

Original Article

Emerging trends in multidrug-resistant ESBL-producing *Escherichia coli* in urinary tract infections: a clinical and genomic observational study from Kunshan city

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Abstract: Objective: With the global spread of extended-spectrum β -lactamase-producing *Escherichia coli* (ESBL-EC), treatment of urinary tract infections (UTIs) has become increasingly challenging. This study aimed to investigate the antimicrobial resistance patterns, genotypic characteristics, and clinical risk factors associated with ESBL-EC in UTI patients in Kunshan City. Methods: This retrospective observational study included 421 patients with culture-confirmed UTIs admitted to two hospitals in Kunshan (2022-2024). Clinical records and 465 bacterial isolates were analyzed. Bacterial identification and susceptibility testing were performed using standard culture and molecular genotyping. Resistance genotypes were identified via polymerase chain reaction (PCR)-based sequencing. Multivariate logistic regression and receiver operating characteristic (ROC) curve analysis were used to assess risk factors and treatment efficacy. Outcomes included resistance rates, ESBL genotype distribution, independent predictors of multidrug resistance (MDR), and treatment efficacy measured by area under the curve (AUC). Results: ESBL-EC was the predominant pathogen, with high resistance to ampicillin (71.65%) and ciprofloxacin (38.19%), while retaining susceptibility to cefoperazone/sulbactam and piperacillin/tazobactam. CTX-M (7.87%) and PASE+CASE (36.61%) were the dominant genotypes. Independent MDR predictors included age ≥ 54 years, body mass index (BMI) ≥ 22.5 kg/m², prior UTI, antibiotic use, prolonged hospitalization, and diabetes (OR=7.985). The aminoglycoside-cephalosporin combination showed the highest predictive value for recovery (AUC=0.872). Conclusions: ESBL-EC in UTIs shows increasing multidrug resistance and a unique local genotype landscape dominated by PASE+CASE (36.61%). Early risk stratification and region-specific empirical therapy are critical for resistance containment in this locality.

Keywords: ESBL-producing *Escherichia coli*, urinary tract infection (UTI), antimicrobial resistance, multidrug resistance, CTX-M genotype, risk factors

Introduction

Urinary tract infections (UTIs) remain a major clinical burden globally, with *Escherichia coli* as the leading pathogen [1]. However, the escalating prevalence of extended-spectrum β -lactamase-producing *E. coli* (ESBL-EC) has substantially narrowed therapeutic options, particularly in regions with high antimicrobial consumption [2, 3]. While CTX-M-type ESBLs are recognized as the predominant genotype worldwide [4], mounting evidence indicates that local resistance gene spectra are profoundly shaped by regional antibiotic prescribing practices, population demographics, and healthcare system structures [5-9]. Consequently, global or national surveillance data

often fail to inform empirical therapy at the local level, necessitating region-specific investigations.

Kunshan City, a densely populated county-level municipality in the Yangtze River Delta core economic zone, exemplifies such a locality requiring dedicated surveillance. With a permanent population exceeding 2 million, Kunshan is characterized by three interconnected features that may uniquely influence ESBL-EC epidemiology: (1) High interregional population mobility, which facilitates the import and export of resistant clones; (2) Rapid population aging, with a growing proportion of elderly residents prone to recurrent UTIs and comorbidities; and (3) A dual-tier healthcare system where tertiary

referral hospitals and primary care facilities operate in parallel, potentially creating divergent antibiotic exposure pressures. This distinctive “socio-healthcare ecology” may generate antimicrobial resistance patterns that differ substantially from those reported in major metropolitan centers like Shanghai or Hangzhou, yet no systematic investigation has been conducted to verify this hypothesis.

To date, contemporary antimicrobial resistance data from peer county-level cities within the Yangtze River Delta remain conspicuously sparse. Published surveillance from neighboring secondary cities in Jiangsu Province has suggested variable ESBL-EC prevalence rates and genotype mixtures, but direct comparisons are hampered by methodological heterogeneity and the absence of unified sampling frames. For Kunshan specifically, the baseline resistance profile, dominant ESBL genotypes, and their associations with clinical outcomes have never been characterized. This knowledge gap is particularly problematic given that empirical antibiotic protocols in local hospitals are often extrapolated from tertiary-center guidelines, which may be ill-suited for the local resistance landscape and could inadvertently accelerate the selection of multidrug-resistant (MDR) strains.

In this context, the present study was designed to provide the first comprehensive baseline characterization of ESBL-EC in UTI patients from Kunshan. Specifically, we aimed to: (1) Delineate the temporal resistance trends and genotypic distribution of ESBL-EC in this locality; (2) Identify independent clinical predictors of MDR using multivariate logistic regression; and (3) Evaluate the predictive efficacy of different antibiotic regimens via ROC curve analysis to guide empirical therapy. By filling this critical data void, our findings are expected to establish a region-tailored evidence base for antimicrobial stewardship, enable risk-stratified management of UTI patients, and offer a referable surveillance model for other county-level cities facing similar ecological and healthcare transitions in the Yangtze River Delta.

Materials and methods

Subjects of study

Four hundred and twenty-one patients who visited our hospital from July 2022 to July 2024

who had confirmed urinary tract infections were included. A total of 465 bacterial isolates were obtained from these patients, as some patients had multiple episodes of infection or yielded more than one pathogen. For patients with multiple positive urine cultures, only the first episode was included in the primary analysis; subsequent isolates collected within 30 days of the initial positive culture were considered recurrent and excluded, whereas isolates obtained after 30 days were treated as new episodes and included separately. After excluding strains from repeat infections in the same patient, the primary pathogenic strain (or the isolate with the most extensive resistance profile in cases of polymicrobial infections) was retained for each patient to define their resistance status. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, according to the international consensus standard [10]. The patients were categorized into a MDR group (n=44) and a non-MDR group (n=377) based on the bacterial culture results of urine and drug sensitivity tests using this definition. Importantly, all 44 patients in the MDR group had ESBL-EC as the causative pathogen, and the MDR classification was determined based on the resistance profile of their ESBL-EC isolates. In total, there were 164 males and 257 females; the age of the patients ranged from 25 to 75 years, with a mean age of (45.08±11.43) years; the mean Body Mass Index (BMI) was (22.47±1.79) kg/m²; and the mean number of days of hospitalization was (34.70±2.31) days.

