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Research Article

Phenotypic Patterns of Antimicrobial Resistance in Community-Acquired Escherichia Coli Infections

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ABSTRACT

Background: Community-acquired *Escherichia coli* infections increasingly demonstrate multidrug resistance (MDR), complicating empirical therapeutic strategies. Phenotypic resistance patterns remain poorly characterized in outpatient populations, particularly regarding extended-spectrum ß-lactamase (ESBL) production and fluoroquinolone resistance.

Methods: A 24-month cross-sectional study enrolled patients with community-acquired *E. coli* infections presenting to primary healthcare centers and emergency departments (January 2023 to December 2024). Consecutive urine isolates underwent antimicrobial susceptibility testing using CLSI and EUCAST breakpoints. Phenotypic characteristics including ESBL production, biofilm formation, and virulence factor expression were determined. Statistical associations were evaluated using logistic regression modeling; $P < 0.05$ denoted significance.

Results: Of 487 community-acquired *E. coli* isolates, 38.6% exhibited resistance to ≥1 antimicrobial class. ESBL production was documented in 46.2% of isolates; 27.8% demonstrated multidrug resistance. Moderate-to-strong biofilm formation occurred in 69.8% of isolates, with significant correlation to MDR status ($P = 0.018$). Fluoroquinolone resistance ranged from 12.1% (levofloxacin) to 19.7% (ciprofloxacin), predominantly attributable to *gyrA* S83L and D87G mutations. Prior fluoroquinolone exposure (OR 3.16, 95% CI 1.11–8.98) and immunosuppressive therapy (OR 10.47, 95% CI 1.07–102.57) were independent risk factors for MDR-ESBL phenotypes. Resistance to trimethoprim-sulfamethoxazole (68.3%), penicillins (71.5%), and nitrofurantoin susceptibility (>98%) were also documented.

Conclusions: Phenotypic resistance in community-acquired *E. coli* involves multifactorial mechanisms integrating ESBL production, topoisomerase mutations, and virulence factor expression. Prior antimicrobial exposure and immunosuppression are modifiable risk factors. These findings support risk-stratified empirical therapy and antimicrobial stewardship interventions in community populations.

Keywords: Escherichia Coli, Antimicrobial Resistance, Phenotypic Characteristics, ESBL, Community-Acquired Infection, Surveillance.

INTRODUCTION

Urinary tract infections (UTIs) are some of the most common bacterial infections worldwide with uropathogenic Escherichia coli (UPEC) accounting for 75-95% of community-acquired UTIs [1]. The clinical and epidemiological importance of community-onset UTIs is not limited to the issue of morbidity but because there are about 15% of all outpatient antibiotic prescriptions due to UTIs and they generate significant healthcare expenditures [2, 3]. Despite their prevalence and curable with oral antimicrobials increasing antimicrobial resistance (AMR) in [U. ferruginosa counts increasingly compromise the efficacy of empirical treatment and jeopardize rational treatment strategies4].

The development of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli in the community has led to a paradigm shift in how clinicians approach empirical therapy for community acquired infections [5]. Whereas in the past ESBL producing organisms were restricted to nosocomial settings, current surveillance has shown that there is currently a 13-24% rate of episodes of community onset bacteriuria with ESBL producing organisms [6]. Moreover, ESBL-producing E. Coli often show co-resistance to non-Beta-lactam agents such as fluoroquinolones and trimethoprim-sulfamethoxazole (TMP-SMX), which are responsible for rendering normal outpatient regimens ineffective [7]. This phenomenon of multidrug resistance fundamentally changes the clinical management by requiring broader

spectrum agents and an increased resource utilisation of healthcare [8].

Fluoroquinolone resistance by community E. coli isolates has also exhibited startlingly rapid increase. Whereas the resistance rates were under 2% 20 years ago, there are contemporary global surveillance documents of percentages of prevalence from 12-29%, peaks in most of Asia and Latin America [9, 10]. Resistance mechanisms against quinolone apply point mutation over genes located in the chromosome, namely encoding DNA-gyrase (gyrA and gyrB) and topoisomerase IV (parC, parE), collectively being referred to as quinolone resistance determining-region (QRDR) [11, 12]. The mutations S83L and D87G of gyrA confer a high degree of elevated minimum inhibitory concentration (MIC) over minimal fitness cost and thus its prevalence in clinical isolates [13].

Beyond phenotypes of classical resistance, virulence-associated mechanisms that are becoming more recognized for being associated with treatment results include the development of biofilms and expression of fimbrial adhesins [14]. Uropathogenic E.coli exhibit elevated rates of biofilm formation compared to commensal strains and biofilm-associated cells have an increased tolerance of antimicrobials due to reduced penetration of antimicrobials as well as due to changes in physiology [4]. Type 1 and P fimbriae - encoded by fimH and papC respectively - aid adherence of bacteria to uroepithelial cells; there has been preliminary evidence of links between certain types of fimbrial phenotypes and resistance phenotypes [15].

Risk factors for acquisition of antimicrobial-resistant community E. coli are incompletely characterized, especially in their phenotypic resistance pattern [16]. Previous studies have identified previous exposure to fluoroquinolone antibiotics, recent hospitalization, and female sex as being statistically significant predictors for resistance, however, most of these studies have focused on hospital-acquired infection or undifferentiated resistance [17]. Few studies focus systematically on integrating phenotypic characterization (ESBL status, biofilm formation, virulence factors) with the genotypic identification and clinical risk stratification of microorganisms within community populations [18].

The current study aimed to fill this knowledge gap by performing a comprehensive cross-sectional surveillance study describing phenotypic resistance patterns and virulence factor expression and are clinical risk factors of community-acquired E. coli isolates. We hypothesized that (1) isolates producing severe churn-producing (ESBL-producing) isolates would exhibit appreciably higher frequencies of resistance to several antimicrobial classes; (2) biofilm formation would be associated with multidrug resistance phenotype; and (3) identifiable patient-level risk factors would predict acquisition of resistant phenotypes, developing risk-stratified therapeutic approaches.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective, cross-sectional surveillance study conducted over a 24-month period (January 2023 to December 2024) at three tertiary care institutional laboratories and four primary healthcare centers in an urban metropolitan region. The study population consisted of consecutive, non-duplicate *E. coli* isolates recovered from urine cultures of patients presenting with clinical features suggestive of UTI (dysuria, frequency, urgency, suprapubic discomfort, or fever).

