Brief Report

Trends in Pharmaceutical Sciences and Technologies 2026: 12(1):1-8

Prevalence of Extended-spectrum ß-lactamase-producing *Escherichia coli* (ESBL *E. coli*) Among ICU Personnel's Stool Specimens in Teaching Hospitals in Tehran

Azar Bagheri-Alamooti[1];Ph.D, Mahsa Ghasemi[2];Ph.D, Soheila Abolghasemi[2];Ph.D, Mohammad Darvishi[3];Ph.D, Rahim Mohammadi[1];Ph.D, Amir Homayooni[4];Ph.D, Alireza Ashouri[2];Ph.D, Laila Ejaz[5];Ph.D, Moein Masjedi[1,6*];Ph.D

[1]Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Infectious Diseases, Faculty of Medicine, Islamic Azad University, Tehran, Iran
[3]Infectious Diseases and Tropical Medicine Research Center (IDTMC), School of Aerospace and Subaquatic Medicine, AJA University of Medical Sciences, Tehran, Iran
[4]Department of Pharmacy, School of Applied Sciences, University of Huddersfield, HD1 3DH, UK
[5]College of Pharmacy, RAK Medical & Health Sciences University, Ras Al-Khaimah, United Arab Emirates
[6]Department of Research and Development, DarooSazan Sorena Exir Pharmaceutical Company, Shiraz, Iran

Abstract

Infections among intensive care unit (ICU) staff can lead to significant challenges, making their prevention crucial for improving patient outcomes and reducing healthcare costs. This study aimed to assess the presence of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*) in fecal samples from personnel working in the ICU of Besat Hospital and hospitals affiliated with the Islamic Azad University of Tehran. This descriptive study involved 64 ICU staff members from Besat Hospital and affiliated institutions, selected based on specific inclusion and exclusion criteria. The fecal samples were cultured to test for ESBL *E. coli* across four different stages. The average age of the participants was 37.08 years, with 64.1% were female. Among the ICU staff, 70.3% were nurses. About two-third (70.3%) of participants had ten or more years of work experience. The results showed that 37.5% tested positive for *E. coli*, with 20.3% specifically identified as ESBL *E. coli*. Our findings indicated that fecal samples of about one in five ICU personnel was positive for ESBL *E. coli*. Additionally, the risk of infection appears to be higher among older individuals, those with longer work experience, and nurses compared to other staff.

Keywords: Nosocomial infection; Broad-spectrum beta-lactams; Intensive Care Unit (ICU); ESBL; beta-lactamase.

Please cite this article as: Ghasemi M, Abolghasemi S, Darvishi M, Mohammadi R, Homayooni A, Ashouri A, *et al*. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL *E. coli*) among ICU personnel's stool specimens in teaching hospitals in Tehran. Trends in Pharmaceutical Sciences and Technologies. 2026;12(1):1-8. doi: 10.30476/tips.2025.105123.1275

1. Introduction

Beta-lactam antibiotics include a range of medications such as penicillins, cephalosporins, carbapenems, and monobactams. Some bacteria have developed resistance to these antibiotics by producing an enzyme called beta-lactamase, which can break the amide bond in the beta-lactam ring (1). Recent bacterial mutations have produced diverse β-lactamases, notably ESBLs in *E. coli*, which hydrolyze penicillins, first-/third-/fourth-generation cephalosporins, and aztreonam, rendering β-lactam therapies ineffective (2-4). The first

Corresponding Author: Moein Masjedi, Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
E-mail: Masjedi_m@sums.ac.ir

Received: 21/10/2025; Accepted: 01/03/2026

confirmed case of ESBL-producing *E. coli* was reported in Germany in 1983. Today, these strains are linked to a range of hospital-acquired infections, including urinary tract infections, pneumonia, and catheter-associated infections (4, 5). A cross-sectional study by Loyola *et al*. found that approximately 50% of samples tested positive for ESBL *E. coli* (6). Additionally, a cohort study indicated that 18.8% of rectal swab samples were positive for this resistant strain (7). Shahcheraghi *et al*. reported 60.0% (72/120) fecal carriage of ESBL-producing *E. coli* in Iran, predominantly blaCTX-M-15 (90.2%, 65/72), followed by blaTEM (50.0%, 36/72) and blaSHV (5.5%, 4/72). MLST revealed high diversity with ST769 and ST472 predominant, necessitating enhanced infection control and screening. (8, 9).

