



Clinical Significance of Extended-Spectrum β -Lactamase (ESBL) and AmpC β -Lactamase (AmpC) Coproduction among Uropathogenic *Escherichia coli* from Diabetic and Nondiabetic Patients: Implications for Antimicrobial Resistance and Treatment Outcomes

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Abstract

Background Urinary tract infections (UTIs) caused by *Escherichia coli* remain a major global health burden, particularly among diabetic patients who are prone to recurrent and complicated infections.

Aim This article determines the prevalence, antimicrobial resistance patterns, and clinical predictors of extended-spectrum β -lactamase (ESBL) and AmpC β -lactamase coproduction among uropathogenic *E. coli* isolates.

Methods Cross-sectional study was conducted on 300 adult patients with culture-confirmed *E. coli* UTIs at Misurata Medical Center, including 150 people living with diabetes (PLWD) and 150 nondiabetic participants. Midstream urine specimens were collected and cultured on standard media. Antimicrobial susceptibility testing followed the Clinical and Laboratory Standards Institute 2024 guidelines, while ESBL and AmpC β -lactamases were phenotypically detected using the double-disk synergy and inhibitor-based disk tests. Data were analyzed using SPSS v27 and R v4.3.2. Categorical and continuous variables were compared using chi-square/Fisher's exact and Mann-Whitney *U* tests, respectively. Predictors of coproduction and its impact on treatment failure were identified via multivariate logistic regression; a *p*-value of < 0.05 was considered statistically significant.

Results The total prevalence of ESBL, AmpC, and ESBL + AmpC coproduction was 58.3, 14.3, and 14.0%, respectively. While the prevalence of ESBL-only and AmpC-only phenotypes did not differ significantly between PLWD and nondiabetic patients, coproduction was significantly higher in PLWD (22%) compared with nondiabetic patients (6%; *p* < 0.001) and was associated with extensive multidrug resistance (93%). Diabetes mellitus (adjusted odds ratio [OR] = 3.64; *p* = 0.001) and prior antibiotic

Keywords

- ▶ *Escherichia coli*
- ▶ extended-spectrum β -lactamase (ESBL)
- ▶ ampC β -lactamase
- ▶ antimicrobial resistance
- ▶ diabetes mellitus
- ▶ urinary tract infection
- ▶ Libya

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exposure (adjusted OR=2.18; $p=0.041$) were independent predictors of coproduction.

Conclusion ESBL + AmpC coproduction in uropathogenic *E. coli* represents an emerging therapeutic and epidemiological concern, particularly among PLWD. Routine AmpC screening and rational antibiotic policies are urgently required to mitigate treatment failure and limit resistance.

ملخص المقال باللغة العربية

الأهمية السريرية للإنتاج المشترك لإنزيم بيتا لكتاماز واسع الطيف (ESBL) وإنزيم بيتا لكتاماز (AmpC) بواسطة بكتيريا الإشريكية القولونية المُفْرِضة للمسالك البولية لدى مرضى السكري وغير السكري؛ تداعياته على مقاومة مضادات الميكروبات ونتائج العلاج

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الخلفية: لا تزال التهابات المسالك البولية التي تسببها الإشريكية القولونية تُشكل عبئاً صحياً عالمياً كبيراً، لا سيما لدى مرضى السكري المعرضين لالتهابات متكررة ومعقدة.

الهدف: تحديد معدل انتشار وأنماط مقاومة مضادات الميكروبات، والتنبؤات السريرية للإنتاج المشترك لإنزيم بيتا لكتاماز واسع الطيف (ESBL) وإنزيم بيتا لكتاماز AmpC لدى عزلات الإشريكية القولونية المُفْرِضة للمسالك البولية.

الطريقة: أُجريت دراسة مقطعية على 300 مريض بالغ مصاب بالتهابات المسالك البولية الإشريكية القولونية، مؤكدة بالزرع، في مركز مصراة الطبي (150 مريضاً مصاباً بداء السكري و150 مريضاً غير مصاب به). تم جمع عينات البول من منتصف مجرى البول وزراعتها على وسائط قياسية. اتبع اختبار حساسية مضادات الميكروبات معيار CLSI 2024، بينما تم الكشف عن النمط الظاهري لإنزيم بيتا لكتاماز واسع الطيف (ESBL) وإنزيم بيتا لكتاماز AmpC باستخدام اختبائي التأخر ثنائي القرص والقرص القائم على المشيط. تم تحليل البيانات باستخدام برنامج SPSS. قورنت المتغيرات الفئوية والمستمرة باستخدام اختبار مربع كاي/فيشر الدقيق ومان-ويتني يو، على التوالي. تم تحديد عوامل التنبؤ بالإنتاج المشترك وتأثيره على فشل المعالجة من خلال الانحدار اللوجستي متعدد المتغيرات؛ واعتُبرت قيمة الاحتمالية > 0.05 ذات دلالة إحصائية.