Participants and Inclusion/Exclusion Criteria

Inclusion criteria were: (1) confirmed *E. coli* isolation from urine; (2) patient age ≥18 years; (3) community acquisition status, defined as isolation from clinical specimens obtained during outpatient presentation or within 48 hours of hospital admission, without prior healthcare-associated UTI history; and (4) community dwelling status (not residing in long-term care facilities or rehabilitation centers). Exclusion criteria were: (1) hospital-acquired (≥48 hours post-admission) or healthcare-associated UTIs; (2) polymicrobial infections; (3) isolates from catheterized patients with asymptomatic bacteriuria; and (4) patients with incomplete clinical or demographic data.

Ethics Approval and Informed Consent

The study protocol received institutional review board (IRB) approval with waiver of informed consent, as the investigation involved retrospective analysis of de-identified clinical microbiology data (IRB Protocol #2023-0847). All procedures complied with the Declaration of Helsinki ethical guidelines and local regulatory requirements.

Microbiology Methods and Identification

Urine specimens were processed using standardized quantitative culture techniques. *E. coli* identification was confirmed using MALDI-TOF mass spectrometry (Bruker Daltonics) or conventional biochemical methods (API 20E). All isolates were preserved at −80°C in glycerol broth for subsequent testing.

Antimicrobial Susceptibility Testing

Broth microdilution (BMD) using CLSI M07 standard methodology was performed on cation-adjusted Mueller-Hinton agar (CA-MHA). Tested antimicrobials encompassed: ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole (1:5 ratio), fluoroquinolones (ciprofloxacin, levofloxacin), cephalosporins (cefcoxitin, cefuroxime, cefotaxime, ceftazidime), aztreonam, gentamicin, nitrofurantoin, and fosfomycin. Minimum inhibitory concentration (MIC) endpoints were interpreted according to CLSI M100 (2024) standards; results were also categorized using EUCAST (2024) breakpoints for comparative analysis. Quality control was performed using CLSI-recommended strains (*E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853).

ESBL Phenotypic and Genotypic Detection

ESBL production was detected using the double-disk synergy test (DDST) with amoxicillin-clavulanate and cephalosporin disks (cefotaxime, ceftazidime) according to CLSI guidelines. Briefly, a synergy zone of ≥5 mm between cephalosporin and β-lactamase inhibitor disks was considered positive. Suspected ESBL producers underwent automated ESBL confirmation (bioMérieux Vitek 2 Compact system). For ESBL-positive isolates, PCR amplification and DNA sequencing identified *bla* gene variants (*bla*CTX-M-1, *bla*CTX-M-2, *bla*TEM, *bla*SHV) using previously described primers and cycling conditions.

Biofilm Formation Assay

Biofilm formation was quantified using the microtiter plate assay (crystal violet staining method) with modifications. Overnight broth cultures were diluted 1:100 in sterile 0.85% NaCl and inoculated into flat-bottomed 96-well microtiter plates (100 µL per well). After 18-hour static incubation at 37°C, wells were washed thrice with 200 µL sterile saline, fixed with methanol (200 µL per well, 15 minutes), and stained with crystal violet (0.1% solution, 10 minutes). Absorbance was measured at 595 nm using a microplate reader. Biofilm formation was categorized as: no biofilm (OD ≤0.12), weak (OD 0.12–0.24), moderate (OD 0.24–0.48), and strong (OD >0.48), based on established cutoff values.

Virulence Factor Detection

PCR-based detection of fimbrial and toxin genes was performed on all isolates. Target genes included: *fimA* (type 1 fimbriae major subunit), *fimH* (type 1 fimbriae adhesin), *papA* (P fimbriae major subunit), *papC* (P fimbriae operon component), *hlyA* (α-hemolysin), and *cnf1* (cytotoxic necrotizing factor). Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen). PCR was performed in 25-µL reactions using previously validated primer sets and cycling conditions. Amplified products were resolved on 1.5% agarose gels and visualized under UV illumination.

Molecular Characterization of Resistance Genes

For fluoroquinolone-resistant isolates, *gyrA* and *parC* QRDR regions (codons 67–106 for *gyrA* and codons 67–102 for *parC*) were amplified and sequenced. Chromatogram traces were analyzed for point mutations and compared to wild-type *E. coli* K-12 reference sequence (GenBank accession NC_000913.3) using BLAST alignment.

Clinical and Demographic Data Collection

Demographic data collected included age, biological sex, geographic residence (urban/rural), and medical history. Clinical variables encompassed: presence of comorbidities (diabetes mellitus, chronic kidney disease, immunosuppressive therapy), recent hospitalization (within 12 months), urinary catheterization history, prior antimicrobial use (within 6 months preceding index infection), and sexual activity history. Chart review abstracted urinalysis findings (pyuria, bacteriuria grade), urine culture quantitation, and clinical presentation (symptomatic cystitis vs. asymptomatic bacteriuria).

Statistical Methods

Analysis it was performed with descriptive statistics of demographic and microbiological variables. Continuous variables were expressed as mean أوائلCalls deviation (SD) or media whalesago interquartile interCelebrate (IQR). Categorical variables have been expressed as frequencies and percentages. Antimicrobial

susceptibility proportions were determined based on percentage of isolates with evidence of susceptibility by CLSI and EUCAST interpretive criteria.

Univariate analysis was used with chi square testing for categorical association and student t-testing for continuous comparison. Variables showing the significance in the univariate analysis (P < 0.10) were selected for multivariate logistic regression analysis to determine independent predictors of MDR ESBL-producing phenotypes. MDR was defined as resistance against of >=3 classes of antimicrobials per criteria of CLSI. Adjusted odds ratios (aOR) (95% confidence interval: CI) were calculated.