Inclusion criteria: (1) Patients with typical symptoms of urinary tract infection; (2) Patients with unexplained fever without other local symptoms; (3) Patients with pyuria or hematuria; (4) Patients with indwelling urinary catheters in the hospital with fever symptoms; (5) Patients with impaired bladder emptying function; (6) Patients prior to receiving surgery for urinary tract diseases; (7) Patients whose clinical and microbiological data were available for retrospective analysis. Clinical cure was defined as resolution of UTI-related symptoms (fever, dysuria, frequency, and urgency) with negative follow-up urine culture at day 3-5 after treatment initiation. Exclusion criteria: (1) Patients with combined malignant tumors; (2) Those taking drugs that might disturb this test; (3) Those who had received immunotherapy. This study was approved by the Medical Ethics Committee of

Kunshan First People's hospital (Approval Number: 2025-018). Informed consent was waived due to the retrospective nature of the study, the use of anonymized clinical and microbiological data, and the minimal risk to patients. All patient identifiers were removed prior to data analysis to ensure confidentiality.

Specimen collection and examination

Urine specimens for this study were collected in the morning after the patient had discontinued antibiotic treatment for at least five days or prior to any antibiotic administration. To enhance bacterial growth, it was recommended that the urine remains in the bladder for a duration of 6-8 hours before collection. Before urine collection, patients were instructed to wash their hands thoroughly and clean the skin around the urethra under the guidance of medical personnel. Female patients were advised to avoid collection during menstruation and to discard the initial portion of the urine to minimize contamination from vulvar secretions. Only the mid-stream urine was retained for analysis, ensuring that the specimen remained uncontaminated by external sources.

For patients using indwelling catheters, the exterior of the catheter was disinfected before urine was collected. The catheter was punctured using a sterile syringe to aspirate urine, taking care to avoid any mixing of disinfectants with the sample. The use of collection bags was strictly prohibited. For patients who had been catheterized long-term, urine was collected during the replacement of the catheter to minimize contamination risk.

In cases where mid-stream urine could not be obtained or the results of prior cultures were abnormal, suprapubic cystocentesis was performed. This procedure involved sterilizing the suprapubic skin and puncturing the bladder with a sterile syringe to aspirate urine directly from the bladder. If anaerobic infection was suspected, a rubber stopper was inserted into the syringe and the sample was sent for appropriate testing. Suprapubic cystocentesis is considered the gold standard for evaluating intra-vesical infections.

Specimens were transported to the laboratory immediately following collection. If immediate analysis was not possible, the specimens were stored at 4°C for no more than 2 hours. Urine

samples that had been stored for more than 24 hours or collected from multiple voids were not suitable for culture. For point-of-care testing, specimens suspected of containing *Neisseria gonorrhoeae* were prioritized. In cases where immediate processing was not feasible, urine samples were kept warm and sent for testing without refrigeration, as cold storage could negatively affect the detection rate. Specimens were rejected if they lacked identifying information (e.g., patient name, hospitalization number, and department), if they exceeded the allowable storage time, or if they were not collected in sterile containers.

Bacterial identification and antimicrobial susceptibility testing

Bacterial identification and antimicrobial susceptibility testing followed the guidelines outlined in the National Guide to Clinical Laboratory Procedures (4th Edition) [11] and the standard operating procedures of our laboratory. Clean mid-stream urine specimens were collected and placed into sterile transport containers for analysis within 2 hours of collection. Blood Agar Plates and Sabouraud Dextrose Agar (SDA) were inoculated by a 10 µL one-time quantitative inoculation loop, and these plates were then incubated at 35°C for 24-48 hours. Once fastidious organisms were suspected, chocolate agar plates were inoculated and incubated in a 5% CO₂ atmosphere at 35°C for 24-48 hours.

Different colony count criteria were used to assess the bacterial growth: gram-negative bacilli (GNB) were considered significant if present at >10⁵ CFU/mL, gram-positive cocci at >10⁴ CFU/mL, and fungi at >10⁴ CFU/mL. Clinically, relevant infections were distinguished from possible contaminants using these thresholds.

Both automated and manual methods were employed to identify bacteria. The VITEK Compact 2 fully automated system was used for routine bacterial identification and antimicrobial susceptibility testing. Manual methods were utilized to guarantee detecting accuracy regarding more complex cases. For example, a combination of biochemical tests was used to identify oxidase test-positive gram-negative bacilli, including disaccharide fermentation, glucose fermentation (O/F), nitrate reduction, and the API20NE identification card when nec-

essary. Biochemical reactions specific to the Enterobacteriaceae family were applied to identify oxidase test-negative gram-negative bacilli, including tests for disaccharides, methyl red, indole, citrate, various decarboxylation reactions, and the API20E identification card.

On Mueller-Hinton (MH) agar plates, antimicrobial susceptibility testing (AST) was performed. The plates were equilibrated to room temperature (25°C) after removing them from refrigeration. Researchers collected a single pure colony from a 24-48-hour blood agar culture using a sterile cotton swab, and this was then suspended in sterile saline to reach the 0.5 McFarland standard. The suspension was streaked onto the MH agar plate in three perpendicular directions to ensure even distribution. Antibiotic susceptibility disks (OXOID, UK) were then placed on the surface of the agar using sterile forceps, remaining with the agar. The plates were incubated at 35°C for 16-24 hours in an aerobic environment, and the inhibition zones around each disk were measured according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

For strains exhibiting multi-drug resistance, combination therapies were applied and efficacy was evaluated by additional testing. Specifically, researchers used the combination of cefoperazone and sulbactam to assess its in vitro activity against resistant strains. Bacterial resistance profiles were repeatedly checked and verified by clinics and hospital personnel on infection management and control to ensure accuracy and consistency.

DNA extraction, quality control and targeted genotype detection

Bacterial genomic DNA, once obtained from midstream urine specimens of infected individuals in past practices, was isolated from monoclinal *Escherichia coli* colonies cultured on blood agar plates. Single colonies were subcultured in LB median and put in the incubator overnight at 37°C with orbital agitation to achieve optimal bacterial density following standard clinical microbiology protocols broadly implemented in tertiary hospitals across China.

Commercially available TIANamp Bacterial DNA Kit (Tiangen Biotech, Beijing, China) was used

to perform genomic DNA extraction, and the process includes enzymatic lysis and silica membrane-based purification. This method has two advantages: the operational compatibility with routine laboratory workflows and the reproducibility across a broad spectrum of Gram-negative isolates.

