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Phenotypic Detection and Antimicrobial Resistance of ESBL-Producing Escherichia coli Clinical Isolates Using VITEK 2

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Abstract **Objectives:** The public health threat of extended-spectrum \beta-lactamase (ESBL)-producing Escherichia coli continues to escalate, driven by rising resistance to common antibiotics and a dwindling pool of effective treatments. This laboratory-based cross-sectional study was designed to evaluate the phenotypic prevalence and antimicrobial resistance profiles of these clinical isolates using the automated VITEK 2 system. **Methods**: Over a two-month period (from February 18 to April 15, 2026), we collected and analyzed 75 unique clinical isolates of *E. coli* using the VITEK 2 platform integrated with the Advanced Expert System (AES). All statistical computations, including Pearson's chi-square and Fisher's exact tests, were carried out using IBM SPSS Statistics (v32), with significance set at $P < 0.05$. **Results**: Of the 75 isolates evaluated, a striking 89.3% (67 isolates) were confirmed as ESBL producers. The vast majority of these samples originated from urine specimens (86.7%, 65/75). We observed that ESBL-positive strains carried significantly higher resistance rates to several key antibiotics, including piperacillin/tazobactam ($P = 0.022$), cefotaxime ($P < 0.001$), ceftazidime ($P < 0.001$), cefepime ($P = 0.007$), and ciprofloxacin ($P = 0.003$). Additionally, multidrug resistance (MDR) was highly prevalent, affecting 66.7% (50/75) of the isolates. On a positive note, imipenem, meropenem, and amikacin maintained excellent in vitro efficacy against almost all tested strains. **Conclusion**: Our findings highlight a worrying prevalence of both phenotypic ESBL production and multidrug resistance among local clinical isolates of *E. coli*. While carbapenems and amikacin remain highly reliable therapeutic options, these results must be interpreted cautiously. The study's scope was bounded by certain limitations, such as a relatively small sample size, a heavy reliance on urine samples, geographical restriction to a single region, and the lack of molecular assays to identify specific ESBL resistance genes.

Key Words Extended-spectrum beta-lactamase, Enterobacterales, Antimicrobial susceptibility testing, Multidrug resistance, Clinical isolates, Iraq

INTRODUCTION

Escherichia coli is the most predominant Gram-negative bacteria that is associated with diverse infections, including urinary tract infections (UTIs), bloodstream infections, wound infections, and intra-abdominal infections [1,2]. Extraintestinal pathogenic Escherichia coli (ExPEC) contributes to various infections due to the presence of several virulence factors, which help its adherent properties [3]. The constant emergence of antibiotic-resistant *E. coli* strains, particularly those producing extended-spectrum β-lactamases (ESBLs) is a problem which should be counteracted nowadays as it limits the therapeutic choices, prolongs the duration of hospitalization, raises the expenditures of healthcare, and increases morbidity and mortality rates worldwide [4,5].

Extended-spectrum β-lactamases (ESBLs) are enzymes that can cleave penicillins, extended-spectrum cephalosporins, and monobactams, thus impairing the action of these antimicrobial drugs. Nevertheless, ESBLs' actions are inhibited in the presence of β-lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam [5,6]. The principal ESBL families are represented by CTX-M, TEM, and SHV, which are mostly found on the transferable plasmids, which are capable of spreading germs quickly among Enterobacterales in terms of antimicrobial resistance. Besides, these plasmids carry extra genes that give rise to resistance

against aminoglycosides, fluoroquinolones, and sulfonamides and contribute to the emergence of multidrug-resistant (MDR) phenotype [7-10]. The issue is aggravated by the global spread of *E. coli*, which creates significant problems for clinical microbiology and health care systems since this bacterium is strongly linked to treatment failures, prolongation of hospital stay, and increased mortality [11,12].