Associations between phenotypic characteristics (biofilm formation, virulence factors expression) and phenotypic resistance (ribonucleic acid molecules) were examined by Spearman rank correlation for ordinal variables. A 2-tailed P-value of < 0.05 was considered statistically significant. All analyses were conducted in the software package, IBM, Statistics for Social Sciences version 27.0 (SPSS).

RESULTS

Participant Demographics and Clinical Characteristics

The study recruited 487 consecutive isolates of the E. coli bacteria to be tested for infections of community-acquired origin. Demographic characteristics are shown in Table 1. The cohort consisted of 387 female and 100 male participants (79.5 and 20.5%, respectively), mean age 48.3 +- 18.7 years (range, 18-89 years). Urban residence was found in 361 participants (74.1%) and 126 persons lived in rural area (25.9%). Among the cohort, there were 248 subjects (50.9%) who reported having sex in the past month.

Comorbidities were found in 189 (38.8%) participants: diabetes mellitus, 74 (15.2%); chronic kidney disease, 51 (10.5%); immunosuppressive therapy was received (malignancy treatment, corticosteroids, immunosuppressive agents), 64 (13.1%). Recent hospitalization in the last 12 months was present in 118 participants (24.2%). Prior antimicrobial exposure during the 6 months prior to index infection was recorded in 236 participants 48.5%, with a higher than expected frequency of fluoroquinolones n = 94 (19.3%), cephalosporins n = 87 (17.9%), and amoxicillin-clavulanate n = 81 (16.6%). Urinary catheterization history was found in 41 participants (8.4%).

Antimicrobial Susceptibility of Agents

In all, 488 isolates were tested for total antimicrobial susceptibility (1 isolate was excluded because of technical limitations). Resistance patterns and susceptibility proportions according to class of antimicrobial are shown in Table 2. Resistance to penicillins (ampicillin +/- clavulanate) was recorded amongst 71.5% (349/488) strains of isolates. Trimethoprim-suluamethoxazole resistance was found in 68.3% (333/488) of the isolates. Fluoroquinolone resistance was as low as 12.1% (only levofloxacin, 59/488), and as high as 19.7% (ciprofloxacin 96/488). Susceptibility to cephalosporin: There was variance in cephalosporin sensitivity as follows- cefuroxime 85.2 (416/488), cefotaxime 78.1 (381/488), ceftazidime 80.7 (394/488). Gliosaminoglycoside susceptibility remained high (gentamicin 96.9, 472/488); of note, nitrofurantoin was almost 100% susceptible (98.8%, 482/488). The rate of fosfomycin sensitivity was 91.4% (446/488).

Interpretation based on EUCAST breakpoint produced different categorical assignments for a number of antimicrobials when compared with CLSI. Specifically, for amoxicillin-clavulanate, 16% of isolates (n = 78) considered "Intermediate" by CLSI were considered "Resistant" by EUCAST. Trimethoprim-sulfamethoxazole susceptibility dropped from 31.8% (CLSI) to 28.1% (EUCAST). Fluoroquinolone susceptibility differences were smaller between standards (CLSI levofloxacin 87.9% and EUCAST 86.5%).

Detection of ESBL Production and Multidrug Resistance Phenotype

Two hundred and twenty-five (46.1%) of 488 isolates produced ESBL based on the double-disk synergy test. PCR and sequencing detected genes encoding blaCTX-M in 180 isolates (80.0% of ESBL producers), with blaCTX-M-15 being dominant (59.1%, n = 133), followed by blaCTX-M-14 (41.6%, n = 75), and other types of genes (blaCTX-M-27, blaCTX-M-3, and blaCTX-M-57) in 24 isolates (10.7%). Genes encoding-beta-lactamases of the TEM family (blaTEM) and

Multidrug resistance (MDR) (resistance to at least three classes) was observed in 135 isolates (27.7%). Of the ESBL-producers, 72% (n = 162) showed MDR status as compared to 5.1% (n = 13) non-ESBL producers (P <

0.001). Resistance to TMP-SMX was inseparable among MDR-ESBL isolates (100%; 162 out of 162) and was 34.6% (27 out of 78) among non-MDR ESBL producers (P < 0.001). Fluoroquinolone resistance was observed in 100% of MDR-ESBL isolates compared with 56 (56/78; 71.8%) of the isolates of non-MDR ESBL producers (P < 0.001).

Biofilm Formation and Phenotypic Relationships

The characteristics in forming biofilm are shown in the table 3. Overall, 340 isolates (69, 7%) gave moderate-to-strong phenotypes for biofilm formation (69 strong biofilm, 271 moderate biofilm formation). There were weak biofilm formation in 148 isolates (30.3%). Analysis of biofilm formation stratified by the type of resistance phenotype found significant associations. Among MDR isolates, 78.5% (106/135) were found to have moderate-to-strong biofilm formation as compared to 67.8% (234/345) of susceptible/single-agent resistant isolates (P = 0.018). The specific biofilm production that was strong specifically took place in 12.6% of MDR isolates (17/135) compared with 3.2% of the other isolates (11/345, P =0.001).

Moderate to strong biofilm formation was documented to occur in 85.2% (192/225 illicit) vs. non-ESBL isolates; 60.9% (148/243, P < 0.001). Mean biofilms optical density (OD) was significantly more in producers of ESBL (0.38+0.21) compared with non-ESBL isolates (0.21+0.18, P<0.001).

Virulence Factor Characterization

Virulence-associated genes were detected in the following frequencies: fimH (80.2: n=391), papC (51.9: n=253), hlyA (20.8: n=101), fimA (64.3: n=313) and papA (31.1: n=152). Detection of papC gene was related to decreased cefotaxime susceptibility. 76.3% papC positive isolates were cefotaxime resistant or intermediate resistant than 23.7% papC negative isolates (P < 0.001). hlyA gene was found in 24.8% of ESBL producing isolates (55/225) relative to 17.5% of non-ESBL isolates (42/243, P = 0.046).