To ensure analytical validity of downstream PCR and genotyping assays, rigorous quality assessment was conducted on the extracted DNA. Researchers used NanoDrop 2000 system (Thermo Fisher Scientific, USA) to perform spectrophotometric analysis, and samples exhibiting A260/A280 ratios between the threshold of 1.8 and 2.0 were regarded acceptable. Moreover, the overall and comprehensive structure of DNA was identified and visualized via electrophoretic separation on a 1% agarose gel stained with ethidium bromide. To preserve data reliability and robustness, the samples showing visible shearing structure or excessive degradation were excluded from subsequent assays and molecular experiment.

All procedures were executed in compliance with biosafety level 2 protocols and under standard aseptic conditions to prevent cross-contamination.

Following DNA extraction and quality confirmation, targeted PCR assays were performed to detect blaCTX-M (universal, group 1, and group 9), blaSHV, blaTEM, plasmid-mediated AmpC beta-lactamase families (MOX, CIT, DHA, ACC, EBC, and FOX), carbapenemase genes (blaKPC, blaNDM, blaIMP, blaVIM, and blaOXA-48-like), and blaOXA-1-like. The complete primer sequences and expected amplicon sizes are provided in **Table 1**.

Unless otherwise specified, each singleplex PCR was performed in a final volume of 25 µL containing 12.5 µL of 2× Taq PCR MasterMix, 1.0 µL each of the forward and reverse primers (10 µmol/L; final concentration, 0.4 µmol/L each), 2.0 µL of genomic DNA (10-50 ng), and 8.5 µL of nuclease-free ddH₂O. For the six-plex pAmpC assay, 0.5 µL of each primer (10 µmol/L; final concentration, 0.2 µmol/L each) was added; the MasterMix and template volumes were unchanged, and nuclease-free water was adjusted to 4.5 µL. Thermal cycling was performed in a calibrated thermocycler using the protocols shown in **Table 2**.

Table 1. Summary of ESBL PCR primers sequences

Primer name		Sequence (5'→3')	Size of amplified fragment
CTX-M-U	F	ATGTGCAGYACCAGTAARGTKATGGC	593 bp
	R	TGGGTRAARTARGTSACCAGAAYCAGCGG	
CTX-M-G1	F	AAAAATCACTGCGCCAGTTC	415 bp
	R	AGCTTATTCATCGCCACGTT	
CTX-M-G9	F	CAAAGAGAGTGCAACGGATG	205 bp
	R	ATTGGAAAGCGTTCATCACC	
SHV	F	GGGTTATTCTTATTGTGCGC	861 bp
	R	TTAGCGTTGCCAGTGCTC	
TEM	F	ATGAGTATTCAACATTCCGTGTCGCC	1080 bp
	R	CCAATGCTTAATCAGTGAGGCACC	
MOX	F	GCTGCTCAAGGAGCACAGGAT	520 bp
	R	CACATTGACATAGGTGTGGTGC	
CIT	F	TGGCCAGAACTGACAGGCAAA	462 bp
	R	TTTCTCCTGAACGTGGCTGGC	
DHA	F	AAC TTTCACAGGTGTGCTGGGT	405 bp
	R	CCGTACGCATACTGGCTTTGC	
ACC	F	AACAGCCTCAGCAGCCGGTTA	346 bp
	R	TCGCCGCAATCATCCCTAGC	
EBC	F	TCGGTAAAGCCGATGTTGCGG	302 bp
	R	CTTCCACTGCGGCTGCCAGTT	
FOX	F	AACATGGGGTATCAGGGAGATG	190 bp
	R	CAAAGCGCGTAACCGATTGG	
KPC	F	ATGCACTGTATCGCCGTCT	893 bp
	R	TTTTCAGAGCCTTACTGCCC	
NDM	F	GGTTTGGCGATCTGGTTTTC	621 bp
	R	CGGAATGGCTCATCACGATC	
IMP	F	GGAATAGAGTGCGTTAAYTCTC	232 bp
	R	GGTTTAAYAAAACAACCACC	
VIM	F	GATGGTGTTTGGTCGCATA	390 bp
	R	CGAATGCGCAGCACCAG	
OXA1	F	GGCACCAGATTTTGAAGC	813 bp
	R	GAAAGCACACCTTGGTTCAG	
OXA48	F	GCGTGTTAAGGATGAACAC	438 bp
	R	CATCAAGTTCAACCCAACCG	

Note: PCR, polymerase chain reaction; bp, base pair; IUPAC, International Union of Pure and Applied Chemistry; CTX-M, cefotaximase-Munich beta-lactamase; SHV, sulfhydryl variable beta-lactamase; TEM, Temoniera beta-lactamase; MOX, moxalactam-type Ambler class C beta-lactamase; CIT, Citrobacter freundii-type Ambler class C beta-lactamase; DHA, Dhahran Hospital-type Ambler class C beta-lactamase; ACC, Ambler class C beta-lactamase; EBC, Enterobacter cloacae-type Ambler class C beta-lactamase; FOX, cefoxitin-type Ambler class C beta-lactamase; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-beta-lactamase; IMP, imipenemase; VIM, Verona integron-encoded metallo-beta-lactamase; OXA, oxacillinase; pAmpC, plasmid-mediated Ambler class C beta-lactamase. IUPAC ambiguity codes: R = A/G, Y = C/T, K = G/T, and S = G/C.

Amplification products were verified by electrophoresis on 1.5% agarose gels containing 0.5 µg/mL ethidium bromide in 1× TAE buffer. Five

microliters of PCR product were mixed with 1 µL of 6× loading buffer, electrophoresed at 120 V for 30 min together with a 100 bp DNA ladder, and visualized under 302-nm ultraviolet illumination. Products showing a single band at the expected size were purified using a silica membrane-based PCR purification kit and subjected to bidirectional Sanger sequencing with the corresponding forward and reverse primers. Consensus sequences were compared with GenBank reference sequences using NCBI BLAST; an identity of at least 98% across the aligned amplicon was required for genotype assignment. Each run included an appropriate known positive control, a no-template control, and a DNA-extraction blank. Pre-PCR preparation, amplification, and post-PCR analysis were performed in physically separated areas to minimize cross-contamination.