النتائج: أظهرت النتائج أن 58.3% من العزلات البكتيرية كانت منتجة لإنزيم ESBL، و14.3% منتجة لإنزيم AmpC، بينما أنتجت 14% منهما كلا الإنزيمين معاً. وعلى الرغم من عدم وجود فرقي كبير في انتشار كل إنزيم على حدة بين مرضى السكري وغير المصابين به، لوحظ وجود فرقي جوهري في نمط الإنتاج المشترك، حيث بلغت نسبته بين مرضى السكري (22%) ما يقارب أربعة أضعاف نسبته بين غير المصابين (6%)، وهو فرقي ذو دلالة إحصائية عالية ($p < 0.001$). وارتبط هذا الإنتاج المشترك - كذلك بدلالة إحصائية عالية ($p < 0.001$) - بمقاومة واسعة النطاق للمضادات الحيوية المتعددة (93%). وأخيراً، حدد التحليل الإحصائي كلاً من مرض السكري والتعرض السابق للمضادات الحيوية بوصفهما عاملين مستقلين يتنبآن بحدوث هذا الإنتاج المشترك.

الاستنتاجات: يُعكّل الانتشار المشترك لإنزيم ESBL وإنزيم AmpC في بكتيريا الإشريكية القولونية المسببة لعدوى المسالك البولية مصدر قلق علاجي ووبائي بارز، لا سيما بين مرضى السكري. وتؤكد هذه النتائج على ضرورة اعتماد فحص روتيني للإنتاج المشترك لهذه الإنزيمات، إلى جانب تطبيق سياسات رشيدة لاستخدام المضادات الحيوية، بهدف التخفيف من حالات فشل العلاج والحد من تفاقم مشكلة مقاومة مضادات الميكروبات.

الكلمات المفتاحية: الإشريكية القولونية؛ بيتا لكتاماز واسع الطيف؛ إنزيم بيتا لكتاماز/AmpC؛ مقاومة مضادات الميكروبات؛ داء السكري؛ عدوى المسالك البولية؛ ليبيا.

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, accounting for significant morbidity, antibiotic consumption, and health care costs. *Escherichia coli* is the leading etiological agent of community-acquired UTIs (CA-UTIs).¹ Over the past two decades, the rapid evolution of β -lactamase-mediated resistance has severely undermined the clinical utility of β -lactam antibiotics, particularly the third-generation cephalosporins that form the backbone of empirical therapy.²

Extended-spectrum β -lactamases (ESBLs) and AmpC β -lactamases represent two major enzyme families responsible for hydrolyzing β -lactam antibiotics. While ESBLs confer resistance to oxyimino-cephalosporins and monobactams but remain inhibited by clavulanate, AmpC enzymes hydrolyze cephamycins and are poorly inhibited by β -lactamase inhibitors.³ The coproduction of ESBL and AmpC enzymes within the same *E. coli* isolate creates a particularly worrisome phenotype that can evade both inhibitor combinations and extended-spectrum cephalosporins, resulting in multidrug-resistant (MDR) profiles and frequent therapeutic failure.^{4,5}

Although ESBL production has been extensively reported worldwide, the simultaneous presence of ESBL and AmpC

β -lactamases has received far less attention, especially in developing regions. Recent surveillance data indicate that coproducers may represent up to 10 to 15% of *E. coli* isolates in some hospital settings and are associated with increased mortality, prolonged hospital stay, and higher rates of carbapenem consumption.^{3,6} Failure to detect AmpC activity in ESBL-positive isolates can therefore lead to inappropriate therapy and foster silent dissemination of MDR strains.⁷

This study specifically targeted CA-UTIs among adults presenting with acute urinary symptoms, excluding recent hospitalizations or indwelling devices to ensure a well-defined outpatient-based population.

Diabetes mellitus (DM) further complicates this scenario. People living with diabetes (PLWD) are predisposed to recurrent and complicated UTIs due to glycosuria, impaired neutrophil function, and reduced uroepithelial defense mechanisms.⁸ Evidence suggests that *E. coli* isolates from PLWD exhibit higher resistance rates, a greater likelihood of ESBL production, and less favorable treatment outcomes compared with people without diabetes counterparts.⁹ However, the interaction between host metabolic status (diabetes) and bacterial resistance mechanisms, specifically ESBL + AmpC coproduction, remains insufficiently explored, particularly across North African and Middle Eastern health care settings.

In light of these gaps, this study aimed to (1) determine the prevalence of ESBL + AmpC coproduction among uropathogenic *E. coli* isolates, (2) compare clinical and microbiological characteristics between PLWD and people without diabetes, (3) identify independent clinical predictors of coproduction, and (iv) assess its impact on therapeutic failure and hospital stay. By integrating clinical and microbiological parameters, this work aims to provide region-specific evidence supporting optimized empiric antibiotic policies and improved management of high-risk UTI patients.