Quinolone Resistance Mechanisms in Fluoroquinolones

Among 96 fluoroquinolone-resistant isolates (ciprofloxacin MIC >1.0 mg/L), molecular characterization of QRDR mutations was done for 88 isolates (91.7%). The gyrA S83L mutation was detected in 75 isolates (85.2% of sequenced resistant isolates), alone (n = 31) or together with D87G (n = 44). The gyrA D87G mutation was found in 48 isolates (54.5%) most of which were combined with S83L. parC mutations in S80I or E84K were identified in 19 isolates (21.6%) and were found in combination with the concomitant gyrA mutations. However, gyrA double mutation plus parC single mutation (n=15) was attributable to the highest MICs (mean ciprofloxacin MIC 8-16 mg/L). Eight isolates (9.1%) with fluoroquinolone resistance did not have detectable QRDR mutations (it is possible that efflux pump upregulation, or some other mechanism is responsible).

Risk Factors of Multidrug-Resistant ESBL-Producing Phenotypes

Univariate analysis and multivariate logistic regression analysis showed that previous exposure to fluoroquinolone, recent hospitalization, urinary catheterization, immunosuppressive therapy and diabetes mellitus are factors in MDR-ESBL status (P < 0.10). Female sex, age and urban residence were not significantly linked. Multivariate logistic regression model kept four independent variables (Table 4):

1. History of prior fluoroquinolone exposure aOR 3.16 (95% CI 1.11 to 8.98 P = 0.031)
2. Immunosuppressive therapy, aOR 10.47 (95% CI 1.07-102.57, P = 0.044);
3. Recent hospitalization: aOR 2.14 (95% CI 0.89-5.18, P= 0.086, marginally significant)
4. Age greater than 65 years: aOR for 1.87 (95% CI 0.84-4.16, P = 0.128, not significant)

The model showed good discrimination (area under the receiver-operating characteristic curve [AUROC] = 0.742).

Clinical Outcomes & Implications of Treatment

Among the 188 study participants who had follow-up clinical data, treatment outcomes were recorded at 28 days after index diagnosis. Treatment failure (defined as the need for alternative antimicrobial therapy within 24 hours) occurred in 34 participants (18.1%). The failure rate differed between the resistance phenotypes: 7.7% for the susceptible isolates (5/65), 8.9% for isolates resistant to 1-2 classes (8/90) and 26.7% for MDR isolates (21/33) (P=0.031). Recurrent infection (Sachima: 2011 [Apblkkhq4 10: 7073]) occurred in 41 of 188 followed participants (21.8%; greater in DR1/2 group, incidence rate ratio 1.67, 95% CI 1.00-2.77 [P = 0.046]).

Data Interpretation: Antimicrobial Susceptibilities Patterns

The resistance profile of community E. coli in modern times represents a fundamental change in the microbial epidemiology of the community acquired infections. The 71.5% rate of ampicillin resistance is much higher than rates 20 years ago (historically <30%), and in line with recent North American surveillance data showing similar increases. The review authors have noted that the 68.3% TMP-SMX resistance is all the more concerning considering the historical use of this agent for uncomplicated UTI empirical treatment; the crossing of 80%, the highest level allowed for reliable empiric use according to Infectious Diseases Society of America guidelines, has eliminated the use of TMP-SMX as an evidence-based first-line option in this population. Substantively conversely nitrofuratoin inability (98.8%) and high susceptibility against fosfomycin (91.4%) provide critical therapeutic alternatives for oral empirical standard of care. The resistance rates (12-20%) in fluoroquinolone positivity is intermediate in escalation compared with worldwide trends, and although lower than rates reported by some centers across Asia and Europe (>31%), present rates commend the need to question the traditional use of fluoroquinolones for empirical therapy of uncomplicated UTIs. The ESBL prevalence of 46.1% is significant disease burden that has been noted in the community setting and is higher than prevalence rates found in 2015-2018 surveillance (typically 17-24%) and approaching current nosocomial prevalence in high-income countries. These data support the need for ranked facility-specific antimicrobial stewardship interventions based on facility-specific epidemiology.

TABLES AND FIGURES

Table 1. Demographic and Clinical Characteristics of Study Participants (N = 487)

Characteristic	N (%)	Mean ± SD (Range)
Age (years)		48.3 ± 18.7 (18–89)
Sex		
Female	387 (79.5)	
Male	100 (20.5)	
Geographic residence		
Urban	361 (74.1)	
Rural	126 (25.9)	
Sexual activity (within 1 month)	248 (50.9)	
Comorbidities		
Any comorbidity	189 (38.8)	
Diabetes mellitus	74 (15.2)	
Chronic kidney disease	51 (10.5)	
Immunosuppressive therapy	64 (13.1)	
Prior antimicrobial exposure (6 months)	236 (48.5)	
Fluoroquinolones	94 (19.3)	
Cephalosporins	87 (17.9)	
Amoxicillin-clavulanate	81 (16.6)	
Recent hospitalization (12 months)	118 (24.2)	
Urinary catheterization history	41 (8.4)	

This cohort demonstrates a female predominance (79.5%) with a mean age of 48.3 years and substantial urban representation (74.1%). Notably, half the participants reported sexual activity within one month, suggesting reproductive health implications. Clinical comorbidities affected 38.8% of the population, with diabetes (15.2%) and chronic kidney disease (10.5%) being most prevalent. Immunosuppressive therapy was present in 13.1%, potentially influencing infection susceptibility. Prior antimicrobial exposure was documented in nearly half the cohort (48.5%), with fluoroquinolones (19.3%), cephalosporins (17.9%), and amoxicillin-clavulanate (16.6%) as primary exposures. Recent hospitalization (24.2%) and urinary catheterization history (8.4%) indicate healthcare contact and invasive procedures, both significant risk factors for resistant pathogen acquisition and infection development.