Statistical analysis

Data were analyzed using SPSS version 22.0. Categorical variables were expressed as percentages and compared using the χ^2 test. The Cochran-Armitage trend test was used to assess the statistical significance of annual trends in resistance rates from 2022 to 2024. Continuous variables were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$); the independent samples t-test was used for normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data. Multicollinearity among independent variables in the multi-

variate logistic regression model was assessed using variance inflation factor (VIF), with VIF >10 indicating significant collinearity. ROC

Table 2. Thermal cycling protocols used for targeted PCR amplification

Assay group	Initial denaturation	Cycling conditions	Final extension
ESBL/OXA singleplex assays (CTX-M-U, CTX-M-G1, CTX-M-G9, SHV, TEM, OXA-1-like, and OXA-48-like)	95 °C for 5 min	35 cycles: 95 °C for 30 s; 55 °C for 30 s; 72 °C for 60 s	72 °C for 7 min
pAmpC six-plex assay (MOX, CIT, DHA, ACC, EBC, and FOX)	94 °C for 10 min	5 cycles: 94 °C for 30 s; 64 °C for 30 s; 72 °C for 60 s; followed by 30 cycles: 94 °C for 25 s; 60 °C for 30 s; 72 °C for 90 s	72 °C for 10 min
Carbapenemase singleplex assays (KPC, NDM, IMP, and VIM)	95 °C for 5 min	35 cycles: 95 °C for 30 s; 52 °C for 45 s; 72 °C for 45 s	72 °C for 10 min

Note: PCR, polymerase chain reaction; ESBL, extended-spectrum beta-lactamase; OXA, oxacillinase; CTX-M, cefotaximase-Munich beta-lactamase; SHV, sulfhydryl variable beta-lactamase; TEM, Temoniera beta-lactamase; pAmpC, plasmid-mediated Ambler class C beta-lactamase; MOX, moxalactam-type Ambler class C beta-lactamase; CIT, Citrobacter freundii-type Ambler class C beta-lactamase; DHA, Dhahran Hospital-type Ambler class C beta-lactamase; ACC, Ambler class C beta-lactamase; EBC, Enterobacter cloacae-type Ambler class C beta-lactamase; FOX, cefoxitin-type Ambler class C beta-lactamase; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-beta-lactamase; IMP, imipenemase; VIM, Verona integron-encoded metallo-beta-lactamase; min, minutes; s, seconds. All reactions were held at 4 °C after final extension. The pAmpC assay used a two-stage annealing strategy to improve specificity during the first five cycles and analytical sensitivity during the subsequent 30 cycles.

curve analysis was employed to evaluate the diagnostic efficacy of different antibiotic regimens. The optimal cut-off values for continuous variables (age, BMI, and hospitalization duration) were determined using the maximum Youden index. To assess the stability of these post-hoc derived cut-off values, bootstrap resampling (1,000 iterations) was performed to generate 95% confidence intervals for the optimal thresholds. A two-sided *P*-value of <0.05 was considered statistically significant.

Results

Distribution of pathogenic bacteria and drug resistance profiles

Among 421 patients, 465 bacterial strains were cultured, with 254 ESBL-EC isolates identified. ESBL-EC was the predominant pathogen, and all MDR cases (*n*=44) were defined based on ESBL-EC resistance profiles. Gram-negative bacteria predominated, with *Escherichia coli* and *Proteus mirabilis* most frequently identified, while *Enterococcus faecalis* and *Staphylococcus epidermidis* were the main gram-positive organisms (Table 3). Resistance rates among ESBL-EC isolates were highest for ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole, and ceftriaxone, whereas susceptibility to cefoperazone/sulbactam, piperacillin/tazobactam, and amikacin remained high (Table 4).

Genomic characterization of ESBL-EC bacteria

Among the 254 ESBL-EC isolates, acquired cephalosporinases (42.13%) and penicillinases

(40.94%) were the most frequently detected genotypes, with PASE+CASE accounting for 36.61% of all isolates - representing the dominant genotype combination. CTX-M, the most prevalent ESBL type, was detected in only 7.87% of isolates. Carbapenemase-associated genotypes were rare (3.54% +OR-ESBL, 1.18% +ESBL OR+HL AmpC), and OXA-type enzyme inhibitor resistance was observed in 10.24% (Table 5).

Phenotypic analysis of drug resistance in multi-drug resistant and non-multi-drug-resistant groups

The MDR group (*n*=44) exhibited significantly higher resistance rates than the non-MDR group across all antibiotic categories: quinolones (93.18% vs. 52.25%), ultra-broad-spectrum cephalosporins (63.64% vs. 50.40%), aminoglycosides (63.64% vs. 8.22%), and sulfonamides (56.82% vs. 24.14%) (Table 6). Notably, no carbapenem resistance was detected in either group, though 18 carbapenem-resistant strains were observed in the non-MDR group.

Comparison of clinical factors between the two groups

A detailed comparison of clinical factors between the MDR and non-MDR groups revealed statistically significant differences in several factors, including age, body mass index (BMI), duration of hospitalization, prior history of urinary tract infections (UTIs), history of antibiotic

Table 3. Analysis of the composition of pathogenic bacteria in UTI patients

Pathogenic bacteria	Number of strains	Composition ratio (%)
Gram-negative bacteria	290	62.37
ESBL-EC	254	54.62
<i>Proteus mirabilis</i>	21	4.52
<i>Pseudomonas aeruginosa</i>	5	1.08
<i>Enterobacter cloacae</i>	2	0.43
<i>Klebsiella pneumoniae</i>	2	0.43
<i>Enterobacter aerogenes</i>	1	0.22
<i>Achromobacter xylosoxidans</i> oxidizing subspecies	1	0.22
<i>Alcaligenes xylosoxidans</i> denitrifying subspecies	1	0.22
<i>Burkholderia cepacia</i>	1	0.22
<i>Moraxella catarrhalis</i>	1	0.22
Certain species of the genus <i>Pantoea</i>	1	0.22
Gram-positive bacteria	147	31.61
<i>Enterococcus faecalis</i>	48	10.32
<i>Staphylococcus epidermidis</i>	32	6.88
<i>Streptococcus agalactiae</i>	17	3.66
Gram-positive bacilli	13	2.80
<i>Staphylococcus hemolyticus</i>	9	1.94
<i>Staphylococcus aureus</i>	7	1.51
<i>Staphylococcus saprophyticus</i>	6	1.29
<i>Enterococcus faecalis</i>	5	1.08
<i>Enterococcus casseliflavus</i>	1	0.22
<i>Enterococcus gallinarum</i>	1	0.22
<i>Staphylococcus sciuri</i>	1	0.22
<i>Staphylococcus lugdunensis</i>	1	0.22
<i>Staphylococcus warneri</i>	1	0.22
<i>Staphylococcus hominis</i> human subspecies	1	0.22
<i>Streptococcus viridans</i>	1	0.22
<i>Streptococcus agalactiae</i> (hemolytic)	1	0.22
<i>Streptococcus mitis</i>	1	0.22
<i>Streptococcus thoraltensis</i>	1	0.22
Fungus	28	6.02
<i>Candida albicans</i>	21	4.52
<i>Candida glabrata</i>	5	1.08
<i>Torulopsis glabrata</i>	1	0.22
Other <i>Candida</i>	1	0.22
Total	465	100

Note: UTI, urinary tract infection; ESBL-EC, extended-spectrum β -lactamase-producing *Escherichia coli*.

use, and presence of diabetes ($P < 0.05$). These differences are summarized in **Table 7**.