Hence proper early screening as well as the accurate phenotype definition of ESBL production is essential in avoiding inappropriate empirical treatment and to control horizontal transmission of these resistant characteristics in wards of hospitals [13]. Although the DDST and CDT methods are both recommended by reference guidelines and very reliable the procedures for both testing methods are manual and require an incubation time of as much as 24 hours delaying essential clinical decisions [14, 15]. To remove these bottlenecks in the diagnostic process, modern clinical microbiology laboratories have utilized computerized automated susceptibility platforms such as the VITEK 2 system (bioMérieux) [16]. The VITEK 2 system provides an effective and reliable method of assessing the growth rate of the isolates against cephalosporin and cephalosporin + clavulanic acid(s) to obtain an accurate phenotype for detecting the presence of ESBL organisms and resistance mechanisms in hours; therefore, there is a significant improvement in infection control practice and antimicrobials stewardship due to the integration of the Advanced Expert System (AES) with the VITEK 2 system [17, 18].

The worldwide dissemination of Escherichia coli that produces extended-spectrum beta-lactamases (ESBLs) has emerged to be a significant concern to public health since these bacteria are linked with resistance to multiple drugs and have fewer treatments available. Increasing occurrence of bacteria that produce ESBLs has been a main contributing factor to treatment failure, prolonged hospitalization and raise of costs associated with treatment. Recent global surveys show that antimicrobial resistance is among the main cause of death associated with infections in humans and require more full time monitoring and application of effective treatment programs [19,20].In Iraq, getting current information on ESBL-producing *E. coli* is especially significant because of regional differences in antimicrobial resistance patterns, differences in how antibiotics are used, and the need to direct local empirical treatment and infection control guidelines.

Regardless, despite the growing incidence of ESBL-producing Escherichia coli, continuous tracking of antibiotic resistance is necessary, particularly on the regional level. Prior research in Iraq indicates the detection of ESBL-producing *E. coli* in clinical samples; however, there are still very few updated reports that include a typing and antibiotic susceptibility profile using automated methods like VITEK 2. Consequently, more studies are needed to provide current data regarding epidemiology and to support rational usage of antibiotics and infection control efforts [21,22]. Hence, this study sought to phenotypically identify and characterize the particular antimicrobial resistance profiles of clinical isolates of ESBL-producing Escherichia coli via the automated VITEK 2 system in order to expand regional epidemiological databases and to facilitate targeted prescribing guidelines.

Objectives of the Study

The primary objectives are:

To phenotypically distinguish between the clinical isolates of Escherichia coli that produced the extended-spectrum beta-lactamase (ESBL) enzyme, along with testing their antibacterial susceptibility patterns by making use of the automated system called VITEK® 2.

The secondary objectives are:

- To find out the prevalence of ESBL-producing *E. coli* clinical isolates found in the study population.
- To analyze the different kinds of antimicrobial susceptibility patterns of the ESBL-producing *E. coli*.
- To find out whether the VITEK® 2 Advanced Expert System (AES) is efficient in interpreting antimicrobial susceptibility data.
- To provide evidence for local epidemiological data such that it can become useful in the effective management of the antimicrobial molecule.

METHODS

Ethical Considerations

The research was conducted using anonymous *E. coli* clinical isolates obtained from ordinary laboratory samples. It was not related to direct patient care, identifying patient data, or any identifiable information about patients.

Thus, ethical approval and informed consent were not required as per institutional protocol as this was an anonymous laboratory research.

Collection of Samples and Population Studied

The investigators confirmed cases of clinical Escherichia coli between 18 February and 15 April 2026. A total of seventy-five clinical isolates were collected from various hospitals and central diagnostic laboratories that provided clinical services. The specimens included urine, stool, wound swabs, and high vaginal swabs (HVS). The specimens were transported to the laboratory in sterile containers and processed in line with the requirements of the Clinical and Laboratory Standards Institute (CLSI)[23].The means of obtaining clinical isolates was the convenience sampling technique. Non-repeating Escherichia coli isolates were collected used for sampling purposes in this study.

- **Inclusion criteria:** The confirmed amounts of the *E. coli* isolates collected from multiple clinical samples, involved urine, stool samples, wound swabs, and

high vaginal swabs (HVS) were collected from 18 February 2026 to 15 April 2026. Only those isolates were selected that were completely identified.