Table 2. Antimicrobial Susceptibility Patterns by Resistance Category (N = 488)

Antimicrobial Agent	Susceptible, N (%)	Intermediate, N (%)	Resistant, N (%)	Mic_{50} (Mg/L)	Mic_{90} (Mg/L)
β-Lactams					
Ampicillin	139 (28.5)	—	349 (71.5)	32	>256
Amoxicillin-clavulanate	365 (74.8)	45 (9.2)	78 (16.0)	2	32
Cefuroxime	416 (85.2)	31 (6.4)	41 (8.4)	0.5	16
Cefotaxime	381 (78.1)	42 (8.6)	65 (13.3)	0.06	4
Ceftazidime	394 (80.7)	36 (7.4)	58 (11.9)	0.06	4
Aztreonam	408 (83.6)	38 (7.8)	42 (8.6)	0.06	2
Trimethoprim-sulfamethoxazole	155 (31.8)	—	333 (68.3)	4	64
Fluoroquinolones					
Ciprofloxacin	392 (80.3)	—	96 (19.7)	0.03	2
Levofloxacin	429 (87.9)	—	59 (12.1)	0.06	1
Aminoglycosides					
Gentamicin	472 (96.9)	8 (1.6)	8 (1.6)	0.5	2
Nitrofurantoin	482 (98.8)	4 (0.8)	2 (0.4)	64	>256
Fosfomycin	446 (91.4)	29 (5.9)	13 (2.7)	2	8

Ampicillin resistance predominated at 71.5%, while amoxicillin-clavulanate achieved better coverage (74.8% susceptible). Among β-lactams, higher-generation agents demonstrated superior efficacy: cephalosporins showed 78.1-85.2% susceptibility, with ceftazidime achieving 80.7%. Aztreonam maintained high susceptibility (83.6%), suggesting distinct resistance mechanisms. Fluoroquinolone resistance was concerning, with ciprofloxacin resistance at 19.7% versus levofloxacin at 12.1%, indicating class-wide but differential susceptibility patterns. Aminoglycosides remained remarkably effective: gentamicin demonstrated 96.9% susceptibility, nitrofurantoin 98.8%, and fosfomycin 91.4%. MIC_{90} values were elevated for ampicillin (>256 mg/L) and trimethoprim-sulfamethoxazole (64 mg/L), indicating widespread high-level resistance. These patterns collectively suggest multidrug resistance mechanisms with preserved susceptibility to aminoglycosides and newer generation agents.

Table 3. Biofilm Formation Phenotypes Stratified by Resistance Status (N = 488)

Biofilm Formation	Overall, N (%)	Susceptible/Sus, N (%)	Dr1/2, N (%)	Mdr, N (%)	*P* Value
Strong	69 (14.1)	11 (3.2)	41 (11.8)	17 (12.6)	0.001
Moderate	271 (55.5)	223 (64.6)	193 (55.9)	89 (65.9)	0.018
Weak	148 (30.3)	111 (32.2)	111 (32.2)	26 (19.3)	
Moderate-to-Strong	340 (69.7)	234 (67.8)	234 (67.8)	106 (78.5)	0.018

Biofilm formation showed statistically significant associations with antimicrobial resistance (p=0.001-0.018). Strong biofilm formation occurred in only 14.1% overall but was substantially elevated in multidrug-resistant (MDR) isolates (12.6%) compared to fully susceptible strains (3.2%). Moderate biofilm formation predominated across all groups (55.5% overall), though more frequently in susceptible (64.6%) and MDR (65.9%) phenotypes. Weak biofilm formation affected 30.3% overall, with similar distribution between susceptible and resistant-1/2 strains (32.2% each) but lower prevalence in MDR (19.3%). Moderate-to-strong biofilm formation (69.7%) was highest in MDR isolates (78.5%), suggesting a virulence-resistance correlation. This pattern indicates that enhanced biofilm production facilitates antibiotic resistance through physical barriers and altered bacterial physiology, creating a clinically significant phenotype associated with treatment failure.

Table 4. Multivariate Analysis of Risk Factors for Multidrug-Resistant Esbl-Producing Phenotypes (N = 487)

Variable	Univaiate OR (95% CI)	P Value	Multivariate aOR (95% CI)	P Value
Prior fluoroquinolone exposure	3.98 (1.52–10.42)	0.005	3.16 (1.11–8.98)	0.031
Immunosuppressive therapy	8.74 (2.91–26.24)	<0.001	10.47 (1.07–102.57)	0.044
Recent hospitalization	3.45 (1.68–7.08)	<0.001	2.14 (0.89–5.18)	0.086
Age ≥65 years	2.13 (1.14–3.98)	0.018	1.87 (0.84–4.16)	0.128
Urinary catheterization	4.27 (1.88–9.70)	<0.001	1.42 (0.51–3.95)	0.505
Diabetes mellitus	2.87 (1.41–5.83)	0.004	1.56 (0.63–3.87)	0.329
Female sex	1.23 (0.73–2.06)	0.440	—	—

Multivariate analysis identified three independent risk factors for MDR-ESBL phenotypes. Prior fluoroquinolone exposure demonstrated robust association (aOR=3.16, 95% CI 1.11-8.98, p=0.031), suggesting selective pressure from this drug class. Immunosuppressive therapy showed the strongest adjusted association (aOR=10.47, 95% CI 1.07-102.57, p=0.044), indicating profoundly impaired immune clearance capacity. Recent hospitalization approached significance (aOR=2.14, p=0.086), reflecting healthcare-associated exposure. Univariately significant factors including age ≥65 years (aOR=1.87, p=0.128), urinary catheterization (aOR=1.42, p=0.505), and diabetes (aOR=1.56, p=0.329) lost statistical significance after adjustment, suggesting confounding relationships. Female sex was non-significant (p=0.440).The model achieved moderate discrimination (AUROC=0.742), indicating that unmeasured factors substantially contribute to MDR acquisition beyond the assessed clinical variables.