Methods

Study Design and Setting

This analytical cross-sectional study was conducted over an 8-month period (March–October 2023). This was a single-center study conducted at Misurata Medical Center, a tertiary care referral hospital in Misurata, Libya, in collaboration with the Central Microbiology Laboratory. The study population included adult patients clinically diagnosed with UTI who yielded pure growth of *E. coli* on culture. Both inpatient and outpatient cases were enrolled; however, only CA-UTIs were included, and inpatients were eligible solely if they presented with CA-UTI criteria.

Sampling Method

A consecutive convenience sampling strategy was used, whereby all eligible patients presenting during the study period were enrolled to minimize selection bias.

Ethical Approval and Informed Consent

Ethical approval was obtained from the Ethics Committee of the Faculty of Pharmacy, Misurata University (Ref. 49/2023), which serves as the institutional review authority for student-led clinical research. Misurata Medical Center did not require a separate institutional review board submission at the time of data collection. All data were fully anonymized, and no patient identifiers were recorded.

Verbal informed consent was used in accordance with institutional regulations for minimal-risk observational research. Participants were informed about study purpose and confidentiality measures. The use of verbal consent is acknowledged as a limitation.

Inclusion and Exclusion Criteria

All included participants presented with clinical symptoms consistent with acute UTI (dysuria, urgency, frequency, suprapubic discomfort, or flank pain) and demonstrating significant bacteriuria ($\geq 10^5$ CFU/mL) due to *E. coli* in urine culture. Both PLWD and people without diabetes were eligible. DM was confirmed by medical records, ongoing antidiabetic therapy, or glycated hemoglobin (HbA1c $\geq 6.5\%$).

Inpatients were included only when their infection met criteria for community acquisition (no hospitalization in the preceding 14 days). Patients admitted for management of UTI and meeting CA-UTI criteria were therefore eligible.

Patients were excluded if they met any of the following criteria:

- (1) Polymicrobial growth in urine culture.
- (2) Recent hospitalization within the preceding 14 days.
- (3) Indwelling catheterization or recent urinary instrumentation.
- (4) Incomplete clinical or microbiological data.
- (5) Prior inclusion (duplicate isolates).
- (6) Initiation of antibiotic therapy more than 48 hours before specimen collection.

These criteria ensured the inclusion of well-documented, monomicrobial *E. coli* infections and reduced confounding factors related to prior treatment or nosocomial exposure.

Sample Size and Patient Groups

A total of 300 nonduplicate urine isolates were analyzed, including 150 PLWD and 150 people without diabetes. This sample size was designed to achieve $\geq 80\%$ statistical power to detect a 15% difference in coproduction prevalence between groups at a 95% confidence level.

Specimen Collection and Culture

Midstream clean-catch urine specimens were collected in sterile containers under aseptic conditions and transported to the laboratory within 1 hour of collection. Samples were cultured on MacConkey agar and CLED (Cystine-Lactose-Electrolyte-Deficient) agar (Oxoid, United Kingdom) and incubated aerobically at 37°C for 24 hours.

Significant bacteriuria was defined as $\geq 10^5$ CFU/mL of a single organism. Identification was based on standard biochemical reactions and confirmed by API 20E (bioMérieux, France) or VITEK 2 automated systems when available (instrument availability during routine laboratory workflow); otherwise, biochemical testing was used (IMViC, triple sugar iron, citrate, urease).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) 2024 performance standards.¹⁰ The antibiotic panel included cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), ceftiofloxacin (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), gentamicin (10 µg), amikacin (30 µg), piperacillin–tazobactam (100/10 µg), ertapenem (10 µg), imipenem (10 µg), and meropenem (10 µg). All antibiotic discs and media were obtained from Oxoid, United Kingdom.

Only *E. coli* American Type Culture Collection (ATCC) 25922 was available locally during the study period; the absence of an additional resistant-quality control strain (e.g., ATCC 35218) is acknowledged as a methodological limitation.

Isolates resistant to three or more antibiotic classes were categorized as MDR, this definition aligns with the international MDR criteria described by Magiorakos et al.¹⁰

Detection of ESBL Production

Screening for ESBL activity was performed according to the CLSI 2024 guidelines using reduced susceptibility to cefotaxime (30 µg) and/or ceftazidime (30 µg). Confirmatory testing employed the phenotypic confirmatory method (double-disk synergy test, DDST), where disks of cefotaxime (30 µg) and ceftazidime (30 µg) were placed 20 mm (center-to-center) from their respective clavulanate-containing disks (cefotaxime–clavulanate 30/10 µg and ceftazidime–clavulanate 30/10 µg).

An increase of ≥ 5 mm in inhibition zone diameter for either cephalosporin–clavulanate combination, compared with the corresponding cephalosporin alone, was interpreted as ESBL production.¹¹

Cefotaxime and ceftazidime were selected for ESBL screening and confirmation because they are the primary oxyimino-cephalosporins recommended by the CLSI for detecting plasmid-mediated ESBL activity in Enterobacterales. Clavulanate-containing disks were included to differentiate ESBLs known to be inhibited by β -lactamase inhibitors from AmpC enzymes, which are not significantly inhibited.