ESBL-producing *E. coli* challenges public health systems by complicating treatments, elevating costs through prolonged hospitalizations and costly alternatives, and enabling outbreaks that heighten morbidity, mortality, and resistance spread. Assessing ICU personnel prevalence informs epidemiology, infection control, and stewardship (9, 10). ESBL-producing bacteria resist β-lactam antibiotics, with rapid plasmid-mediated ESBL gene transfer among Enterobacteriaceae complicating treatment. Gene accumulation within strains yields broader, potent β-lactam resistance (11).

The rising prevalence of ESBL-producing *E. coli* poses a major public health threat, especially in ICUs where vulnerable patients face heightened risks from comorbidities and invasive procedures. This global issue, including in Iran, results from antibiotic overuse, over-prescription, and poor infection control (9, 10).

Investigating ESBL-producing *E. coli* prevalence among Tehran's ICU healthcare workers is crucial, as they serve as reservoirs and vectors for hospital transmission. Stool surveillance reveals cross-contamination risks to patients/staff, amid Iran's high multidrug-resistant organism burden, demanding urgent surveillance and interventions (12, 13). This study assesses ESBL-producing *E. coli* prevalence in stool from Tehran's central hospital ICU personnel to quantify carriage, inform infection control strategies, and enhance patient safety via surveillance, antibiotic education, and hygiene protocols. (12, 13).

2. Materials and Methods

2.1. Study Type and Duration

This cross-sectional study took place at Besat Hospital and Tehran Islamic Azad University (IAU)-affiliated hospitals, focusing on screening for ESBL-producing *E. coli* in stool samples from ICU staff.

2.2. Sample Size Calculation

To determine the appropriate sample size, the following equation was used (Eq. 1), resulting in the random selection of at least 64 staff members to participate in the study.

$$n^2 = \frac{p(1-p)^* z_2^1 - a/2}{d^2} \qquad \text{(Eq. 1)}$$

Where n represents the required sample size, (1-p) represents the proportion of the population that does not have the characteristic. Z_(1-α⁄2) represents Z-score corresponding to the desired confidence level and d^2 represents the square of the margin of error.

2.3. Inclusion/Exclusion Criteria

Being a part-time ICU staff and/or experiencing gastrointestinal symptoms such as abdominal pain, nausea, vomiting, diarrhea, dark stools (melena), heartburn, gas, inflammatory bowel diseases (like Crohn's disease and ulcerative colitis), irritable bowel syndrome, constipation, or gastroesophageal reflux disease (GERD) in the previous six months were considered as exclusion criteria. No specific criteria regarding age, sex, type of ICU, and duration of word experience were considered to include participants.

2.4. Data collection

2.4.1. Stool Sampling and Examination

Detecting *E. coli* and ESBL-producing

E. coli through stool examinations involves several steps and methodologies aimed at identifying the presence of these bacteria and their resistance to antibiotics.

In this study, 5-gram stool samples were collected from included ICU staff in the morning.

2.5. Gram Staining and Culture for Isolation

Stool samples were initially inoculated onto MacConkey agar and blood agar plates to isolate Gram-negative lactose-fermenting bacilli. Following incubation at 37 °C for 18-24 hours, isolated colonies were subjected to standard Gram staining, which confirmed the presence of Gram-negative, rod-shaped bacteria. Colonies exhibiting morphology consistent with *Escherichia coli* (pink, lactose-fermenting colonies on MacConkey agar with a distinctive metallic sheen on EMB agar) were selected for further biochemical testing. Primary identification was confirmed using a panel of standard biochemical tests, including positive reactions for indole, methyl red, and β-glucuronidase (using MUG assay), and negative reactions for urease, citrate utilization, and hydrogen sulfide production (14).

2.6. Screening and Phenotypic Confirmation of ESBL Production

Presumptive *E. coli* isolates were screened for ESBL production by culturing them on MacConkey agar supplemented with cefotaxime (2 µg/mL) or via a disk diffusion assay using cefpodoxime (10 µg) according to CLSI guidelines. Isolates showing reduced susceptibility were further tested for phenotypic confirmation using the combination disk diffusion method. This involved testing the isolate against cefotaxime (30 µg) and ceftazidime (30 µg) disks alone and in combination with clavulanic acid (cefotaxime/clavulanic acid and ceftazidime/clavulanic acid). An increase in zone diameter of ≥5 mm for the antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was confirmed as an ESBL producer (14).

2.7. Ethical Considerations

In this research, ICU staff were not pressured to take part in the study. To adhere with ethical standards, we explained the study's purpose and methods to them beforehand, and they were only included if they chose to participate. We also assured the staff that any information collected would remain confidential and that there would be no costs associated with their participation.