- **Exclusion criteria:** Samples with contamination, mixed bacterial culture, duplicate isolates, and isolates with incomplete identification or missing results of susceptibility tests were not taken into consideration when conducting the study.

Identification and Characterization of the Bacteria

The isolates were first cultured on standard media for identification and characterization. Identification of *E. coli* isolates was performed using the VITEK® 2 identification system (bioMérieux, Marcy-l'Étoile, France). Identification cards were prepared and processed according to the manufacturer's instructions. The VITEK® 2 system identified the isolates with confidence levels ranging from 87% to 99%.

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing was performed using the VITEK® 2 automated system to determine the minimum inhibitory concentrations (MICs). The tested antimicrobial agents included beta-lactam/beta-lactamase inhibitor combinations (Ampicillin/Sulbactam and Piperacillin/Tazobactam), cephalosporins (Cefotaxime, Ceftazidime, and Cefepime), carbapenems (Imipenem and Meropenem), aminoglycosides (Amikacin and Gentamicin), fluoroquinolones (Ciprofloxacin), Trimethoprim/Sulfamethoxazole, and Colistin.

The antimicrobial susceptibility profiles were automatically analyzed using the VITEK® 2 Advanced Expert System (AES) and interpreted according to CLSI standards. The results were categorized as Susceptible (S), Intermediate (I), Resistant (R), or Susceptible-Dose Dependent (SDD), when applicable.

Quality Control

The quality control procedures for VITEK® 2 identification and antimicrobial susceptibility testing were performed according to the manufacturer's recommendations and Clinical and Laboratory Standards Institute (CLSI) guidelines. Reference strains were used to verify the accuracy and reliability of the identification and antimicrobial susceptibility results.

Statistical Analysis

Frequency and percentages were used to summarize categorical data. Antimicrobial susceptibility results were interpreted according to the VITEK® 2 AST outputs and reported as Susceptible (S), Intermediate (I), Resistant (R), and Susceptible-Dose Dependent (SDD) when applicable. Pearson's chi-square test or Fisher's exact test was used for categorical comparisons, and the odds ratio (OR) was reported when Fisher's exact test was applied. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics version 32, and graphical representations were prepared using GraphPad Prism version 11.0.2. This study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies.

RESULTS

Table 1 presents the demographic and clinical characteristics of the study population. A total of 75 Escherichia coli isolates were included in the analysis. Urine was the predominant sample source, accounting for 86.7% of isolates, while the AES confidence was reported as consistent in 90.7% of cases. Additionally, 81.3% of the reports did not include a susceptible-dose dependent (SDD) note.

Among the 75 *E. coli* isolates included in this study, 67 (89.3%) were classified as phenotypic ESBL producers, while 8 (10.7%) were classified as non-ESBL isolates according to the VITEK® 2 AES interpretation. The high proportion of ESBL-producing isolates indicates a high prevalence of ESBL-associated resistance phenotypes among the studied *E. coli* isolates (Table 2).

Among all isolates and ESBL-positive isolates, urine was the most frequent sample source. The distribution of ESBL-positive isolates did not show a significant difference according to sample source (urine vs. non-urine) ($p = 0.587$). However, this comparison should be interpreted with caution due to the limited number of non-urine isolates (Table 3).

The susceptibility profile revealed high levels of resistance to several beta-lactam and cephalosporin agents, particularly ampicillin, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, and cefepime. A clinically significant resistance rate was also observed for ciprofloxacin. In contrast, imipenem, meropenem, amikacin, ceftazidime/avibactam, and ceftolozane/tazobactam remained highly active in vitro against the majority of isolates (Table 4).