Detection of AmpC β -Lactamase Production

Isolates showing resistance to cefoxitin (zone ≤ 18 mm) were further tested for AmpC production using the inhibitor-based AmpC disk test described by Coudron et al.²⁰ A sterile filter paper disk impregnated with phenylboronic acid (PBA, 400 µg) or cloxacillin (200 µg) was placed adjacent to a cefoxitin (30 µg) disk on Mueller–Hinton agar inoculated with the test organism.

An increase of ≥ 5 mm in the inhibition zone around the inhibitor-containing disk compared with cefoxitin alone indicated AmpC β -lactamase activity.

Cefoxitin resistance is a well-established phenotypic marker for screening AmpC-producing Enterobacterales, as AmpC enzymes hydrolyze cephamycins while ESBLs do not. The use of PBA or cloxacillin enhances detection by inhibiting AmpC enzymes, enabling differentiation from other cefoxitin-resistant mechanisms.

Isolates positive for both ESBL and AmpC phenotypic tests were classified as ESBL/AmpC coproducers.

Clinical Data Collection

Demographic and clinical data were obtained from medical records. Variables included age, sex, diabetic status, hospitalization status (inpatient/outpatient), recent antibiotic exposure (within 90 days), and clinical outcome.

Treatment success was defined as symptom resolution within 5 days without change of antibiotic regimen. Treatment failure was defined as persistence or worsening of symptoms requiring therapy modification or readmission. For hospitalized patients, length of stay (in days) was recorded.

Statistical Analysis

Data were analyzed using SPSS v27 (IBM, United States) and R v4.3.2. Continuous variables were expressed as medians

(interquartile range [IQR]) and compared using the Mann–Whitney *U* test. Categorical variables were summarized as frequencies and compared by chi-square or Fisher's exact test.

Binary logistic regression identified independent predictors of ESBL + AmpC coproduction (age, gender, diabetes, prior antibiotics, hospitalization). Odds ratios (ORs) with 95% confidence intervals (CIs) were reported.

A multivariate model was constructed to assess the effect of coproduction on treatment failure, controlling for confounders. Model discrimination was evaluated by the receiver operating characteristic (ROC) curve using the pROC package in R, with area under the curve (AUC) reported. A *p*-value of < 0.05 was considered statistically significant.

Results

As illustrated in ►Fig. 1, coproducing isolates were predominantly detected in PLWD, and their presence was associated with increased rates of treatment failure and prolonged hospitalization.

Demographic and Clinical Characteristics

►Table 1 presents a total of 300 nonduplicate *E. coli* isolates, including 150 from PLWD and 150 from people without diabetes. The mean age was 53.4 ± 14.2 years (range: 18–86 years), with a female predominance (67.3%).

Fever and dysuria were the most common clinical symptoms in both groups. Prior antibiotic exposure within 3 months was significantly higher among PLWD (44.7%) than people without diabetes (25.3%, $p = 0.002$), and the mean hospital stay was longer (6.4 ± 2.8 vs. 4.3 ± 1.9 days, $p < 0.001$).

Prevalence of β -Lactamase Phenotypes

The distribution of β -lactamase phenotypes revealed a significantly higher prevalence of ESBL and AmpC coproduction among isolates from PLWD (22.0%) compared with nondiabetic patients (6.0%; $p < 0.001$), while nonproducers were significantly more common in the nondiabetic group (42.0% vs. 12.6% in PLWD; $p < 0.001$). However, the proportions of isolates producing ESBL only (48.7% vs. 40.0%; $p = 0.14$) or AmpC only (16.7% vs. 12.0%; $p = 0.29$) were not significantly

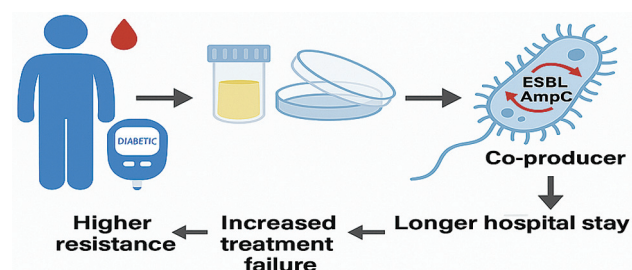


Fig. 1 Graphical summary illustrating coproduction of extended-spectrum β -lactamase (ESBL) and AmpC β -lactamases in *Escherichia coli* isolated from diabetic urinary tract infection patients, showing its association with increased resistance, treatment failure, and longer hospital stay.

Table 1 Demographic and clinical characteristics of patients with *E. coli* UTI

| Variable | PLWD (n = 150) | People without diabetes (n = 150) | p-Value |
|--|-----------------|-----------------------------------|---------|
| Mean age (\pm SD), y | 56.2 \pm 13.6 | 50.6 \pm 14.8 | 0.008 |
| Female sex, n (%) | 104 (69.3) | 98 (65.3) | 0.47 |
| Prior antibiotic use (< 3 months), n (%) | 67 (44.7) | 38 (25.3) | 0.002 |
| Inpatient cases, n (%) | 61 (40.7) | 43 (28.7) | 0.04 |
| Mean hospital stay (\pm SD), d | 6.4 \pm 2.8 | 4.3 \pm 1.9 | < 0.001 |
| Treatment failure, n (%) | 37 (24.7) | 18 (12.0) | 0.009 |

Abbreviations: PLWD, people living with diabetes; SD, standard deviation; UTI, urinary tract infection.