Effect of clinical factors on drug resistance

Multivariate logistic regression identified age ≥ 54 years, BMI ≥ 22.5 kg/m², hospitalization ≥ 38.5 days, prior UTI, prior antibiotic use, and diabetes as independent risk factors for MDR

($P < 0.05$) (**Tables 8, 9**). VIF values were below 2.5 for all variables, excluding significant collinearity. ROC-derived optimal cut-offs (maximum Youden index) were age ≥ 54.0 years, BMI ≥ 22.5 kg/m², and hospitalization ≥ 38.5 days; bootstrap validation (1,000 resamples) confirmed internal stability (95% CI: age, 53.2-55.1; BMI, 22.1-23.0; hospitalization, 37.8-39.4).

Table 4. Analysis of resistance of ESBL-EC to common antimicrobial drugs

Antimicrobial drugs	Number of resistant strains	Resistance rate (%)
Cefepime	9	3.54
Ceftazidime	25	9.84
Cephazolin	96	37.80
Ceftriaxone	76	31.02
Cefoperazone/Sulbactam	0	0.00
Ampicillin	182	71.65
Piperacillin and Tazobactam	2	0.79
Gentamicin	57	22.44
Tobramycin	19	7.48
Ciprofloxacin	97	38.19
Levofloxacin	75	29.53
Amikacin	3	1.18
Trimethoprim-sulfamethoxazole	89	35.04
Imipenem	5	1.97

Note: ESBL-EC, extended-spectrum β -lactamase-producing *Escherichia coli*.

Emergence and trends of multi-drug-resistant ESBL-EC

ESBL-EC isolates increased from 62 (2022) to 98 (2024). Resistance to ultra-broad-spectrum cephalosporins showed the most pronounced increase (45.16% to 55.10%), followed by quinolones (48.39% to 55.10%) and aminoglycosides (17.74% to 25.51%) (**Table 11**). The Cochran-Armitage trend test confirmed significant upward trends for cephalosporins ($\chi^2=6.810$, $P=0.009$), quinolones ($\chi^2=4.520$, $P=0.034$), and aminoglycosides ($\chi^2=4.010$, $P=0.045$), but not for sulfonamides ($P=0.144$) (**Figure 1**). Pooled data across the study period showed 100% susceptibility to carbapenems and consistently high susceptibility to cefoperazone/sulbactam (95.3%) and piperacillin/tazobactam (98.1%).

Relationship between antibiotic efficacy and clinical therapeutic effects

ROC curve analysis evaluated the predictive efficacy of antibiotic regimens for clinical recovery. Aminoglycosides showed the highest AUC among monotherapies (0.806), followed by ultra-broad-spectrum cephalosporins (0.785), quinolones (0.742), and sulfonamides (0.687). Notably, the aminoglycoside-cephalosporin combination achieved the highest AUC (0.872), significantly outperforming all monotherapies ($P<0.05$) (**Table 10**; **Figure 2**).

Analysis of the rationality of antibiotic use

In this study, UTI patients were categorized into acute and chronic infection groups based on their clinical conditions. The association between the rationality of antibiotic use and the risk of drug resistance was assessed across five dimensions: regimen standardization, precise drug selection, combination drug strategy, antibiotic rotation, and resistance monitoring/intervention. The results indicated that patients with acute infections had better adherence to precise drug selection and combination strategies, while patients with chronic infections

exhibited more favorable outcomes in terms of antibiotic rotation and resistance monitoring (**Figure 3**).

Discussion

The increasing prevalence of ESBL-producing *E. coli* has substantially complicated the treatment of UTIs by limiting the effectiveness of commonly used antibiotics [13, 14]. Previous antibiotic exposure, advanced age, chronic diseases, and prolonged hospitalization are established contributors to ESBL infection and multi-drug resistance [15, 16]. However, resistance phenotypes and β -lactamase genotypes vary considerably among regions. The present study therefore provides locally relevant evidence from Kunshan, a highly urbanized county-level city characterized by substantial population mobility, frequent intercity healthcare contact, and heterogeneous antibiotic exposure. These local conditions may facilitate both antimicrobial selection pressure and the circulation of mobile resistance determinants.

Consistent with previous international surveillance reports [17-19], our data showed a year-on-year increasing trend in resistance to broad-spectrum cephalosporins, quinolones, and sulfonamides from 2022 to 2024. Nevertheless, the genotype distribution in our cohort differed markedly from the CTX-M-dominant pattern

Table 5. ESBL-EC gene detection results

ESBLs	Genotype	Strains (n=254)	Composition rate
ESBL		42	16.54
	CTX-M	20	7.87
	SHV	1	0.39
Cephalosporinase		107	42.13
	Acquired PASE+CASE	93	36.61
Carbapenemases	+OR-ESBL	9	3.54
	+ESBL OR+HL AmpC	3	1.18
Penicillinase	Acquired Penicillinase	104	40.94
Enzyme Inhibitor Resistance	OXA	26	10.24

Note: ESBL-EC, extended-spectrum β -lactamase-producing *Escherichia coli*; ESBL, extended-spectrum β -lactamase; SHV, sulfhydryl variable; CTX-M, cefotaximase-Munich; OXA, oxacillinase; PASE, plasmid-encoded AmpC-type cephalosporinase; CASE, cephalosporinase; AmpC, ampicillinase C; HL AmpC, hyperproduced AmpC.