A total of 75 Escherichia coli isolates identified by the VITEK® 2 system were included in this study. Urine was

Table 1: Demographic and Clinical Characteristics of the Study Population

Characteristic	Category	n	%
Total records / isolates	Microbiology chart reports	75	100.0
Organism	Escherichia coli	75	100.0
Sample source	urine	65	86.7
	Wound swab	4	5.3
	Stool	4	5.3
	HVS	2	2.7
AES confidence	Consistent	68	90.7
	Not recorded	7	9.3
AES/User modified flag	No/Not stated	51	68.0
	Yes	24	32.0
SDD note in report	No/Not stated	61	81.3
	Yes	14	18.7

AES: Advanced Expert System, HVS: High Vaginal Swab, SDD: Susceptible-Dose Dependent. "Not recorded" and "Not stated" indicate that the corresponding information was unavailable in the original VITEK® 2 report

Table 2: Frequency and Prevalence of ESBL-Producing Escherichia coli among Clinical Isolates

ESBL phenotype category	n	Total isolates (%)	Assessed isolates (%)
Positive	67	89.3	89.3
Negative	8	10.7	10.7
Not assessed	0	0.0	
Total	75	100.0	100.0

Table 3: Distribution of Escherichia coli Isolates by Sample Source and ESBL Status

Sample source	Total n (%)	ESBL-positive n (%)	ESBL-negative n (%)	Not assessed n (%)	OR by Fisher's exact test	p-value
urine	65 (86.7)	57 (87.7)	8 (12.3)	0 (0.0)	0.32	0.587
Wound swab	4 (5.3)	4 (100.0)	0 (0.0)	0 (0.0)		
Stool	4 (5.3)	4 (100.0)	0 (0.0)	0 (0.0)		
HVS	2 (2.7)	2 (100.0)	0 (0.0)	0 (0.0)		
Urine vs. non-urine	-	57 vs. 10	8 vs. 0	0 vs. 0		

Table 4. Antimicrobial Susceptibility Profile of Escherichia coli Isolates Detected by VITEK 2 System

Antimicrobial agent	Antibiotic class	Total isolates n	Tested n (%)	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	SDD n (%)	Not assessed n (%)
Ampicillin	Penicillin	75	17 (22.7)	0 (0.0)	0 (0.0)	17 (100.0)	0 (0.0)	58 (77.3)
Ampicillin/Sulbactam	Penicillin/β-lactamase inhibitor	75	58 (77.3)	12 (20.7)	6 (10.3)	40 (69.0)	0 (0.0)	17 (22.7)
Piperacillin/Tazobactam	Penicillin/β-lactamase inhibitor	75	75 (100.0)	47 (62.7)	0 (0.0)	28 (37.3)	0 (0.0)	0 (0.0)
Cefuroxime	Second-generation cephalosporin	75	14 (18.7)	0 (0.0)	0 (0.0)	14 (100.0)	0 (0.0)	61 (81.3)
Cefuroxime Axetil	Second-generation cephalosporin	75	14 (18.7)	0 (0.0)	0 (0.0)	14 (100.0)	0 (0.0)	61 (81.3)
Cefoxitin	Cephamycin	75	17 (22.7)	14 (82.4)	0 (0.0)	3 (17.6)	0 (0.0)	58 (77.3)
Cefixime	Third-generation cephalosporin	75	14 (18.7)	0 (0.0)	0 (0.0)	14 (100.0)	0 (0.0)	61 (81.3)
Cefotaxime	Third-generation cephalosporin	75	58 (77.3)	12 (20.7)	0 (0.0)	46 (79.3)	0 (0.0)	17 (22.7)
Ceftriaxone	Third-generation cephalosporin	75	17 (22.7)	0 (0.0)	0 (0.0)	17 (100.0)	0 (0.0)	58 (77.3)
Ceftazidime	Third-generation cephalosporin	75	75 (100.0)	13 (17.3)	12 (16.0)	50 (66.7)	0 (0.0)	0 (0.0)
Ceftazidime/Avibactam	Cephalosporin/β-lactamase inhibitor	75	58 (77.3)	56 (96.6)	0 (0.0)	2 (3.4)	0 (0.0)	17 (22.7)
Ceftolozane/Tazobactam	Cephalosporin/β-lactamase inhibitor	75	58 (77.3)	52 (89.7)	2 (3.4)	4 (6.9)	0 (0.0)	17 (22.7)
Cefepime	Fourth-generation cephalosporin	75	75 (100.0)	27 (36.0)	0 (0.0)	34 (45.3)	14 (18.7)	0 (0.0)
Imipenem	Carbapenem	75	61 (81.3)	61 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (18.7)
Meropenem	Carbapenem	75	72 (96.0)	70 (97.2)	0 (0.0)	2 (2.8)	0 (0.0)	3 (4.0)
Amikacin	Aminoglycoside	75	73 (97.3)	73 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.7)
Gentamicin	Aminoglycoside	75	75 (100.0)	52 (69.3)	0 (0.0)	23 (30.7)	0 (0.0)	0 (0.0)
Ciprofloxacin	Fluoroquinolone	75	75 (100.0)	8 (10.7)	20 (26.7)	47 (62.7)	0 (0.0)	0 (0.0)
Levofloxacin	Fluoroquinolone	75	3 (4.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	72 (96.0)
Nitrofurantoin	Nitrofuran	75	17 (22.7)	14 (82.4)	2 (11.8)	1 (5.9)	0 (0.0)	58 (77.3)
Colistin	Polymyxin	75	58 (77.3)	0 (0.0)	58 (100.0)	0 (0.0)	0 (0.0)	17 (22.7)
Trimethoprim/ Sulfamethoxazole	Folate pathway inhibitor	75	75 (100.0)	37 (49.3)	1 (1.3)	37 (49.3)	0 (0.0)	0 (0.0)

