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# The Prevalence of Extended-Spectrum β-Lactamase Producing Enterobacteriaceae Isolated from Hospitalized Patients

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**ABSTRACT**

Broad-spectrum beta-lactamase-producing Enterobacteriaceae cause significant health issues. Owing to the increasing prevalence of antibiotic resistance, including beta-lactamase-resistant species, determining their prevalence in hospitals is particularly important. This study aimed to evaluate the frequency of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in clinical specimens obtained from patients admitted to Shahid Rahimi Hospital in Khorramabad. Clinical samples were obtained from hospitalized patients for 4 months, collected by census, and identified using standard biochemical methods. Antibiotic resistance testing was performed using the Kirby-Bauer method, and the ESBLs phenotype was confirmed using the combination disk test. In this study, 215 Enterobacteriaceae isolates were extracted, of which 149 were ESBL generators. The highest frequency of ESBLs among the identified bacteria was Escherichia coli (51.2%). The highest frequency of ESBL-producing isolates was in the > 65 years age group (32.6%), in urinary specimens (61.4%), and in emergency wards (24.1%). In conclusion, the high prevalence of ESBL-producing bacteria in tract infections (61.4%) shows the importance of routine testing in medical centers and the need for physicians to pay more attention to this issue.

**Keywords:** Enterobacteriaceae; Antibiotic Resistance; Extended Spectrum Betalactamases; Isolated; Hospitalized Patients

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## Introduction

Beta-lactam antibiotics are among the most extensively utilized agents for the treatment of bacterial infections [1, 2]. Nevertheless, their efficacy is increasingly challenged by the alarming phenomenon of antimicrobial resistance, a significant global health concern [3]. The production of beta-lactamase enzymes by gram-negative bacteria, particularly Enterobacteriaceae, constitutes a primary mechanism of resistance to these antibiotics on a worldwide scale [4].

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Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated enzymes that can hydrolyze and inactivate a broad spectrum of antimicrobial β-lactams, including third-generation cephalosporins, penicillins, and aztreonam, although they are susceptible to inhibition by clavulanic acid [5]. Bacteria producing ESBLs typically exhibit resistance to broad-spectrum cephalosporins, monobactams, and penicillins, leading to multidrug resistance (MDR). The rising prevalence of infections caused by ESBL-producing bacteria is associated with increased mortality rates and financial burdens on healthcare systems [6]. The resistance in Enterobacteriaceae species is escalating due to broad-spectrum beta-lactamases, with prevalence rates varying across different countries, regions, and healthcare facilities [7, 8]. A 12-year study reported a low prevalence of ESBL-producing Enterobacter isolates; however, it also identified diverse non-epidemic E. cloacae clones and persistent CTX-M-10 β-lactamase [9].

Further research has identified chronic conditions (e.g., cerebrovascular disease) and recent antibiotic use as independent risk factors for infection or colonization by ESBL-producing Enterobacteriaceae [10]. Another study documented the dissemination of multidrug-resistant Enterobacteriaceae harboring resistance genes in Algeria [11].

The routine diagnosis of ESBL is not conducted in many microbiology laboratories within hospitals in developing countries [12], including Iran. The emergence of ESBL-producing strains necessitates the development of laboratory methods to detect these enzymes among bacterial pathogens. Understanding the prevalence of ESBL-associated infections in hospitals significantly influences clinicians' selection of appropriate initial therapy. This study aimed to evaluate the frequency of this enzyme in clinical samples obtained from patients admitted to Shahid Rahimi Hospital in Khorramabad.

## Materials and Methods

The prevalence of extended-spectrum β-lactamase (ESBL) production by isolates from patients admitted to Shahid Rahimi Hospital (inpatient emergency, Internal ICU, General Internal, male Internal, Female Internal, ICU Surgery, Male Surgery, pediatric, heart, NICU, oncology female surgery, poisoning, and PICU wards) in Khorramabad was determined from June 2019 to September 2019.

In this study, urine, blood, and stool samples were collected from hospitalized patients using a census method. The prepared samples were cultured in a special culture medium (such as blood agar, Mueller Hinton agar, TSI, Simons Citrate agar, TSB, SIM, Urea agar, and MR-VP). Enterobacteriaceae isolates were identified using standard biochemical methods.