2.8. Statistical Analyses

All the data collected during this study were analyzed using SPSS software (version 26). For quantitative variables, we calculated the mean and standard deviation (SD). For qualitative variables, we looked at both absolute and relative frequencies. To evaluate the study's hypotheses, we used the chi-square test, considering a p-value of 0.05 as the threshold for statistical significance.

3. Results and Discussion

Table 1. Demographic data of ICU staff included in the study.

		Frequency (%)	Age average(mean±SD)
Sex	Female	41 (64.1%)	36.68±5.52
	Male	23 (35.9%)	37.78±4.77
Job role	Nurse	70.3%	
	Paramedic (practical nurse)	29.7%	
work experience	<10years	19 (29.7%)	
	≥10years	45 (70.3%)	

Table 2. Prevalence of *E. coli* and ESBL *E. coli* in ICU staff.

Species tested	Test result	Prevalence	Frequency (%)
E. coli	Positive	24	37.5
	Negative	40	62.5
ESBL-producing *E. coli*	Positive	13	20.3
	Negative	51	79.7

The demographic data of included ICU staff is represented in Table 1. More than half (64.1%) of the cohort were female. Most of the studied ICU staff (70.3%) were nurses. About two-third (70.3%) of the participants had ten or more years of experience.

As shown in Table 2, laboratory tests indicated that *E. coli* was present in 24 out of 64 ICU staff. The prevalence of ESBL *E. coli* was found to be 20.3% (n=13). Notably, more than half of the staff (54.2%) who tested positive for *E. coli* also had positive results for ESBL *E. coli* ($p<0.001$). Over the past few decades, there has been a global increase in bacterial species capable of producing beta-lactamases like ESBL and AmpC, which are significant contributors to resistance against beta-lactam antibiotics such as penicillin and cephalosporin, ultimately diminishing the effectiveness of antibacterial treatments. Trick *et al.* found that 15% of ICU employees in the United States had ESBL *E. coli* from rectal, nasal, gastrotomy-tube site, wound and axillary samples (15). In another study by Trubiano *et al.*, the prevalence among ICU staff was 19.15%, which aligns closely with our findings (16). Given that the number of positive ESBL-producing *E. coli* cases in our study is relatively higher than in previous research, it suggests that additional educational interventions are needed in healthcare settings to help reduce these cases. The considerable variation in reported ESBL prevalence rates between studies is not indicative of error but rather a consequence of divergent methodological and contextual factors. Key reasons for this heterogeneity include differences in the microbiological methods used for ESBL screening and phenotypic confirmation, which can vary in sensitivity and specificity, as well as the demographic and clinical characteristics of the sampled population (e.g., ICU patients versus community volunteers), which directly influence colonization pressure. Furthermore, the local epidemiology, including the background rate of antibiotic consumption that drives resistance selection and the presence of highly transmissible clones, coupled with the effectiveness of existing infection control measures and general sanitation levels, creates vastly different environments for ESBL transmission and detection, making direct comparisons between studies challenging (17).

The analysis of the possible relationship between age and the prevalence rates of *E. coli* and ESBL *E. coli*, as shown in Figure 1, revealed a statistically significant association between age and the prevalence of ESBL *E. coli* among ICU staff ($p=0.028$). However, this correlation was not significant for the overall prevalence of *E. coli* ($p=0.331$). Similar to our findings, a study by Abdulrahman *et al.* in Egypt found that older ICU staff with more work experience had a higher number of ESBL-producing *E. coli*-positive cases (8). Additionally, an investigation by Haller *et al.* in Germany indicated that ICU staff with more work experience are at a higher risk of being infected by resistant bacteria like ESBL-producing *Klebsiella pneumoniae*, which aligns with our results as well (18).

Our study found that having more than 10 years of work experience is significantly associated with the prevalence of ESBL-producing *E. coli* ($p=0.047$), however there was no significant link with the overall prevalence of *E. coli* ($p=0.587$). Additionally, our analysis showed no significant relationship between