Not assessed indicates that susceptibility testing for the corresponding antimicrobial agent was not performed or the result was unavailable in the VITEK® 2 report. Abbreviations: S, susceptible; I, intermediate; R, resistant; SDD, susceptible-dose dependent

Table 5: Comparison of Antimicrobial Resistance Rates between ESBL-Positive and ESBL-Negative Escherichia coli Isolates

Antimicrobial agent	Antibiotic class	ESBL-positive resistant/tested n (%)	ESBL-negative resistant/tested n (%)	OR by Fisher's exact test	p-value	Not assessed n (%)
Ampicillin/Sulbactam	Penicillin/β-lactamase inhibitor	37/50 (74.0)	3/8 (37.5)	4.37	0.093	17 (22.7)
Piperacillin/Tazobactam	Penicillin/β-lactamase inhibitor	28/67 (41.8)	0/8 (0.0)	12.27	0.022	0 (0.0)
Cefotaxime	Third-generation cephalosporin	46/50 (92.0)	0/8 (0.0)	175.67	<0.001	17 (22.7)
Ceftazidime	Third-generation cephalosporin	50/67 (74.6)	0/8 (0.0)	49.06	<0.001	0 (0.0)
Ceftazidime/Avibactam	Cephalosporin/β-lactamase inhibitor	2/50 (4.0)	0/8 (0.0)	0.88	1.000	17 (22.7)
Ceftolozane/Tazobactam	Cephalosporin/β-lactamase inhibitor	4/50 (8.0)	0/8 (0.0)	1.65	1.000	17 (22.7)
Cefepime	Fourth-generation cephalosporin	34/67 (50.7)	0/8 (0.0)	17.51	0.007	0 (0.0)
Imipenem	Carbapenem	0/53 (0.0)	0/8 (0.0)	0.16	1.000	14 (18.7)
Meropenem	Carbapenem	2/64 (3.1)	0/8 (0.0)	0.68	1.000	3 (4.0)
Amikacin	Aminoglycoside	0/65 (0.0)	0/8 (0.0)	0.13	1.000	2 (2.7)
Gentamicin	Aminoglycoside	22/67 (32.8)	1/8 (12.5)	2.47	0.422	0 (0.0)
Ciprofloxacin	Fluoroquinolone	46/67 (68.7)	1/8 (12.5)	10.81	0.003	0 (0.0)
Colistin	Polymyxin	0/50 (0.0)	0/8 (0.0)	0.17	1.000	17 (22.7)
Trimethoprim/Sulfamethoxazole	Folate pathway inhibitor	33/67 (49.3)	4/8 (50.0)	0.97	1.000	0 (0.0)