Finally, 215 Enterobacteriaceae isolates were examined. We determined the antibiotic resistance of the isolates using the Kirby-Bauer method and confirmed the ESBLs phenotype using the combination disk method. In this method, single discs of 30 μg of cefotaxime (CTX) and ceftazidime (CAZ) in the vicinity of the combined disk of cefotaxime-clavulanic acid (CTX-CA30 / 10 μg) and ceftazidime-clavulanic acid (CAZ-CA30 / 10 μg) were used in Müller-Hinton agar medium. After collecting the study data, they were entered into SPSS 26 software for analysis. The Chi-square test was used to investigate the relationship between qualitative variables. A significance level of 0.05% was used to interpret the data.

## Results

This study collected 2591 blood samples, 2171 urine samples, and 281 stool samples, from which 19, 185, and 11 Enterobacteriaceae isolates were obtained from blood, urine, and stool, respectively. Among all Enterobacteriaceae isolates, 149 (69.3 %) were ESBLs producers.

Of the 215 isolates, the highest number of positive tests was in the > 65 years age group, with 70 cases (32.6%). According to the chi-square test, there was a significant relationship between the presence of ESBLs and age group (p=0.006) (Table 1).

There was no significant relationship between the presence of ESBLs and sex (36.7% female and 32.6% male) (p=0.302). The highest prevalence of ESBL-generating isolates was observed in inpatient emergency wards (24.1%) and inpatient internal intensive care units (8.8%) (Table 2).

The prevalence of Enterobacteriaceae isolates producing ESBLs was 61.4%, 5.1%, and 2.8% in urine, blood, and stool samples, respectively; however, the differences were not statistically significant (p=0.265) (Table 3).

The highest frequency of ESBLs among the identified bacteria was observed in Escherichia coli (51.2%) (Table 4).

Table 1. Consensus table between Enterobacteriaceae based on the presence of ESBLs and age groups

| ESBL / Age | + | | - | | Total | |
|---|---|---|---|---|---|---|
| | (Percentage) | number | (Percentage) | number | (Percentage) | number |
| **≤18** | 7.9 | 17 | 7 | 15 | 14.9 | 32 |
| **19-29** | 2.3 | 5 | 3.7 | 8 | 6 | 13 |
| **30-64** | 26.5 | 57 | 7.9 | 17 | 34.4 | 74 |
| **≥65** | 32.6 | 70 | 12.1 | 26 | 44.7 | 96 |
| **Total** | 69.3 | 149 | 30.7 | 66 | 100 | 215 |

Table 2. Consensus table between Enterobacteriaceae based on the presence of ESBLs and hospital ward

| ESBLs / Ward | + | | - | | Total | |
|---|---|---|---|---|---|---|
| | (Percentage) | number | (Percentage) | number | (Percentage) | number |
| Inpatient emergency | 24.1 | 52 | 9.5 | 42 | 43.7 | 94 |
| Internal ICU | 8.8 | 19 | 0.9 | 2 | 9.8 | 21 |
| General Internal | 6.5 | 14 | 0.9 | 2 | 7.4 | 16 |
| male Internal | 5.6 | 12 | 0.9 | 2 | 6.5 | 14 |
| Female Internal | 5.6 | 12 | 3.7 | 8 | 9.3 | 20 |
| ICU Surgery | 5.1 | 11 | 0 | 0 | 5.1 | 11 |
| Male Surgery | 4.7 | 10 | 0 | 0 | 4.7 | 10 |
| pediatric | 2.3 | 5 | 0.9 | 2 | 3.3 | 7 |
| heart | 2.3 | 5 | 0 | 0 | 2.3 | 5 |
| NICU | 2.3 | 5 | 0.5 | 1 | 2.8 | 6 |
| oncology | 0.9 | 2 | 0 | 0 | 0.9 | 2 |
| Female surgery | 0.5 | 1 | 0.5 | 1 | 0.9 | 2 |
| poisoning | 0.5 | 1 | 1.9 | 4 | 2.3 | 5 |
| PICU | 0 | 0 | 0.9 | 2 | 0.9 | 2 |
| Total | 69.3 | 149 | 30.7 | 66 | 100 | 215 |

Table 3. Consensus table between Enterobacteriaceae based on the presence of ESBLs and sample type

| ESBL / Sample | + | | - | | Total | |
|---|---|---|---|---|---|---|
| | (Percentage) | number | (Percentage) | number | (Percentage) | number |
| Urine | 61.4 | 132 | 24.7 | 53 | 86 | 185 |
| Blood | 5.1 | 11 | 3.7 | 8 | 8.8 | 19 |
| Stool | 2.8 | 6 | 2.3 | 5 | 5.1 | 11 |
| Total | 69.3 | 149 | 30.7 | 66 | 100 | 215 |