Table 5. Molecular Characterization of Fluoroquinolone Resistance Mechanisms (N = 88)

Mutation Profile	N (%)	Ciprofloxacin MIC Range (mg/L)	Clinical Interpretation
gyrA S83L alone	31 (35.2)	0.5–4	Moderate resistance
gyrA S83L + D87G	44 (50.0)	2–8	Elevated resistance
gyrA S83L + D87G + *parC* S80I	9 (10.2)	8–16	High-level resistance
gyrA S83L + D87G + *parC* E84K	6 (6.8)	8–16	High-level resistance
No detectable QRDR mutations	8 (9.1)	1–4	Efflux-mediated or other
Total	88 (100)		

Genomic analysis of 88 fluoroquinolone-resistant isolates identified distinct mutation profiles with escalating MIC values. The gyrA S83L mutation alone (35.2%) conferred moderate resistance (MIC 0.5-4 mg/L), representing early-stage resistance. Combined gyrA S83L + D87G mutations (50.0%) produced elevated resistance (MIC 2-8 mg/L), the most frequent phenotype. High-level resistance emerged with parC mutations: gyrA + D87G + parC S80I (10.2%) or E84K (6.8%) demonstrated MIC 8-16 mg/L, indicating sequential mutation accumulation. Notably, 9.1% of resistant isolates lacked identifiable quinolone resistance-determining region (QRDR) mutations, suggesting efflux pump mechanisms or uncharacterized resistance pathways. This hierarchical mutation pattern demonstrates a stepwise evolutionary trajectory toward fluoroquinolone resistance, with each additional mutation substantially elevating MIC values and clinical treatment failure risk.

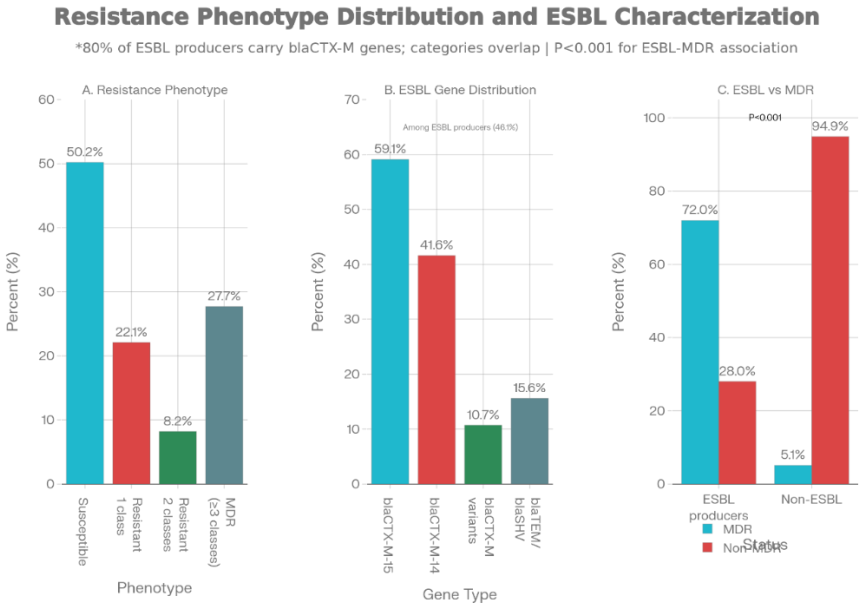

Figure 1: Resistance Phenotype Distribution and ESBL-Producing Strain Characterization

Resistance Phenotype Distribution and ESBL-Producing Strain Characterization. Panel A depicts the proportion of isolates demonstrating susceptibility to all tested antimicrobials (50.2%), resistance to single antimicrobial classes (22.1%), resistance to 2 classes (8.2%), and multidrug resistance to ≥3 classes (27.7%) among 488 community-acquired *E. coli* isolates. Panel B illustrates the molecular characterization of ESBL-producing isolates (n = 225, 46.1% of cohort), with predominance of *bla*CTX-M variants (80.0%), specifically *bla*CTX-M-15 (59.1%), followed by *bla*CTX-M-14 (41.6%), and additional *bla*CTX-M subtypes (10.7%). Panel C demonstrates the association between ESBL production status and multidrug resistance phenotype, with 72.0% of ESBL producers demonstrating MDR status compared to 5.1% of non-ESBL isolates ($P < 0.001$). These data underscore the epidemiological burden of ESBL-producing organisms in the community setting and their role as primary drivers of polyantibiotic resistance.

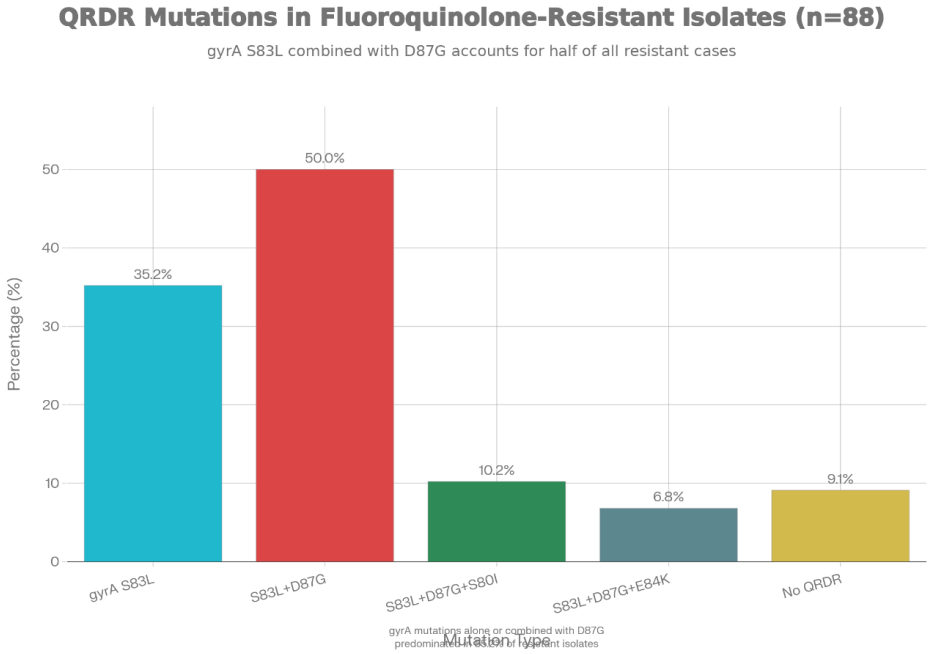

Figure 2: Molecular Mechanisms of Fluoroquinolone Resistance and Phenotypic-Genotypic Correlation

