)
Sulfamethoxazole/ Trimethoprim	54	3 (5.6)	51 (94.4)	0 (0.0)
Cefotaxime	60	6 (10.0)	54 (90.0)	0 (0.0)
Ceftazidime/avibactam	67	7(10.4)	60 (89.6)	0 (0.0)
Ciprofloxacin	74	8 (10.8)	61 (82.4)	5 (6.8)
Minocycline	59	6 (10.2)	49 (83.1)	4 (6.8)
Cefixime	28	2 (7.1)	25 (89.3)	1 (3.6)
Ertapenem	58	31 (53.4)	27 (46.6)	0 (0.0)
Cefazolin	62	1 (1.6)	61 (98.4)	0 (0.0)
Tetracycline	74	3 (4.1)	70 (94.6)	1 (1.4)
Imipenem	80	41 (51.2)	37 (46.3)	2 (2.5)
Nalidixic acid	7	1 (14.3)	5 (71.4)	1 (14.3)
Amoxicillin/clavulanic acid	77	1 (1.3)	73 (94.8)	3 (3.9)
Ceftazidime	75	3 (4.0)	71 (94.7)	1 (1.3)
Cefoxitin	1	0 (0.0)	1 (100.0)	0 (0.0)
Nitrofurantoin	7	4 (57.1)	3 (42.9)	0 (0.0)
Ampicillin	70	0 (0.0)	69 (98.6)	1 (1.4)
Chloramphenicol	27	5 (18.5)	22 (81.5)	0 (0.0)
Gentamicin	78	25 (32.1)	52 (66.7)	1 (1.3)
Amikacin	80	41 (51.2)	33 (41.3)	6 (7.5)

Note:

Percentages were calculated based on the number of isolates tested for each antibiotic.

N varies due to selective testing; nalidixic acid was tested only on urine isolates, and cefoxitin was primarily tested for Gram-positive bacteria

MDR is defined as resistance to ≥3 antimicrobial classes (see Methods)

3.6 Prior antibiotic use

Of the 496 patients, 76 (15.4%) reported prior antibiotic use, while 417 (84.4%) had not used antibiotics previously. Among 83 Enterobacteriaceae isolates with complete data on prior antibiotic use, ESBL production was observed in 25.0% of isolates from patients without prior antibiotic exposure and in 40.0% of isolates from patients with prior antibiotic exposure. This difference was not statistically significant (Fisher's exact test, $p = 0.338$).

The Association between previous antibiotic use and imipenem susceptibility among Enterobacteriaceae isolates was also assessed. No statistically significant relationship was observed ($p = 0.368$), suggesting that prior antibiotic exposure did not significantly influence imipenem resistance in this dataset (Table5. Imipenem **susceptibility of Enterobacteriaceae isolates according to prior antibiotic use**).

Table5. Imipenem susceptibility of Enterobacteriaceae isolates according to prior antibiotic use

Previous antibiotic use	n	Sensitive n (%)	Resistant n (%)	Intermediate n (%)
Not used	66	33 (50%)	32 (48.5%)	1 (1.15%)
Used	14	8 (57.1%)	5 (35.7%)	1 (7.1%)
Total	80	41 (51.2%)	37 (46.3%)	2 (2.5%)

p- value = 0.368

3.7 Logistic Regression Analysis of Prior Antibiotic Use and ESBL Production

A binary logistic regression analysis was performed to evaluate the association between prior antibiotic use and ESBL production among Enterobacteriaceae isolates with complete data (n = 83). The model was not statistically significant (Omnibus test: $\chi^2 = 1.306$, $p = 0.253$), indicating that prior antibiotic use did not significantly improve the prediction of ESBL production.

Patients with a history of antibiotic use had higher odds of ESBL production compared to those without prior exposure (OR = 2.00, 95% CI: 0.621-6.443); however, this association was not statistically significant (p = 0.245). The model explained only a small proportion of the variance in ESBL production (Nagelkerke R2 = 0.023) (Table 6).

Table 6. Logistic regression analysis of the association between prior antibiotic use and ESBL production among Enterobacteriaceae isolates

Variable	B	SE	Odds Ratio (exp(B))	95% CI	p-value
Prior antibiotic use	0.693	0.597	2.00	0.621-6.443	0.245

Note: Model statistics: $\chi^2 = 1.306$, $p = 0.253$; Nagelkerke $R^2 = 0.023$; n = 83.

4. Discussion

4.1 Prevalence of ESBL-Producing Enterobacteriaceae

This study evaluated the prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and their carbapenem susceptibility among clinical isolates in Kandahar, Afghanistan. ESBL-producing isolates accounted for 26.3% of Enterobacteriaceae, indicating a considerable burden of resistance in this setting. The observed prevalence likely results from the combined impact of antimicrobial misuse and limited infection prevention infrastructure. In Afghanistan, antibiotics are frequently obtained without prescription, self-medication is common, and microbiological diagnostics are not routinely used to guide therapy, creating selective pressure that favours ESBL-producing strains. In addition, inadequate infection control practices in healthcare facilities may facilitate the horizontal transmission of resistant organisms between patients (H. Ehsan et al., 2025; Laxminarayan et al., 2013).