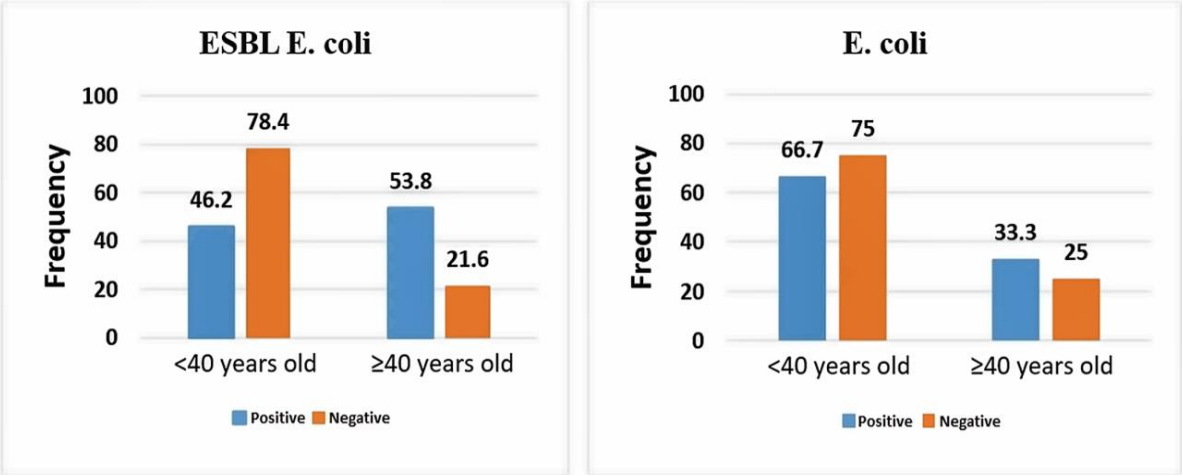

Figure 1. Bar chart of the effect of ICU staff age on the prevalence of *E. coli* and ESBL *E. coli*.

the prevalence rates of *E. coli* or ESBL-producing *E. coli* and the sex of ICU staff.

Interestingly, a parallel survey (unpublished data) conducted during our study examined patients admitted to the ICU in March and April 2021 for positive cases of *E. coli* and ESBL in sputum, blood, and urine. Despite finding that 37.5% of staff tested positive for *E. coli* and 20.3% for ESBL *E. coli*, none of the patients were positive for either strain. This suggests that short-term exposure may not pose a significant transmission risk, but it could be concerning for patients who are hospitalized for extended periods. Regarding job role the present study indicated that nurses had a higher prevalence of ESBL *E. coli* compared to paramedic staff ($p=0.047$).

4. Conclusion

Antibacterial resistance is a significant public health issue and a major concern in hospital settings, and it should not be overlooked. With the rise in antibiotic resistance, it's crucial for hospitals to have a clear understanding of the organisms causing nosocomial infections, along with their sensitivity and resistance patterns. This knowledge is not only beneficial for the local hospital but also serves as an important regional resource.

Given that antimicrobial resistance in ESBL *E. coli* is linked to higher rates of morbidity and mortality in ICUs, monitoring these trends should be a priority in healthcare settings. Since the causes, locations, and rates of nosocomial infections can vary from one center/ward to another, it's essential for hospitals to identify their specific risk factors. Regular surveillance of infectious agents and the development of strategies for preventing and treating these infections are crucial for enhancing the quality of care.

In summary, conducting periodic screenings for microbial species and updating sensitivity patterns to antimicrobial agents are vital steps in preventing potential nosocomial infections and reducing patient morbidity and mortality in the ICU.

Authors contributions

M.D. and S.A.: Conceptualization and the study supervision; M.Gh.: Data gathering, coordination and statistical analyses; M.M.: Conceptualization, statistical analyses, writing the manuscript draft, final proofreading;, A.A., R.M., L.E. and A.H.: Revision.