Not assessed indicates that susceptibility testing for the corresponding antimicrobial agent was not performed or the result was unavailable in the VITEK® 2 report. OR, odds ratio

the most common sample source (86.7%, 65 isolates), followed by wound swabs, stool, and high vaginal swabs (HVS). AES confidence was reported as consistent in the majority of isolates, supporting the reliability of the automated identification and susceptibility results (Table 3).

Table 6: Distribution of Multidrug Resistance Phenotypes among Escherichia coli Isolates

Resistance phenotype	Operational definition	n	Percentage
Non-MDR phenotype	Resistance detected in fewer than three antimicrobial classes	25	33.3
MDR phenotype	Resistance detected in three or more antimicrobial classes	50	66.7
Third-generation cephalosporin-resistant phenotype	Resistance to cefotaxime and/or ceftazidime and/or ceftriaxone/cefixime	63	84.0
Fluoroquinolone-resistant phenotype	Resistance to ciprofloxacin and/or levofloxacin	47	62.7
Aminoglycoside-resistant phenotype	Resistance to gentamicin and/or amikacin	23	30.7
Carbapenem-resistant phenotype	Resistance to imipenem and/or meropenem/ertapenem	2	2.7
Trimethoprim/Sulfamethoxazole-resistant phenotype	Resistance to trimethoprim/sulfamethoxazole	37	49.3

Table 7: Minimum Inhibitory Concentration Distribution of Selected Antimicrobials among Escherichia coli Isolates

Antimicrobial agent	Total isolates n	Tested n (%)	MIC range	MIC50	MIC90	Resistant n (%)	Dominant interpretation	Not assessed n (%)
Ampicillin	75	17 (22.7)	≥32–≥32	≥32	≥32	17 (100.0)	R	58 (77.3)
Ampicillin/Sulbactam	75	58 (77.3)	≤2–≥32	≥32	≥32	40 (69.0)	R	17 (22.7)
Piperacillin/Tazobactam	75	75 (100.0)	≤4–≥128	≤4	64	28 (37.3)	S	0 (0.0)
Cefuroxime	75	14 (18.7)	≥64–≥64	≥64	≥64	14 (100.0)	R	61 (81.3)
Cefuroxime Axetil	75	14 (18.7)	≥64–≥64	≥64	≥64	14 (100.0)	R	61 (81.3)
Cefoxitin	75	17 (22.7)	≤4–≥64	≤4	≥64	3 (17.6)	S	58 (77.3)
Cefixime	75	14 (18.7)	≥4–≥4	≥4	≥4	14 (100.0)	R	61 (81.3)
Cefotaxime	75	58 (77.3)	≤0.25–≥64	≥64	≥64	46 (79.3)	R	17 (22.7)
Ceftriaxone	75	17 (22.7)	32–≥64	≥64	≥64	17 (100.0)	R	58 (77.3)
Ceftazidime	75	75 (100.0)	≤0.12–≥64	32	≥64	50 (66.7)	R	0 (0.0)
Ceftazidime/Avibactam	75	58 (77.3)	≤0.12–≥16	≤0.12	0.5	2 (3.4)	S	17 (22.7)
Ceftolozane/Tazobactam	75	58 (77.3)	≤0.25–≥32	≤0.25	4	4 (6.9)	S	17 (22.7)
Cefepime	75	75 (100.0)	≤0.12–≥32	8	16	34 (45.3)	R	0 (0.0)
Imipenem	75	61 (81.3)	≤0.25–1	≤0.25	≤0.25	0 (0.0)	S	14 (18.7)
Meropenem	75	72 (96.0)	≤0.25–≥16	≤0.25	≤0.25	2 (2.8)	S	3 (4.0)
Amikacin	75	73 (97.3)	≤1–4	≤2	4	0 (0.0)	S	2 (2.7)
Gentamicin	75	75 (100.0)	≤1–≥16	≤1	≥16	23 (30.7)	S	0 (0.0)
Ciprofloxacin	75	75 (100.0)	≤0.06–≥4	≥4	≥4	47 (62.7)	R	0 (0.0)
Levofloxacin	75	3 (4.0)	≥8–≥8	≥8	≥8	3 (100.0)	R	72 (96.0)
Nitrofurantoin	75	17 (22.7)	≤16–128	≤16	64	1 (5.9)	S	58 (77.3)
Colistin	75	58 (77.3)	≤0.5–1	≤0.5	≤0.5	0 (0.0)	I	17 (22.7)
Trimethoprim/ Sulfamethoxazole	75	75 (100.0)	≤20–≥320	≤20	≥320	37 (49.3)	S	0 (0.0)