Table 2 Distribution of β -lactamase phenotypes among *E. coli* isolates

| β -lactamase phenotype | PLWD (n = 150) | People without diabetes (n = 150) | Total (n = 300) | p-Value |
|------------------------------|----------------|-----------------------------------|-----------------|---------|
| ESBL only | 73 (48.7%) | 60 (40.0%) | 133 (44.3%) | 0.14 |
| AmpC only | 25 (16.7%) | 18 (12.0%) | 43 (14.3%) | 0.29 |
| ESBL + AmpC (coproducers) | 33 (22.0%) | 9 (6.0%) | 42 (14.0%) | < 0.001 |
| Nonproducers | 19 (12.6%) | 63 (42.0%) | 82 (27.3%) | < 0.001 |

Abbreviations: ESBL, extended-spectrum β -lactamase; PLWD, people living with diabetes.

different between the diabetic and nondiabetic groups, respectively (**Table 2**).

Antimicrobial Resistance Profiles

Antimicrobial susceptibility patterns of the *E. coli* isolates are presented in **Table 3**. Overall resistance was highest to third-generation cephalosporins and fluoroquinolones, whereas carbapenems and nitrofurantoin remained largely active.

Coproducers exhibited the broadest resistance spectrum, with \geq 85% resistance to cefotaxime, ceftazidime, cefepime, and ciprofloxacin, and 93% qualifying as MDR ($p < 0.001$).

Predictors of ESBL + AmpC Coproduction

Univariate analysis identified DM, prior antibiotic exposure, and inpatient status as significant predictors ($p < 0.01$).

In multivariable logistic regression, only diabetes (OR = 3.64, 95% CI 1.74–7.61, $p = 0.001$) and prior antibiotic use (OR = 2.18, 95% CI 1.03–4.62, $p = 0.041$) remained independent predictors as shown in **Table 4**.

Impact on Treatment Outcomes

Coproducers were associated with a significantly higher treatment failure rate (35.7%) compared with

Table 3 Antimicrobial resistance rates (%) among *E. coli* isolates according to β -lactamase phenotype

| Antibiotic | ESBL only (n = 133) | AmpC only (n = 43) | ESBL + AmpC (n = 42) | Nonproducers (n = 82) | p-Value |
|-------------------------------|---------------------|--------------------|----------------------|-----------------------|------------------|
| Cefotaxime (CTX) | 92.5 | 79.1 | 97.6 | 11.0 | < 0.001 |
| Ceftazidime (CAZ) | 88.7 | 74.4 | 95.2 | 9.8 | < 0.001 |
| Cefepime (FEP) | 84.2 | 65.1 | 92.9 | 6.1 | < 0.001 |
| Cefoxitin (FOX) | 38.3 | 88.4 | 91.0 | 8.5 | < 0.001 |
| Piperacillin–tazobactam (TZP) | 53.4 | 62.8 | 81.0 | 12.2 | < 0.001 |
| Ciprofloxacin (CIP) | 77.4 | 69.8 | 88.1 | 18.3 | < 0.001 |
| Gentamicin (GEN) | 62.4 | 58.1 | 79.0 | 20.7 | < 0.001 |
| Amikacin (AMK) | 21.8 | 18.6 | 35.7 | 6.1 | 0.004 |
| Nitrofurantoin (NIT) | 12.0 | 16.3 | 9.5 | 5.0 | 0.18 nanoseconds |
| Ertapenem (ETP) | 5.3 | 7.0 | 9.5 | 2.4 | 0.29 nanoseconds |
| Imipenem (IMP) | 4.5 | 4.7 | 7.1 | 1.2 | 0.37 nanoseconds |
| Meropenem (MEM) | 3.8 | 4.7 | 7.1 | 0 | 0.31 nanoseconds |
| Multidrug resistance (MDR) | 76.7 | 68.4 | 92.8 | 19.5 | < 0.001 |

Abbreviation: ESBL, extended-spectrum β -lactamase.