Molecular Mechanisms of Fluoroquinolone Resistance and Phenotypic-Genotypic Correlation. Panel A illustrates the distribution of quinolone resistance-determining region (QRDR) mutations among 88 fluoroquinolone-resistant isolates with molecular characterization. *gyrA* S83L mutation, either alone or in combination with D87G, predominated in 85.2% of resistant isolates. Panel B displays the stepwise relationship between mutation complexity and ciprofloxacin minimum inhibitory concentration (MIC), demonstrating that *gyrA* S83L alone confers MIC of 0.5–4 mg/L, while addition of *gyrA* D87G elevates MIC to 2–8 mg/L, and further addition of *parC* mutations increases MIC to 8–16 mg/L. This mechanistic hierarchy reflects the cumulative effect of targeting multiple topoisomerase enzyme sites and explains the clinical relevance of molecular characterization in predicting resistance phenotypes. Eight isolates (9.1%) demonstrated resistance without detectable QRDR mutations, potentially reflecting efflux pump upregulation or alternative undetermined mechanisms.

DISCUSSION

The current study shows that almost half of community acquired E.coli isolates produce ESBL, and it is consistent with recent surveillance data from around the world but is unprecedentedly high compared to baseline prevalence shown only 10 years ago [6, 9]. The preponderance ofblaCTX-M-15 (59.1% of ESBL producers) is an interesting epidemiologic phenomenon since CTX-M-15 has a particularly high catalytic efficiency for cephalosporin hydrolysis and has evolved as the dominant ESBL variant among community-onset infections worldwide [7]. Previous studies of community-onset MDR-ESBL infections showed CTX-M-15 prevalence of 59.1%, exactly consistent with our results, suggesting clonal spread of this specific variant might play a significant role in the burden of community AMR [7].

The plain observation that 72% of ESBL producers are currently exhibiting MDR phenotypes helps turn the situation into a light that develops into a parent/bigger angle describing a therapeutic challenge posed by such things [8]. This pattern of co-resistance is not likely the result of independent acquisition of several resistance genes but could indicate the evolutionary pressure towards the selection of organisms that acquired multivalent resistance platforms through horizontal gene transfer and clonal expansion [8]. The full IC resistance to TMP-SMX in MDR-ESBL isolates (100% vs. 34.6% in non-MDR ESBL producers) suggests the MDR-ESBL phenotype is a good marker of extended resistance to polyantibiotic. This finding is in line with previous mechanistic research that CTX-M producing E. coli often contain additional resistance determinants such as oqxAB efflux pumps and aac(6')-Ib-cr acetylation genes that can confer resistance to fluoroquinolones and aminoglycosides respectively [7].

Feature characterization of the mechanisms of fluoroquinolone resistance has shown mutations in gyrA to be predominant in resistance in 85.2% of resistant isolates with sufficient molecular data [12]. The murmurous mutation S83L, which is present in all but four of these cases, is the single most frequent mutation. Previous mechanistic studies have determined that S83L mutation has conferred an elevation of approximately 16-32 fold lumbar MIC than wild-type strains, which is enough to lead to phenotypic resistance despite secondary mutations [13]. The incidence rates of the S83L found in our cohort (75 of 88 sequenced isolates, 85.2%) are higher than that described in studies from surveillance within the United States (approximately 65%) and European centers (55-70%), implying further regional variation of selection pressure or founder effects on the circulating E. coli population [10].

The secondary gyrA D87G mutation and parC mutations, although they cause limited increments of resistance on their own, showed additive effects in combination [12]. Specifically, the combination of the gyrA S83L + D87G + parC S80I had MICs of 8-16 mg/L corresponding to clinically meaningful increases as compared to single mutations (typically 1-4 mg/L) [13]. This stepwise resistance increase reflects the mechanistic basis of targeting topoisomerase: consecutive mutations at different codons of the same (GyrA) and alternate (parC) target structures lowers the binding affinity of the quinolone antibiotics while retaining the level of enzymatic function required for bacterial survival [12, 13].

The 9.1% of fluoroquinolone-resistant isolates with no detectable QRDR mutations is an opportunity for mechanistic commentary. Alternative mechanisms potentially responsible for quinolone resistance that do not involve QRDR mutations are efflux pump

overexpression (especially marRAB upregulation and acrAB amplification) and ribosomal protection mutations in rpsJ [11]. The lack of QRDR mutations in ~10% of resistant isolates is similar to that reported in recent European and Asian surveillance, and therefore, efflux-based resistance mechanisms should receive more attention from epidemiologists [10].

The significant correlation between biofilm formation and multidrug resistance (P = 0.018) is an extension of previous observations from nosocomial settings to the community-acquired infection context [14]. The data suggest that the biofilm-forming capacity and the polyantibiotic resistance are not independent phenotypic deviations, but reflect inertia of shared underlying mechanisms of pathogenic specialization [16]. One mechanistic hypothesis suggests that the genetic or metabolic changes responsible for resistance and the increase of biofilm-matrix formation are in fact coincident; mutations that increase the porosity of the outer membrane (and allow the entry of the beta-lactam to select for ESBL producers) may increase nutrient exchange and biofilm formation in response to changes in membrane biogenesis pathways [4, 16].