MIC50 = minimum inhibitory concentration required to inhibit 50% of isolates; MIC90 = minimum inhibitory concentration required to inhibit 90% of isolates

ESBL-positive isolates showed significantly higher resistance rates to piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, and ciprofloxacin compared with ESBL-negative isolates. These findings indicate that the ESBL-positive phenotype was not limited to resistance against third-generation cephalosporins but was also associated with resistance to other antimicrobial classes. Most non-significant differences were observed among agents that retained good activity or had a limited number of resistant isolates (Table 5).

Multidrug resistance was prevalent, with 50 isolates (66.7%) classified as multidrug-resistant (MDR). Resistance to third-generation cephalosporins and fluoroquinolones represented the most common resistance phenotypes, followed by trimethoprim/sulfamethoxazole resistance. Carbapenem resistance remained rare, indicating that carbapenems retained high in vitro activity against the studied isolates (Table 6).

The categorical susceptibility results were supported by the MIC distribution analysis. Several cephalosporins and ciprofloxacin showed higher MIC50 and MIC90 values, reflecting reduced susceptibility among the studied isolates. In contrast, the lower MIC values observed for imipenem, meropenem, amikacin, and some beta-lactam/beta-lactamase inhibitor combinations provided further evidence of their higher in vitro activity against the tested *E. coli* isolates (Table 7).

DISCUSSION

The existence of ESBL-producing *E. coli* strains on a global scale poses a significant challenge to clinicians and substantially reduces the availability of empirical treatment methods. The results of the analysis of *E. coli* strains showed that the majority of the strains in question were ESBL producers (89.3%, or 67/75). This knowledge corresponds well with the recent data published discussing ESBL production rates and the growing problem with the emergence of resistant strains in hospitals across the world [19,4]. The high levels of ESBL production can result from massive and wrongful use of third-generation cephalosporins in the community and health care facilities, which creates selection pressure for resistant strains [5,6]. Moreover, antibiotic misuse and unregulated access to antibiotics, as well as the lack of control over the availability of antibiotics contribute to the ongoing exposure of bacteria to antibiotics thus speeding up ESBL-producing *E. coli* spread [18,12].

Most *E. coli* isolates in this study were obtained from different urine sample types (86.7%), with nearly all wounds having a total of 5.3%, feces having 5.3%, and vaginal swabs having 2.7%. This finding is consistent with results that indicated high prevalence of *E. coli* as a causative agent of both community-acquired and hospital-acquired urinary tract infections [3,25]. Uropathogenic *E. coli* (UPEC) have different virulence traits such as fimbrial adhesins and other factors through which the strains gain access to the urinary tract [26]. The combination of virulence factor characteristics and ESBL resistance traits causes difficulties in treating the infections as well as facilitating recurrent infections and long hospitalization [27,29]. Moreover, the identification of *E. coli* strains from wound swabs and high vaginal swabs proves this organism has potential to cause opportunistic infections at various body locations [28].