Table 4 Predictors of ESBL + AmpC coproduction among *E. coli* isolates

| Variable | Unadjusted OR (95% CI) | <i>p</i> | Adjusted OR (95% CI) | <i>p</i> |
|----------------------|------------------------|----------|----------------------|----------|
| Age > 60 years | 1.34 (0.65–2.79) | 0.42 | 1.21 (0.52–2.80) | 0.64 |
| Female sex | 1.19 (0.58–2.43) | 0.63 | 1.12 (0.50–2.51) | 0.77 |
| Diabetes mellitus | 4.45 (2.12–9.33) | < 0.001 | 3.64 (1.74–7.61) | 0.001 |
| Prior antibiotic use | 2.72 (1.39–5.34) | 0.003 | 2.18 (1.03–4.62) | 0.041 |
| Inpatient status | 1.98 (1.01–3.87) | 0.046 | 1.53 (0.73–3.21) | 0.26 |

Abbreviations: CI, confidence interval; ESBL, extended-spectrum β -lactamase; OR, odds ratio.

noncoproducers (14.6%, $p=0.002$). Median hospital stay was 6 days (IQR=4–8) among patients infected with coproducer isolates versus 4 days (IQR=3–6) in those with noncoproducers ($p=0.004$, Mann–Whitney *U*). This difference is illustrated in ►Fig. 2, which demonstrates a visibly longer hospitalization period in the coproducer group.

The logistic regression model was designed to predict treatment failure, integrating microbiological and clinical variables (adjusted OR = 2.81, 95% CI 1.21–6.52, $p=0.016$). The overall performance of the predictive model was robust, with an AUC = 0.82 (95% CI 0.75–0.88), confirming good discriminatory ability (►Fig. 3).

Discussion

This study provides new insights into the coexistence of ESBLs and AmpC β -lactamases among uropathogenic *E. coli* in PLWD and people without diabetes. The findings highlight both the clinical relevance and the public health implications of β -lactamase coproduction in a resource-limited setting such as Libya.

Demographic and Clinical Characteristics

The present study demonstrates that DM remains a major determinant of complicated UTIs caused by *E. coli*. The longer hospital stays and higher treatment failure rates among PLWD reflect a complex interplay between host metabolic dysregulation and bacterial virulence. Persistent hypergly-

cemia impairs neutrophil chemotaxis, oxidative burst, and phagocytic efficiency, while glucosuria provides a nutrient-rich environment that promotes bacterial adhesion and biofilm formation.¹² These pathophysiological mechanisms explain the clinical observation that PLWD often experience delayed recovery and frequent relapse.

Comparable evidence has been reported globally. In Ethiopia, researchers documented delayed clinical resolution and increased MDR among diabetic UTI patients,⁹ while similar findings were observed in Tunisian hospitals.¹³ A meta-analysis of 35 studies estimated that DM nearly doubles the odds of infection with resistant organisms.¹⁴ Such findings underscore the need for early, targeted antimicrobial therapy in PLWD, particularly in North Africa, where metabolic disorders and unregulated antibiotic use coexist.

Prevalence of β -Lactamase Phenotypes

The observed coproduction rate of 14% places Libya within the regional cluster of high-burden settings. Comparable figures have been reported in neighboring countries,^{15,16}

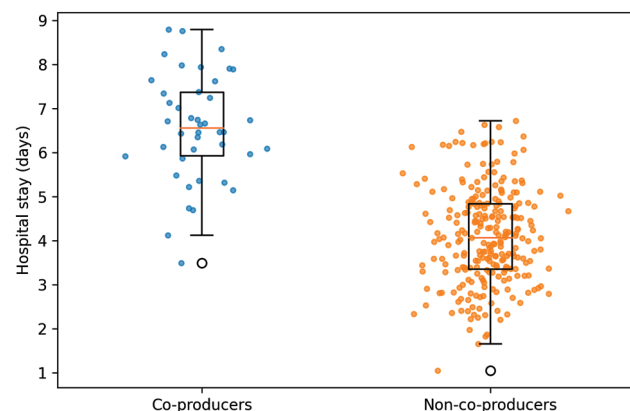


Fig. 2 Boxplot showing longer hospital stay among coproducer infections (median 6 vs. 4 days, $p=0.004$).

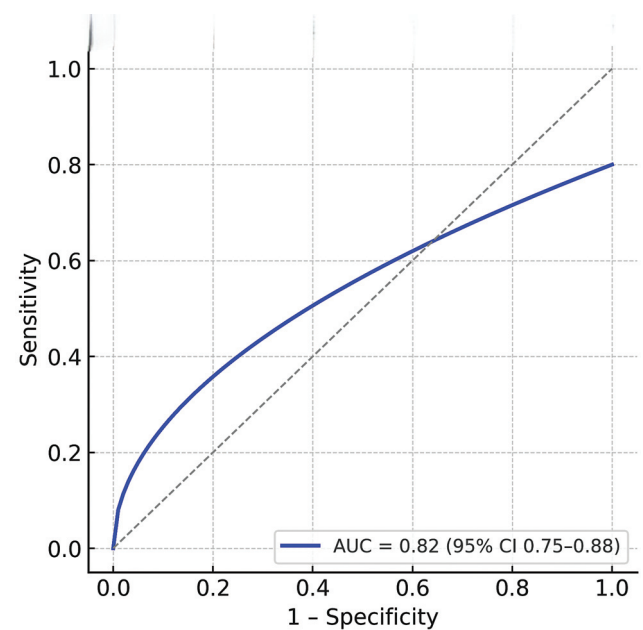


Fig. 3 Receiver operating characteristic (ROC) curve of the multi-variable model predicting treatment failure (area under the curve [AUC] = 0.82).

contrasting sharply with southern Europe (6–8%)^{6,16} and East Asia (5–6%).² The convergence across North African countries likely reflects similar antibiotic use patterns and widespread over-the-counter access to antibiotics. Hospitals in this region often act as amplification hubs for plasmid-mediated resistance, driven by empirical cephalosporin use and limited microbiological capacity.