Table 6. Phenotypic analysis of drug resistance in the two groups

Antimicrobial resistance phenotypes	Non-MDR group (n=377)	MDR group (n=44)
Aminoglycosides	31	28
Ultra-broad-spectrum cephalosporins	190	28
Quinolones	197	41
Sulfonamides	91	25
Carbapenems	18	0

Note: MDR, multidrug resistance.

commonly reported in the literature [20-22]. CTX-M accounted for only 7.87% of isolates, whereas acquired cephalosporinases and penicillinases accounted for 42.13% and 40.94%, respectively. Most notably, PASE+CASE was detected in 36.61% of isolates, representing the dominant genotype combination in this cohort. Although CTX-M variants remained associated with third-generation cephalosporin resistance [20], their relatively low frequency contrasts with the 50%-70% predominance reported in other Chinese UTI populations [21, 22].

This high prevalence of PASE+CASE may reflect a distinct local resistance ecology rather than a simple extension of national or global ESBL trends. Kunshan is closely integrated into the Yangtze River Delta through commuting, patient referral, and healthcare exchange, yet its genotype landscape appears to diverge from patterns described in other settings. Possible explanations for this predominance include differences in local prescribing practices, transmission of specific resistance plasmids, and repeated patient movement between community and hospital settings. However, MLST and

plasmid typing were not performed; therefore, we could not determine whether this pattern resulted from clonal expansion, horizontal plasmid transmission, or independent acquisition events. The absence of these data also prevented reliable tracing of transmission among patients, wards, hospitals, and study years.

These interpretations should therefore be regarded as epidemiological hypotheses requiring confirmation by whole-genome sequencing and plasmid analysis.

Phenotypically, the isolates showed high resistance to ampicillin and ceftriaxone but retained excellent susceptibility to cefoperazone/sulbactam and piperacillin/tazobactam. MDR isolates also showed substantially higher resistance to aminoglycosides and quinolones than non-MDR isolates. These findings indicate that empirical treatment based solely on conventional cephalosporins or fluoroquinolones may be unreliable in Kunshan. In clinically stable patients, β -lactam/ β -lactamase inhibitor combinations may therefore be considered when supported by local susceptibility results, while carbapenems should be reserved for severe infection or confirmed resistance.

Age ≥ 54 years, BMI ≥ 22.5 kg/m², hospitalization ≥ 38.5 days, previous UTI, prior antibiotic exposure, and diabetes were independently associated with MDR. These findings are consistent with previous studies [23-25], but their combination provides a practical local frame-

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Table 7. Comparison of clinical factors between the two groups of patients

Clinical factors	Non-MDR group (n=377)	MDR group (n=44)	χ^2/t	P
Age ($\bar{x} \pm s$, years)	44.28 $\pm$ 10.52	51.91 $\pm$ 15.98	4.273	<0.001
BMI ($\bar{x} \pm s$, kg/m ²)	22.38 $\pm$ 1.78	23.19 $\pm$ 1.73	3.149	0.005
Hospitalization duration ($\bar{x} \pm s$, d)	37.11 $\pm$ 1.38	40.11 $\pm$ 1.85	13.171	<0.001
Gender [n (%)]			2.521	0.112
Male	142 (37.67)	22 (50.00)		
Female	235 (62.33)	22 (50.00)		
Prior UTI history [n (%)]			18.567	<0.001
Yes	124 (32.89)	29 (65.91)		
No	253 (67.11)	15 (34.09)		
Antibiotic use history [n (%)]			23.611	<0.001
Yes	98 (25.99)	27 (61.36)		
No	279 (74.01)	17 (38.64)		
Smoking history [n (%)]			0.558	0.455
Yes	84 (22.28)	12 (27.27)		
No	293 (77.72)	32 (72.73)		
History of alcohol intake [n (%)]			0.203	0.652
Yes	116 (30.77)	15 (34.09)		
No	261 (69.23)	29 (65.91)		
Cerebrovascular disease [n (%)]			0.029	0.865
Yes	48 (12.73)	6 (13.64)		
No	329 (87.27)	38 (86.36)		
Hypertension [n (%)]			1.662	0.197
Yes	126 (33.42)	19 (43.18)		
No	251 (66.58)	25 (56.82)		
Cardiovascular disease [n (%)]			0.988	0.320
Yes	41 (10.88)	7 (15.91)		
No	336 (89.12)	37 (84.09)		
Diabetes [n (%)]			7.038	0.008
Yes	129 (34.22)	24 (54.55)		
No	248 (65.78)	20 (45.45)		

Note: MDR, multidrug resistance; UTI, urinary tract infection; BMI, body mass index; SD, standard deviation. Data are presented as mean $\pm$ SD or n (%).

Table 8. Assignment of clinical factor variables

Influence factors	The assignment of value
Age (years)	<54.0=0, $\geq$ 54.0=1
BMI (kg/m ²)	<22.5=0, $\geq$ 22.5=1
Hospitalization duration (d)	<38.5=0, $\geq$ 38.5=1
Prior UTI history	No = 0, Yes = 1
Antibiotic use history	No = 0, Yes = 1
Diabetes	No = 0, Yes = 1

Note: UTI, urinary tract infection; BMI, body mass index.

work for early risk stratification. Diabetes showed the strongest association with MDR (OR=7.985), possibly because of impaired host defense, recurrent urinary infection, and re-

peated healthcare exposure [26, 27]. Patients with these characteristics may benefit from earlier culture testing, closer infection-control precautions, and empirical regimens selected according to the local antibiogram.

The ROC analysis showed that aminoglycosides had the highest predictive performance among single-drug categories, whereas the aminoglycoside-cephalosporin combination produced the highest AUC (0.872), sensitivity (0.933), and specificity (0.847) [28].

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Table 9. Multivariate logistic regression analysis of the effect of clinical factors on drug resistance

Influence factors	β	SE	Wald χ^2	P	OR (95% CI)
Age	1.150	0.426	7.299	0.007	3.159 (1.371-7.276)
BMI	1.058	0.381	7.715	0.005	2.880 (1.365-6.074)
Hospitalization duration	1.312	0.617	4.512	0.034	3.712 (1.107-7.449)
Prior UTI history	0.902	0.405	4.507	0.034	2.464 (1.072-5.664)
Antibiotic use history	0.779	0.289	7.273	0.007	2.178 (1.237-3.836)
Diabetes	2.078	0.922	5.077	0.024	7.985 (1.311-8.645)

Note: MDR, multidrug resistance; UTI, urinary tract infection; BMI, body mass index; OR, odds ratio; CI, confidence interval; SE, standard error.