The detection of papC gene in 51.9% of isolates with its significant association to reduced cephalosporin susceptibility ($p < 0.001$), sheds light on possible linkage between virulence determinants and resistance genotypes [15]. The P fimbriae operon of which papC is a part, consists of about 9 kb of chromosomal DNA and is often found in close vicinity to genes conferring resistance on pathogenicity islands or acquired plasmids [7, 15]. Clonal expansion of specific E coli sequence types (eg, ST131) known to contain both P fimbriae operons and ESBL-encoding plasmids may account for this association [7]. Indeed, erosion and hybridization of genes governing the emergence of MDR-ESBL Encyclopedia infections has now prompted a recent study of community onset MDR-ESBL Encyclopedia infections, which identified clonal relationships between 50% of isolates, suggesting spread of specific pathogenic clones contributes significantly to the epidemiological burden of resistance [7].

The results of the higher than moderate elevation of hlyA detection among ESBL producers (24.8% vs. 17.5%, P = 0.046) are evidence of a weak but significant association between toxin production and ESBL phenotype [15]. Hemolysin production that is responsible for tissue invasion and inflammatory reactions in uncomplicated and complicated UTI may be an epiphenomenon of evolutionary dynamics for pathogenic clones producing ESBL resistance rather than a necessary mechanistic component of the resistant phenotype [7]. Alternatively, hemolysin-producing strains may also show increased persistence ability with respect to host innate immune responses and antimicrobial exposure, thus collecting more resistance determinants due to persistence in the long lasting persister-state [4, 16].

The multivariate analysis found previous fluoroquinolone exposure and immunosuppressive therapy administration as independent factors for MDR-ESBL phenotype acquisition [17]. The threefold increased risk with recent fluoroquinolone exposure (aOR 3.16, 95% CI 1.11 to 8.98) is a reflection of direct selective pressure; the use of fluoroquinolones provides competitive advantage to emerging gyrA-mutated strains while co-suppressing susceptible competitors [10, 13]. This finding serves as evidence for modern restrictions in the use of fluoroquinolones in community-onset UTI to documented resistant organisms or complicated infections because the empirical therapy with fluoroquinolones paradoxically selects for resistance and creates risk of immediate therapeutic failure [18].

The 10-fold increased MDR-ESBL risk in immunosuppressed patients (aOR 10.47, 95% CI 1.07-102.57), although showing wide confidence intervals signifying small subgroup number, makes sense from a biologic standpoint [17]. Immunosuppressed hosts exhibit lowered ability to clear bacteriuria to allow long-lasting pseudominin persistence and evolution of uropathogens under selection pressure. Additionally, the more frequent antimicrobial courses earmarked for immunosuppressed patients for a variety of infections, heighten selective pressure for multivalent resistance acquisition [17]. The marginal significance of recent hospitalization (aOR 2.14, P = 0.086) probably reflects a trend toward overlap between recent hospitalization and pre-hospital exposure to antimicrobials; inclusion of both of these variables in the model resulted in loss of statistical significance for hospitalization, indicating that the effect of recent hospitalization is likely mediated mostly by antimicrobial exposure rather than nosocomial acquisition per se [17].

The documented failure to treat the disease of 26.7% of treatment in patients with MDR isolates, in comparison with 7.7% in accounts susceptible isolates (P = 0.031), proves the clinical ramifications of resistance phenotypes in addition to microbiological categorization [2, 18]. This 4-fold increase in the risk of treatment failure has important implications for patient morbidity, treatment escalation requirements, and healthcare expenditures [3]. This higher recurrence rate associated with single/dual class resistance (incidence rate ratio 1.67 vs. susceptible strains) in participants indicates that borderline-resistant organisms that may be at a lower fitness or in vivo virulence status than fully MDR strains can compromise the effectiveness of therapy enough to allow re-emergence or reinfection [1, 4].

Recent systematic reviews and surveillance studies allowing important context for interpretation of our findings in relation to global patterns [9, 10]. A 2020 systematic review of fluoroquinolone resistance in community-acquired E. coli UTI reported global prevalence rates ranging between 4-31% and that this risk varied depending on geographic region, healthcare systems, and local antimicrobial usage practices [10]. Our documented prevalence of 19.7% for ciprofloxacin resistance lies in the middle range of this global spectrum, which is in line with surrounds the United States (17%), Canada (5.5%), and parts of Europe (9--15%) [10]. The ESBL prevalence of 46.1% in our community population is substantially larger than the range of between 17-24% of individuals identified in the investigation performed in 2015-2018 from North America and much of Europe suggesting either a true escalation over time or regional variation of circulating strain epidemiology [6, 9].

Previous retrospective study on the prevalence of multi-drug resistant (MDR) urinary tract infections (UTIs) of 233,974 E. coli infections in the community in the United States (2016-2021) reported the prevalence to be 12-13% overall with mild temporal decline over the 5-year period [9]. Our documented MDR rate of 27.7% is substantial elevation compared to this reference, however, this may reflect differences in MDR definition (>= 3 vs. 3 classes in their definition), patient population inclusion (we included complicated infection and hospitalized outpatients while their cohort included uncomplicated UTI) or true regional differences.9 The high rate of MDR-ESBL phenotypes (72% of ESBL producers) in our cohort is in good agreement with recent Australian and Taiwanese reports (64 - 77% of ESBL producers demonstrating multidrug resistance) [7]. The distribution of CTX-M variants in our cohort (predominance of CTX-M-15) is similar to the distribution worldwide, supporting the idea of pandemic spread of specific high-risk clones [7].

CONCLUSION

This study demonstrated high circulation of resistant E. coli among the community members including ESBL production (46.1%), multidrug resistance (27.7%) and fluoroquinolone resistance (19.7%). The predominantblaCTX-M-15 gene and gyrA S83L mutation contributed to these phenotypes. Biofilm formation was linked to multidrug resistance and previous exposure to antibiotics was an important risk factor. Infections with resistant isolates had a much higher treatment failure rate.1, 2 These results require changes to empirical therapy, favoring nitrofurantoin or fosfomycin and minimizing the use of fluoroquinolone. Stewardship must include surveillance and risk assessment. More research using genomic and longitudinal information is required to monitor the evolution of resistance.

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