Previous Libyan studies identified ESBL prevalence ranging from 30 to 45% but did not routinely screen for AmpC.^{17,18} Consequently, the true magnitude of dual β -lactamase production may have been underestimated. Our data therefore provide the first robust analytical evidence that coproduction is both measurable and clinically meaningful in Libyan UTI isolates.

Antimicrobial Resistance Profiles

The resistance configuration observed, dominant resistance to third-generation cephalosporins and fluoroquinolones, moderate resistance to β -lactam/ β -lactamase inhibitor combinations, and preserved activity of carbapenems and nitrofurantoin, mirrors recent global antimicrobial resistance (AMR) surveillance trends.¹⁹ Dual ESBL + AmpC production enables hydrolysis of nearly all extended-spectrum cephalosporins while simultaneously conferring inhibitor resistance, effectively neutralizing common empirical regimens such as ceftriaxone–clavulanate.^{7,20}

At the molecular level, coharboring of *bla*CTX-M and plasmid-encoded *AmpC* genes frequently occurs on IncF and IncI1 plasmids, often alongside *qnrA/B/S* determinants that mediate quinolone resistance.^{21,22} Such genetic linkage explains the simultaneous resistance to both β -lactams and fluoroquinolones observed in our coproducers. Recent sequencing data from Saudi Arabia revealed identical plasmid backbones among hospital *E. coli* strains,²³ suggesting regional dissemination through health care networks.

Clinically, the preserved sensitivity to carbapenems and nitrofurantoin (> 90%) echoes earlier Libyan findings¹⁷ and indicates that these agents remain reliable last-line options. However, sporadic carbapenem resistance documented locally and in neighboring countries signals the potential emergence of *bla*NDM and *OXA*-48-like genes.²⁴ This trend warrants proactive molecular surveillance and strict enforcement of antibiotic stewardship.

Predictors of ESBL + AmpC Coproduction

Our logistic regression analysis identified DM and prior antibiotic exposure as independent risk factors for coproduction. These associations are consistent with multicentric evidence showing that chronic diseases and repeated antimicrobial exposure facilitate the selection and maintenance of resistant flora.^{6,23} Antibiotic pressure, particularly from third-generation cephalosporins and fluoroquinolones, enriches gut reservoirs of ESBL/AmpC-producing Enterobacterales.⁴

Hospitalization initially appeared significant in univariate analysis but lost significance after adjustment for antibiotic exposure, suggesting that the hospital environment ampli-

fies risk primarily through antimicrobial selection pressure. This reinforces a key stewardship principle: exposure, rather than location, drives coproduction risk.

Impact on Treatment Outcomes

The association between coproduction, treatment failure, and prolonged hospitalization has important therapeutic and economic implications. Coproducers were linked to longer hospital stays and higher relapse rates, consistent with previous observations.²⁴ ESBL/AmpC coproducers were twice as likely to cause recurrent infection compared with single producers.¹⁵

The predictive model's AUC demonstrated strong discriminative performance, confirming the reliability of phenotypic data in forecasting poor outcomes. Such models could be integrated into infection-control dashboards to identify high-risk patients upon admission. Nevertheless, predictive analytics should complement rather than replace laboratory confirmation. Routine AmpC testing in diagnostic protocols remains essential, yet is still absent in most Libyan microbiology laboratories. Establishing this capacity would substantially enhance clinical decision-making and antimicrobial stewardship.

Phenotypic differentiation between ESBL, AmpC, and coproducers remains challenging. Concurrent AmpC expression may obscure ESBL activity, leading to false negatives in standard DDST.¹¹ Recent studies advocate using inhibitor-based assays with cefoxitin and boronic acid or cloxacillin disks to unmask coproducers.⁷

From a systems perspective, Libya's lack of integrated AMR data pipelines hinders national risk assessment. The World Health Organization Global Antimicrobial Resistance and Use Surveillance (WHO GLASS) framework provides a valuable template for harmonizing data on antimicrobial use (AMU) and AMR.¹⁹ Implementing such a model locally would facilitate trend monitoring, benchmarking against regional averages, and adaptive updates to empirical therapy guidelines. Institutional antibiograms, stratified by ward and infection type, should be generated quarterly to maintain therapeutic precision.

Overall Interpretation

Collectively, these findings highlight that coproduction of ESBL and AmpC β -lactamases in uropathogenic *E. coli* is not uncommon and exerts measurable clinical consequences. Diabetic status and prior antibiotic exposure emerge as key risk factors. Routine inclusion of AmpC screening in diagnostic protocols and periodic updates to local antibiograms are essential to optimize empirical therapy, particularly in high-risk diabetic populations.