Table 10. Diagnostic efficacy of different antibiotics in treating UTI patients

Indicators	Cutoff value	AUC	95% CI	Susceptibility	Specificity	Youden index
Aminoglycosides	4 $\mu\text{g/mL}$	0.806	0.748-0.834	0.806	0.754	0.560
Ultra-broad-spectrum cephalosporins	2 $\mu\text{g/mL}$	0.785	0.741-0.826	0.789	0.716	0.505
Quinolones	1 $\mu\text{g/mL}$	0.742	0.712-0.783	0.725	0.694	0.419
Sulfonamides	76 $\mu\text{g/mL}$	0.687	0.654-0.706	0.658	0.527	0.285
Combined regimen	-	0.872	0.851-0.924	0.933	0.847	0.780

Note: UTI, urinary tract infection; AUC, area under the curve; CI, confidence interval.

Table 11. Annual distribution and resistance rates of ESBL-EC isolates (2022-2024)

Year	No. of ESBL-EC isolates	Ultra-broad-spectrum cephalosporins resistance rate (%)	Quinolones resistance rate (%)	Aminoglycosides resistance rate (%)	Sulfonamides resistance rate (%)
2022	62	45.16	48.39	17.74	30.65
2023	94	51.06	53.19	21.28	35.11
2024	98	55.10	55.10	25.51	38.78
χ^2 for trend		6.810	4.520	4.010	2.130
P for trend		0.009	0.034	0.045	0.144

Note: ESBL-EC, extended-spectrum β -lactamase-producing *Escherichia coli*. P values were calculated using the Cochran-Armitage trend test.

However, complete quantitative MIC testing and checkerboard assays were not performed. Thus, the observed association cannot establish in vitro synergy or therapeutic superiority. It may reflect true drug interaction, the activity of at least one agent, or treatment-selection bias. Future studies should include broth microdilution MIC testing and fractional inhibitory concentration index analysis to confirm the activity of this combination.

The apparent paradox that MDR isolates exhibited high aminoglycoside resistance (63.64%) yet the aminoglycoside-cephalosporin combination showed the highest predictive efficacy (AUC=0.872) warrants mechanistic consideration. First, this combination ensures broader

coverage across different resistance genotypes: 36.36% of MDR isolates remained susceptible to aminoglycosides, and a substantial proportion retained susceptibility to cephalosporins, thereby increasing the probability that at least one agent remains active against the infecting strain. Second, the local genotype landscape - dominated by PASE+CASE (36.61%) rather than CTX-M - may render the combination particularly effective, as cephalosporins provide activity against non-ESBL strains while aminoglycosides cover CTX-M-positive isolates. Third, partial synergistic interactions between aminoglycosides and β -lactams, mediated by enhanced bacterial cell wall penetration, may contribute to the observed efficacy even when resistance to one component is present.

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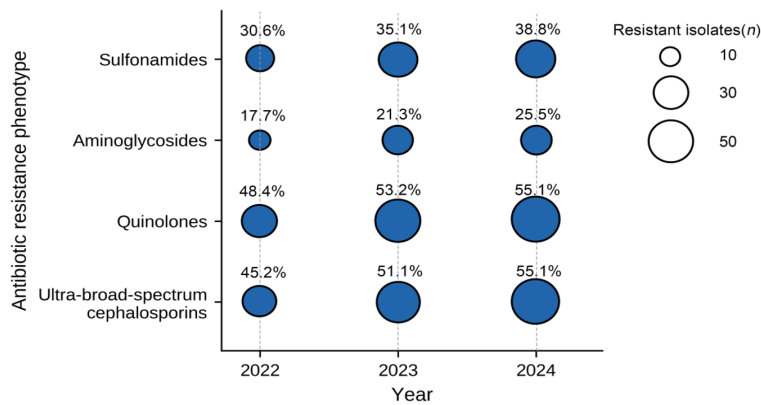


Figure 1. Trends in the emergence of antibiotic resistance in *Escherichia coli* strains over time (2022-2024). The bubble plot shows resistance to sulfonamides, aminoglycosides, quinolones, and ultra-broad-spectrum cephalosporins. Bubble size represents the number of resistant isolates, with larger bubbles indicating greater numbers, while the percentages above the bubbles indicate the corresponding resistance rates. Resistance rates increased across all four antibiotic categories during the study period: from 30.6% to 38.8% for sulfonamides, from 17.7% to 25.5% for aminoglycosides, from 48.4% to 55.1% for quinolones, and from 45.2% to 55.1% for ultra-broad-spectrum cephalosporins. Quinolones and ultra-broad-spectrum cephalosporins exhibited the highest resistance rates in 2024.

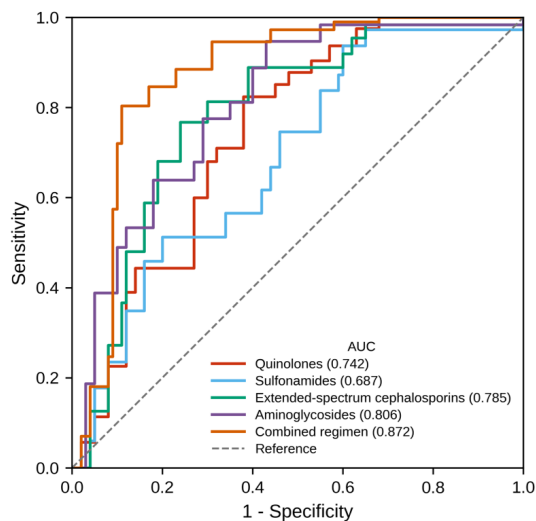


Figure 2. Receiver Operating Characteristic (ROC) curves for antibiotic therapies in predicting patient recovery. The receiver operating characteristic (ROC) curves compare the discriminatory performance of quinolones, sulfonamides, extended-spectrum cephalosporins, aminoglycosides, and a combined antibiotic regimen in predicting recovery among patients with ESBL-producing *Escherichia coli* infections. The corresponding areas under the curve (AUCs) were 0.742 for quinolones, 0.687 for sulfonamides, 0.785 for extended-spectrum cephalosporins, 0.806 for aminoglycosides, and 0.872 for the combined regimen. The combined regimen demonstrated the highest overall predictive performance, whereas sulfonamides showed the lowest. The gray dashed diagonal line represents chance-level discrimination.

However, without chequerboard synergy data, this synergy remains hypothetical. Alternatively, the ROC analysis may reflect that patients receiving combination therapy had earlier culture-directed treatment or more intensive clinical monitoring, rather than purely pharmacodynamic superiority.