Funding

None of the authors of this study have received any fund from any organization.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Jalde SS, Choi HK. Recent advances in the development of β-lactamase inhibitors. *J Microbiol.* 2020 Aug;58(8):633-647. doi: 10.1007/s12275-020-0285-z. Epub 2020 Jul 27. PMID: 32720096.
2. Jeong SH, Song W, Park MJ, Kim JS, Kim HS, Bae IK, *et al*. Boronic acid disk tests for identification of extended-spectrum beta-lactamase production in clinical isolates of Enterobacteriaceae producing chromosomal AmpC beta-lactamases. *Int J Antimicrob Agents.* 2008 May;31(5):467-71. doi: 10.1016/j.ijantimicag.2007.12.014. Epub 2008 Mar 11. PMID: 18337065.
3. Chaudhary U, Aggarwal R. Extended spectrum -lactamases (ESBL) - an emerging threat to clinical therapeutics. *Indian J Med Microbiol.* 2004 Apr-Jun;22(2):75-80. PMID: 17642700.
4. Hadziyannis E, Tuohy M, Thomas L, Procop GW, Washington JA, Hall GS. Screening and confirmatory testing for extended spectrum β-lactamases (ESBL) in Escherichia coli, Klebsiella pneumoniae, and Klebsiella oxytoca clinical isolates. Diagn Microbiol Infect Dis 2000;36(2):113-7.
5. Akbari M, Amir Mozaffari N, Peeri Dogaheh H. Prevalence of extended-spectrum beta lactamase in Entrobacteriaceae isolated from urinary tract infections in Ardabil, Iran. *J Ardabil Uni Med Sci.* 2014;14(3):285-91.
6. Loyola S, Gutierrez LR, Horna G, Petersen K, Agapito J, Osada J, *et al.* Extended-spectrum β-lactamase-producing Enterobacteriaceae in cell phones of health care workers from Peruvian pediatric and neonatal intensive care units. *Am J Infect Control.* 2016 Aug 1;44(8):910-6. doi: 10.1016/j.ajic.2016.02.020. Epub 2016 Apr 7. PMID: 27067520; PMCID: PMC5253370.
7. Abdel Rahman AT, Hafez SF, Abdelhakam SM, Ali-Eldin ZA, Esmat IM, Elsayed MS, *et al.* Antimicrobial resistant bacteria among health care workers in intensive care units at Ain Shams University Hospitals. *J Egypt Soc Parasitol.* 2010 Apr;40(1):71-83. PMID: 20503587.
8. Aghamohammad S, Badmasti F, Shirazi AS, Dabiri H, Solgi H, Sabeti S, *et al*. Considerable rate of putative virulent phylo-groups in fecal carriage of extended-spectrum β-lactamase producing Escherichia coli. *Infect Genet Evol.* 2019 Sep;73:184-9. doi: 10.1016/j.meegid.2019.04.035. Epub 2019 May 2. PMID: 31054921.
9. Aghamohammad S, Nikbin VS, Badmasti F, Shahcheraghi F. High heterogeneity of fecal carriage extended-spectrum beta-lactamase-producing E. coli isolated from iranian community and clinical settings. *BMC Infect Dis*. 2022 Mar 31;22(1):318. doi: 10.1186/s12879-022-07304-7. PMID: 35361158; PMCID: PMC8973555.
10. Singh N, Pattnaik D, Neogi DK, Jena J, Mallick B. Prevalence of ESBL in Escherichia coli Isolates Among ICU Patients in a Tertiary Care Hospital. *J Clin Diagn Res.* 2016 Sep;10(9):DC19-DC22. doi: 10.7860/JCDR/2016/21260.8544. Epub 2016 Sep 1. PMID: 27790433; PMCID: PMC5071933.
11. Stürenburg E, Mack D. Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. *J Infect.* 2003 Nov;47(4):273-95. doi: 10.1016/s0163-4453(03)00096-3. PMID: 14556752.
12. Barzegar S, Arzanlou M, Teimourpour A, Esmaelizad M, Yousefipour M, MohammadShahi J, et al. Prevalence of the integrons and ESBL genes in multidrug-resistant strains of Escherichia coli isolated from urinary tract infections, Ardabil, Iran. *Iran J Med Microbiol.* 2022;16(1):56-65.
13. Yousefipour M, Rasoulinejad M, Hadadi A, Esmailpour N, Abdollahi A, Jafari S, *et al*. Bacteria Producing Extended Spectrum β-lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants. *Iran J Pathol.* 2019 Winter;14(1):61-67. doi: 10.30699/IJP.14.1.61. Epub 2018 Dec 27. PMID: 31531102; PMCID: PMC6708561.
14. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes

to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. *J Clin Microbiol*. 2021 Nov 18;59(12):e0021321. doi: 10.1128/JCM.00213-21. Epub 2021 Sep 22. PMID: 34550809; PMCID: PMC8601225.

15. Trick WE, Weinstein RA, DeMarais PL, Kuehnert MJ, Tomaska W, Nathan C, *et al*. Colonization of skilled-care facility residents with antimicrobial-resistant pathogens. *J Am Geriatr Soc.* 2001 Mar;49(3):270-6. doi: 10.1046/j.1532-5415.2001.4930270.x. PMID: 11300237.
16. Dasgupta S, Das S, Chawan NS, Hazra A. Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian J Crit Care Med.* 2015 Jan;19(1):14-20. doi: 10.4103/0972-5229.148633. PMID: 25624645; PMCID: PMC4296405.
17. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase-Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. *Clin Infect Dis.* 2016 Aug 1;63(3):310-8. doi: 10.1093/cid/ciw283. Epub 2016 May 3. Erratum in: Clin Infect Dis. 2016 Sep 15;63(6):851. doi: 10.1093/cid/ciw497. PMID: 27143671.
18. Haller S, Eller C, Hermes J, Kaase M, Steglich M, Radonić A, *et al.* What caused the outbreak of ESBL-producing Klebsiella pneumoniae in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing. *BMJ Open*. 2015 May 11;5(5):e007397. doi: 10.1136/bmjopen-2014-007397. PMID: 25967999; PMCID: PMC4431171.