Coproduction represents a convergent evolutionary strategy whereby bacteria combine ESBL and AmpC enzymes to overcome both oxyimino-cephalosporins and β -lactamase inhibitors. Horizontal gene transfer via conjugative plasmids facilitates this adaptation.²⁵ In vitro evolution experiments demonstrate that the presence of *bla*CTX-M on mobile elements accelerates the acquisition of AmpC genes through recombination events.²²

In PLWD, the selective advantage of such strains is magnified. Hyperglycemia impairs cytokine signaling (interleukin-8, tumor necrosis factor- α) and disrupts epithelial tight junctions, facilitating bacterial translocation.¹² Furthermore, subinhibitory antibiotic concentrations in chronically treated patients may induce SOS response (bacterial DNA damage - induced global stress response) responses that stimulate horizontal gene transfer. Thus, resistance evolution in diabetics is driven by both pharmacological and immunological factors.

Public Health and Stewardship Significance

The dual enzyme mechanism documented here poses a serious challenge to empirical therapy in resource-limited settings. Unchecked prescription of third-generation cephalosporins and self-medication are major drivers in Libya, where regulatory oversight remains limited. Empirical management of UTIs in PLWD should prioritize nitrofurantoin or carbapenems, reserving fluoroquinolones for documented susceptible isolates.

At the national level, establishing coordinated AMR surveillance within the Ministry of Health, integrated with regional WHO GLASS nodes, would enable real-time tracking of resistance phenotypes. Capacity-building initiatives should focus on equipping provincial laboratories with reliable AmpC detection and molecular screening kits. Public awareness campaigns addressing antibiotic misuse would complement these laboratory interventions.

From a regional standpoint, the North African AMR consortium could harmonize data sharing among Libya, Tunisia, Egypt, and Algeria, similar to the European Antimicrobial Resistance Surveillance Network model. Such cooperation would provide policymakers with early warnings of plasmid-mediated resistance trends and cross-border dissemination.

Strengths, Limitations, and Future Directions

The principal strength of this study lies in its comprehensive integration of clinical, microbiological, and analytical data, enabling direct correlation between phenotypic β -lactamase mechanisms and patient-level outcomes. To the best of our knowledge, this is the first study from Libya to quantify the prevalence of ESBL/AmpC coproduction among uropathogenic *E. coli* and to evaluate its clinical predictors using the CLSI-standardized phenotypic methods, thereby improving reproducibility and facilitating comparison with regional and international studies. The inclusion of both CA diabetic and nondiabetic UTI cases provides a clinically relevant overview of resistance patterns in a high-risk population.

Despite these strengths, several limitations should be acknowledged. First, the study was conducted in a single tertiary care center, which may limit generalizability to other regions of Libya. Second, molecular confirmation of ESBL and AmpC genes (e.g., polymerase chain reaction or whole-genome sequencing) was not performed due to resource

constraints, and phenotypic methods alone may under- or overestimate coproduction in some isolates. Third, important clinical variables were not fully available in patient records, including glycemic control (HbA1c levels), diabetes severity, presence of comorbidities, prior antibiotic classes, and details of empirical or culture-guided treatment regimens. Follow-up cultures after treatment were also unavailable, preventing microbiological confirmation of clinical cure. Fourth, minimum inhibition concentration (MIC) values and resistant quality control strains (e.g., ATCC 35218) were not available during testing, which may influence phenotypic accuracy. Fifth, although we attempted to minimize bias through consecutive sampling, no matching strategy (e.g., propensity score matching) could be implemented to balance age or sex differences between diabetic and nondiabetic groups. Sixth, the lack of EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints and reliance solely on CLSI may limit comparability for some international readers. Seventh, verbal consent rather than written documentation was used due to institutional policy for minimal-risk research, and this is acknowledged as an ethical limitation.

Future studies should incorporate multicenter sampling to improve representativeness, complemented by molecular assays to determine the genetic determinants and transmission dynamics of ESBL and AmpC enzymes. The integration of AMU metrics with resistance profiles would help identify ecological and behavioral drivers of coproduction. Prospective cohort designs with serial sampling may clarify the temporal progression from ESBL to ESBL/AmpC coproduction, especially under antibiotic selective pressure. Additionally, including glycemic control parameters, treatment regimens, comorbidity indices, and follow-up culture data would strengthen clinical interpretations and support the development of optimized, evidence-based management strategies for UTIs particularly among PLWD.

Conclusion

In summary, this study demonstrates that coproduction of ESBL and AmpC β -lactamases among uropathogenic *E. coli* in Libya is frequent and clinically consequential.

The phenomenon is amplified in PLWD and those with prior antibiotic exposure, leading to higher MDR rates and worse therapeutic outcomes.

Addressing this challenge requires integrating AmpC testing into standard diagnostic routines, enforcing antibiotic stewardship, and establishing national AMR monitoring systems to safeguard the efficacy of last-resort agents.

Conflict of Interest

None declared.

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