Although no carbapenem resistance was detected among MDR isolates, unnecessary carbapenem exposure may accelerate the emergence of carbapenem-resistant organisms [29]. The findings therefore support a locally adapted, carbapenem-sparing approach based on susceptibility testing and patient risk. Overall, the predominance of PASE+CASE and the low CTX-M frequency

distinguish Kunshan from the genotype patterns reported elsewhere in China [21, 22, 30]. County-level surveillance should therefore complement national datasets, particularly within the highly interconnected Yangtze River Delta. Future multicenter studies comparing Kunshan with neighboring county-level cities and incorporating whole-genome sequencing, plasmid typing, and antibiotic-use data are needed to determine whether this genotype landscape reflects local clonal expansion, plasmid-mediated dissemination, or differences in antimicrobial selection pressure.

Limitations

This study was restricted to two hospitals in Kunshan, which may limit generalizability. Genetic analysis was based on targeted PCR and Sanger sequencing. MLST and plasmid typing were not conducted because of the retrospective design, incomplete preservation of viable isolates, and limited laboratory resources prevented supplementary testing. Consequently, clonal relationships, plasmid dissemination, and transmission pathways could not be determined.

Complete quantitative MIC testing and antimicrobial chequerboard assays were also unavailable. Therefore, MIC distributions, MIC50/

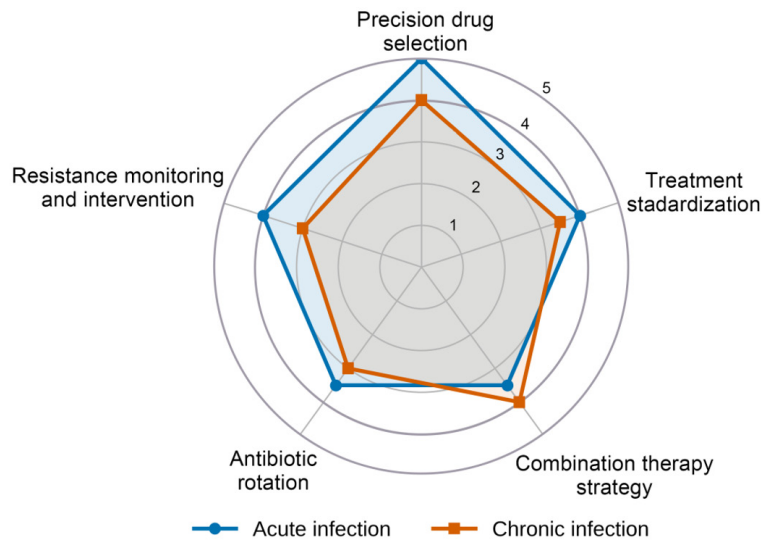


Figure 3. Comparison of antibiotic use strategies in acute versus chronic urinary tract infections. The radar chart compares acute and chronic urinary tract infections across five aspects of antibiotic use: precision drug selection, treatment standardization, combination therapy strategy, antibiotic rotation, and resistance monitoring and intervention. Scores are presented on a scale from 1 to 5, with higher values indicating greater implementation of the corresponding strategy. Acute infections received higher scores for precision drug selection, treatment standardization, antibiotic rotation, and resistance monitoring and intervention, whereas chronic infections received a higher score for combination therapy strategy. Blue circles and orange squares represent acute and chronic infections, respectively.

MIC90 values, and in vitro synergy or antagonism could not be assessed. The higher AUC observed for combination therapy should consequently be interpreted as an observational clinical association rather than experimental evidence of synergistic efficacy.

The optimal cut-off values for age, BMI, and hospitalization duration were derived post-hoc using ROC analysis with maximum Youden index. Although bootstrap resampling confirmed internal stability, these thresholds were optimized within this specific cohort and carry an inherent risk of overfitting. Consequently, they should be interpreted as hypothesis-generating rather than definitive clinical decision boundaries, and external validation in independent prospective cohorts is needed before these cut-offs can be generalized to other populations or clinical settings.

Antibiotic exposure was recorded without detailed information on dose, duration, or route, and residual confounding cannot be excluded. The retrospective design also introduced potential selection and information bias. Future prospective studies should preserve iso-

lates and incorporate MLST, plasmid typing, whole-genome sequencing, MIC testing, and combination assays. Multi-center, longitudinal investigations incorporating pharmacokinetics, host-pathogen interactions, and molecular epidemiology are further needed to reduce these limitations.

Conclusion

This study identified a distinctive ESBL-EC profile in Kunshan, characterized by a high prevalence of PASE+CASE and several clinical predictors of MDR. The aminoglycoside-cephalosporin regimen was associated with recovery, but its superiority and synergistic activity could not be confirmed because quantitative MIC and checkerboard data were unavailable. Similarly, the absence of MLST and plasmid typing prevented determination of whether the local genotype pattern reflect-

ed clonal spread or plasmid-mediated transmission. These findings support local risk stratification and susceptibility-guided therapy while highlighting the need for prospective molecular and pharmacodynamic validation.

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Disclosure of conflict of interest

None.

Abbreviations

ALT, Alanine Aminotransferase; AST, Antimicrobial Susceptibility Testing; AUC, Area Under the Curve; API, Analytical Profile Index; BMI, Body Mass Index; CASE, Chromosomally Encoded AmpC β -lactamase; CFU, Colony-Forming Unit; CLSI, Clinical and Laboratory Standards

Institute; CTX-M, Cefotaximase-Munich (a family of ESBL genes); DNA, Deoxyribonucleic Acid; EC, *Escherichia coli*; ESBL, Extended-Spectrum Beta-Lactamase; ESBL-EC, Extended-Spectrum Beta-Lactamase-producing *Escherichia coli*; HL AmpC, High-Level AmpC β -lactamase; IRB, Institutional Review Board; LB, Luria-Bertani (broth medium); MDR, Multidrug Resistance; MH, Mueller-Hinton (agar); OR, Odds Ratio; OXA, Oxacillinase (class D β -lactamase); PASE, Plasmid-Associated AmpC β -lactamase; PCR, Polymerase Chain Reaction; ROC, Receiver Operating Characteristic; SD, Standard Deviation; SDA, Sabouraud Dextrose Agar; UTI, Urinary Tract Infection; VITEK, VITEK® 2 Compact Automated Identification System.

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