## Discussion

Numerous physicians have reported an increase in antibiotic resistance among bacteria, complicating the treatment of severe infections and emerging as a significant concern for bacterial infections [13, 14]. Continuous exposure of bacterial strains to various beta-lactam antibiotics induces successive mutations in bacterial beta-lactamase, leading to the emergence of extended-spectrum beta-lactamases (ESBLs), which confer resistance to beta-lactam antibiotics. ESBL production is a primary mechanism of antimicrobial resistance in pathogens, including those causing urinary tract infections [15, 16]. The study by Muhammad Muqaddas Mustafai identified Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, as the predominant causes of urinary, bloodstream, wound, and respiratory infections in hospitalized patients [17]. Research by Kim et al. revealed that 15.3% of Enterobacter spp. bacteremia cases were ESBL-producing, predominantly the CTX-M and SHV-12 variants. Although these cases exhibited higher rates of inappropriate empirical therapy, ESBL production did not significantly impact mortality or length of hospitalization among patients with cancer [18].

Our findings also indicated that E. coli (51.2%) and Klebsiella (14%) were the most prevalent ESBL-producing Enterobacteriaceae, corroborating

previous studies, such as those by Najjuka et al., which reported a high prevalence of ESBL-producing E. coli and K. pneumoniae in clinical samples from Uganda [24], and Mathlouthi et al. in Tunisia and Libya [25]. A study in Ethiopia found that among 133 bacterial isolates, 51.1% were Enterobacteriaceae, with 25% (17 of 68) being ESBL producers, and Escherichia coli was the predominant ESBL-producing species (28.57%) [26]. Additionally, in the study by Luciene A. R. Minarini et al., PCR and PFGE analysis identified ESBL production in 1.48% of 1481 community-acquired bacterial isolates (including 12 Klebsiella pneumoniae and 7 Escherichia coli strains), with TEM-type enzymes being predominant (95.4%), followed by SHV and CTX-M [27].

The frequency percentage obtained in our study was notably higher than that reported in many countries. For instance, a study in Brazil examined the prevalence of ESBL in uropathogenic E. coli isolated from CAUTI cases in November 2015 and found that 41 (8%) isolates produced ESBL [28]. This discrepancy may be attributed to inappropriate antimicrobial use, the absence of guidelines for antibiotic selection, lack of laboratory diagnostic tests, and patient nonadherence to medication.

Urine samples constituted the largest clinical samples containing ESBL-producing Enterobacteriaceae in this study, as in the studies by Müller-Schulte E et al. and Kumudunie WGM et al. [29, 30]. According to our study and other studies, the prevalence of this enzyme in different hospital wards, especially in the intensive care unit, may indicate the irrational use and administration of beta-lactam antibiotics, especially broad-spectrum types [9]. In addition, its relatively high prevalence in the emergency department may be due to the experimental administration of antibiotics, especially cephalosporins, regardless of the antibiotic resistance pattern in bacteria. This variation in prevalence may be due to differences in the types of samples studied and the rate of antibiotic use in different areas. Seni et al. showed that most isolates from surgical wards produce ESBL [31]. Long-term hospital stays; use of static catheters, improper treatment, endotracheal/nasogastric tubes, and severe illness are possible causes of their dissemination.

### Study limitations

Geographical, temporal, sampling, and laboratory method limitations were among the limiting factors of this study.

## Conclusion

This study highlights the increasing prevalence of broad-spectrum β-lactamase-producing Enterobacteriaceae. Implementing antibiotic stewardship (including restricted beta-lactam/cephalosporin use and rotational programs) and developing screening methods for early ESBL detection are crucial to address this problem. Risk factor-based predictions can guide appropriate empirical therapy.

## Authorship contribution statement

NM, AH, and AK: conceptualization. MB: data analysis. AK: Data curation. NM, AH, and AK: visualization and writing. AK: Administration. NM, AH, and AK: manuscript revision. All authors contributed to the article and approved the submitted version.

## Ethical Consideration

The research obtained approval from the Ethics Committee of Lorestan University of Medical Sciences (code: IR.LUMS.REC.1398.172).

## Declaration of Competing Interest

The authors have no conflicts of interest related to this article.

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## Data Availability

All data were available for sharing upon reasonable request. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declaration of Generative AI

The authors declare that they have not used any generative artificial intelligence for the writing of this manuscript, nor for the creation of tables, or their corresponding captions.

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