Analysis of susceptibility to antimicrobials revealed the presence of a highly resistant multidrug-resistant (MDR) phenotype in the isolates of the studied pathogen. High levels of resistance were noted against a number of beta-lactam agents, e.g., Ampicillin/Sulbactam, Cefotaxime, Ceftazidime, and Cefepime. The associated process leading to such phenomenon is the enzymatic hydrolysis of beta-lactam antibiotics by ESBL enzymes, namely CTX-M, TEM, and SHV [7,9]. The observed resistance to Cefepime, one of the fourth-generation cephalosporins, is concerning because it indicates evidence for an occurrence of strong beta-lactamase activity, which may lead to failure of treatment [30].

The use of the VITEK® 2 system revealed also co-resistance towards other classes of antimicrobials excluding beta-lactams, in particular, aminoglycosides (gentamicin) and fluoroquinolones (ciprofloxacin). The phenomenon can be explained by co-existence of various determinants of resistance, such as aminoglycoside-modifying enzymes and plasmid-mediated quinolone-resistance genes located on transferable plasmids containing ESBL genes [29,31]. Genetic association in this case provides an opportunity for horizontal transfer of many resistance mechanisms and limits therapeutic options available for outpatient therapies [31].

In the present investigation, the isolates of *E. coli* producing ESBLs were shown to be very susceptible to carbapenems (imipenem and meropenem) and amikacin. The above-mentioned results confirm the importance of carbapenems as treatment options for serious infections caused by ESBL-producing Enterobacterales [33].

Nevertheless, this overuse can lead to the emergence of carbapenem-resistant Enterobacteriaceae (CRE) by way of certain mechanisms such as carbapenemase and porin [34].

In this study, the VITEK® 2 Advanced Expert System (AES) provided reliable interpretation of susceptibility patterns of antimicrobials, attaining 93.3% confidence level. The automated phenotypic systems give quick interpretations of MIC patterns, and minimize manual interpretation mistakes while helping in timely interventions in the antimicrobial stewardship [22].

The results of this study indicating a high occurrence of ESBL-producing *E. coli* are consistent with the reports from the countries around the area where the prevalence of ESBL-producing *E. coli* has been increasing. The case in the Middle East and the neighboring countries, such as Iraq, reflected that there was an increase of ESBL-mediated resistance consistently. This may be attributed to the excessive use of antibiotics, lack of measures to fight resistance, and dissemination of resistance plasmids in Enterobacterales. The conclusions stress the importance of conducting constant regional surveillance [35,11].The high percentage of urine isolates detected in this study (86.7%) can present as a limiting factor in the study as urine samples are the commonly used clinical samples for the detection of *E. coli*. This may also lead to selection bias and can affect the application of the findings to other *E. coli* infection types such as bacteremia, pneumonia, etc. [3].

Although carbapenems were shown to be effective against ESBL-producing *E. coli* isolates in the study, their usage needs to be monitored carefully. It is crucial to use them properly in antimicrobial stewardship programs to minimize the selection pressure and to prevent the occurrences of carbapenem-resistant Enterobacterales [11,34].

Limitations

This study has several limitations. First, it was conducted in a single geographical region with a relatively small sample size, which may limit the generalizability of the findings. In addition, most isolates were obtained from urine specimens, potentially introducing selection bias. Molecular confirmation of ESBL genes (such as blaCTX-M, blaTEM, and blaSHV) was not performed, and patient clinical characteristics were unavailable because only anonymized laboratory isolates were used. Finally, the relatively short study period may not fully reflect long-term antimicrobial resistance patterns.

CONCLUSIONS

In conclusion, this research showed that there is a high rate of ESBL-generating Escherichia coli in the clinical samples analyzed in this research. The VITEK® 2 system was efficient in identifying the samples and determining their susceptibility to antibiotics, hence enabling the identification of the presence of important patterns of drug resistance in the tested samples. The drugs carbapenems and amikacin were effective in treating the studied isolates, but careful treatment choices should be made when using these drugs in order to maintain their efficacy. There is local knowledge available about ESBL-producing *E. coli* and the importance of constant monitoring and antibiotic stewardship has also been identified. Further multicenter studies with larger

sample sizes and molecular characterization are recommended to validate and expand upon these findings.

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