The prevalence of ESBL- producing Enterobacteriaceae observed in this study is consistent with reports from several low and middle-income countries, where ESBL rates typically range between 20% and 40% (Bezabih et al., 2022). In Africa, pooled ESBL prevalence has been estimated at approximately 28%, highlighting significant inter- regional variability in ESBL occurrence across the continent (Abay et al., 2025). Higher prevalence rates exceeding 50% have been reported in some tertiary care settings in South Asia and the Middle East (Romika & Mala, 2012). Country-level studies further illustrate notable heterogeneity, with prevalence values reported from <1% in some settings up to >50% in others (Sangare et al., 2015). Variation in ESBL prevalence across regions may reflect differences in antibiotic prescribing practices, infection prevention measures, healthcare infrastructure, and availability of antimicrobial stewardship programs. In Afghanistan, limited regulation of antibiotic use and over-the-counter availability of antimicrobials may contribute to the emergence and spread of resistant organisms.

4.2 Carbapenem Susceptibility and Emerging Resistance

Carbapenems are widely regarded as the treatment of choice for serious infections caused by ESBL-producing Enterobacteriaceae. Clinical practice guidelines from the IDSA recommend agents such as meropenem, imipenem-cilastatin, and ertapenem for ESBL-E infections, particularly those outside

uncomplicated urinary tract infections (Tamma et al., 2024). Similarly, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines recommend carbapenems as targeted therapy for third-generation cephalosporin-resistant Enterobacteriaceae, including ESBL producers (Paul et al., 2022).

In this study, ESBL- producing isolates showed moderate susceptibility to imipenem (66.7%) and ertapenem (62.5%), lower than rates reported in many other settings, where susceptibility frequently exceeds 80% (Amer et al., 2017; Lee et al., 2012). Regional variability in carbapenem susceptibility is evident: carbapenem resistance in some regions of Saudi Arabia has reached up to 49.5% for imipenem and 57.9% for meropenem (Alshehri & Irekeola, 2024). While studies from Pakistan reported meropenem resistance in around 34% among uropathogenic E. coli (B. Ehsan et al., 2023). In contrast, data from China showed high carbapenem susceptibility (91.7%) among ESBL- producing E. coli (Fu et al., 2024).

The reduced susceptibility observed in this study raises concern regarding additional resistance mechanisms beyond ESBL production. Potential contributors include carbapenemase production (such as OXA-48 and NDM, which are increasingly reported in South Asia), metallo-beta-lactamases (MBLs), altered membrane permeability due to porin loss, and efflux pump overexpression (Aurilio et al., 2022; Caliskan-Aydogan & Alocilja, 2023). Importantly, the lack of a statistically significant association between ESBL production and carbapenem resistance suggests that these mechanisms operate independently of ESBL enzymes, underscoring the complexity of carbapenem resistance.

4.3 Antimicrobial Resistance Patterns

The overall antimicrobial susceptibility profile showed high rates of resistance to commonly used antibiotics, including ampicillin, cefazolin, amoxicillin/clavulanic acid, tetracycline, and sulfamethoxazole-trimethoprim, indicating widespread multidrug resistance in this region.

Such high resistance levels may be attributed to empirical and sometimes inappropriate use of broad-spectrum antibiotics, lack of routine culture-guided therapy, and insufficient antimicrobial stewardship. Although carbapenems and amikacin showed relatively greater activity, the moderate susceptibility rates highlight the need for cautious, rational use to preserve their effectiveness.

.4.4 Association Between Prior Antibiotic Use and ESBL Production

Although ESBL production was more frequent among isolates from patients with prior antibiotic exposure than among those without, the difference was not statistically significant. Similarly, no significant association was found between prior antibiotic use and imipenem susceptibility. The lack of statistical significance may be explained by the relatively small number of patients reporting prior antibiotic use, which may have limited the analysis's statistical power. Nevertheless, prior antibiotic exposure remains an established risk factor for antimicrobial resistance, and larger studies are needed to explore this relationship in the Afghan context further (Omar et al., 2025).

4.5 Study Limitations

This study provides important baseline data on antimicrobial resistance patterns in Kandahar, a region where published data remain limited. However, several limitations should be acknowledged. First, the study was conducted at a single centre, which may limit the generalizability to other regions of Afghanistan. Second, the overall sample size of ESBL-positive isolates was relatively small, potentially affecting statistical power. Third, molecular characterisation of ESBL, carbapenemase, and Metallo-beta-lactamase genes was not performed, preventing the identification of specific resistance mechanisms. Additionally, certified reference strains for external quality control, including standard ESBL-positive and ESBL- negative controls, were not available. Although internal quality control measures were performed, the absence of standardised ATCC strains may have affected the precision of susceptibility test results.

Despite these limitations, the findings highlight a significant burden of ESBL- producing Enterobacteriaceae and emerging carbapenem resistance in Kandahar. Strengthening antimicrobial stewardship programs, improving infection prevention and control practices, and implementing routine surveillance systems are urgently needed. Future multicentre studies incorporating molecular analyses are recommended to understand resistance mechanisms better and guide evidence-based treatment policies.

5. Conclusion

This study demonstrated a high prevalence of ESBL-producing Enterobacteriaceae among clinical isolates in Kandahar, Afghanistan, with more than one-quarter of isolates producing ESBLs. Carbapenems demonstrated moderate activity against ESBL-producing isolates, and susceptibility rates were lower than those commonly reported in other settings. The relatively low carbapenem susceptibility compared with other settings raises concern for emerging resistance mechanisms.

This finding underscores the urgent need for strengthened antimicrobial stewardship, improved infection control measures, and routine antimicrobial surveillance. Routine antimicrobial susceptibility testing for all Enterobacteriaceae isolates is strongly recommended to guide appropriate and effective therapy.

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Conflict of Interest

The authors declare no conflicts of interest.

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