Received: 27 April 2024 | Revised: 1 October 2024 | Accepted: 7 October 2024

DOI: 10.1002/hsr2.70149

ORIGINAL RESEARCH

Health Science Reports Open Access WILEY

Virulence genes and antibiotic susceptibility patterns of *Escherichia coli* isolated from nosocomial urinary tract infections in the northwest of Iran during 2022–2023: A cross-sectional study

Mohammad Yousef Memar[1] | Masoud Vosughi[2,3] | Yalda Rahbar Saadat[2] | Mohammadreza Ardalan[2] | Mina Yekani[1] | Bahram Niknafs[2] | Sepideh Zununi Vahed[2]

[1]Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2]Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence

Bahram Niknafs and Sepideh Zununi Vahed, Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
Email: bahramniknafs@gmail.com, niknafs.b@tbzmed.ac.ir, sepide.zununi@gmail.com, and zununivahed@tbzmed.ac.ir

Abstract

Background and Aims: Urinary tract infections (UTIs) are prevalent among hospitalized patients, constituting the most frequent health-care infections. Uropathogenic *Escherichia coli* (UPEC) is leading causative agent of UTIs. The present study was aimed to examine the susceptibility of UPEC isolates obtained from nosocomial cases to antibiotics, as well as their biofilm formation capability and frequency of virulence genes.

Methods: A total of 100 UPEC isolates were collected from nosocomial UTIs at Imam Reza Hospitals in Tabriz, Iran, spanning from April 2022 to January 2023. The antimicrobial susceptibility patterns were evaluated using the disk diffusion method, along with the detection of broad-spectrum β-lactam enzymes (ESBLs) and carbapenemases. The ability of isolates to form biofilms was assessed using the microtiter-plate method, while the PCR method was employed to identify the presence of virulence genes.

Results: The highest resistance was observed toward piperacillin (82%), followed by aztreonam and ciprofloxacin (81%), while the lowest resistance was found against piperacillin/tazobactam (12%) and meropenem (9%). ESBLs were detected in 62% of the isolates. The microtiter-plate results revealed strong, moderate, and weak biofilm formation abilities in 32%, 33%, and 24% of the isolates, respectively. The most prevalent virulence gene was *fimA* (74%) followed by *hlyF* (68%), *papA* (44%), *papC* (32%), *iroN* (26%), and *cnf* (20%).

Conclusion: The elevated levels of resistance to multiple antimicrobial agents, coupled with the co-presence of virulence genes and biofilm formation abilities, contribute to the persistence of UPEC-related infections, particularly in hospitalized

patients. These findings underscore the necessity of implementing an effective program to control nosocomial UTIs caused by UPEC in the healthcare centers.

KEYWORDS
antibiotics, biofilm, urinary tract infections, uropathogenic *Escherichia coli*, virulence

1 | INTRODUCTION

Escherichia coli is the most common bacterial pathogen isolated from urinary tract infections (UTIs).[1] Among different *E. coli* pathotypes, uropathogenic *E. coli* (UPEC) is responsible for 90% of community—and up to 50% of hospital-acquired UTIs in both men and women.[2] The impact of UPEC strains on public health is considerable. UTIs represent a substantial socioeconomic problem with treatment costs estimated to be several billion dollars annually for national health resources.[3] UTIs are common among women, children, elderly, and immunocompromised individuals,[4] and the risk of recurrence is higher in women.[5]

The occurrence of nosocomial UTIs can be attributed to both typical UPEC strains and atypical *E. coli* strains possessing the certain virulence factors. The plasticity of the *E. coli* genome has facilitated the transmission of various virulence encoding genes among *E. coli* strains.[2] UPEC has various virulence features including adhesins or fimbriae, flagella, iron-acquisition factors, biofilm formation ability, and toxins such as hemolysin.[6,7] These features provide the potential for the pathogen to evade or overwhelm host defense mechanisms, invade host cells, and induce inflammation in the host.[3]

The indiscriminate antibiotic therapy has led to the development of multiple drug-resistance (MDR) strains, which is considered a major challenge in the treatment of UTIs.[3] Although carbapenems have been used to eliminate extended-spectrum β-lactamases (ESBLs)-producing and MDR pathogens, the development of carbapenem-resistant isolates has restricted their efficiency.[8,9] Additionally, the ability to form biofilms supports the growth and persistence of UPEC in the genitourinary tract by providing a nutrient-rich environment and protecting the bacteria from antimicrobial agents and host defense mechanisms.[7,10] Therefore, characterizing the bacteria will enhance our understanding of their pathogenesis and aid in the development of effective therapeutic approaches for controlling UPEC. Undoubtedly, there are differences between UPEC isolates causing hospital-acquired UTIs and those causing community-acquired cases in terms of the level of antimicrobial resistance and the presence of specific virulence factors. In addition, the characteristics of pathogens different regarding the presence of virulence factors and the level of antibiotic resistance between strains in different regions and even different hospitals in the same region. In a few published reports from the present region, most characteristics related to the strains causing community-acquired UTIs or a limited number of virulence factors have been studied. Therefore, studying the antibiotic susceptibility patterns and virulence characteristics of the isolates that cause UTIs obtained from the hospital provides useful information for adopting appropriate strategies for the treatment and control of these infections. Thus, our objective was to assess the antibiotic susceptibility patterns, biofilm formation, and presence of virulence genes in UPEC isolates collected from nosocomial UTI cases hospitalized at Imam Reza hospital in Tabriz, Iran.

2 | METHODS

2.1 | Bacterial isolates

This study carried on 100 UPEC isolates collected from nosocomial UTI patients admitted to Imam Reza Hospital of Tabriz between April 2022 and January 2023. In the present study, nosocomial UTIs were considered as infections acquired after hospital stay at least 48 h after admission. The presence of UTIs clinical sign and the use of antibiotics by patients at the time of admission were considered as exclusion criteria. The urine samples were cultured on blood agar and MacConkey agar and incubated overnight at 35°C. The isolated bacteria with a colony count higher than 10^5 CFU/mL were considered as UTIs agent and identified using Gram-staining, microbiological standards, and biochemical tests. The identified isolates were stocked in the Tryptic Soy Broth (TSB) containing 15% glycerol at −70°C for the next steps.

2.2 | Antibiotics susceptibility testing

2.2.1 | Disk diffusion

Antibiotic susceptibility patterns were determined using the disk diffusion methods conferring to the Clinical and Laboratory Standards Institute (CLSI) guideline.[11] First, a suspension of tested bacteria equivalent to 0.5 McFarland's standard was inoculated on the plates containing Muller-Hinton Agar (MHA). Disks of cefepime (30 μg), piperacillin (100 μg), ceftazidime (30 μg), piperacillin tazobactam (100/10 μg), meropenem (10 μg), aztreonam (30 μg), amikacin (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), fosfomycin (200 μg), and nitrofurantoin (200 μg) were tested. After overnight incubation at 35°C, the inhibition zones around disks were interpreted based on CLSI breakpoints. *E. coli* ATCC 25922 was considered as the quality control strains for the susceptibility testing. In this study, MDR was considered as nonsusceptibility to at least three groups of drugs.[12]

2.2.2 | Detection of extended-spectrum β-lactamases (ESBLs)

The ESBLs-producing isolates were identified using the method described by CLSI using ceftazidime, ceftazidime/clavulanate, and cefotaxime/clavulanate disks.[11] First, a lawn culture was prepared on the MHA using a bacterial suspension equivalent to 0.5 McFarland standard, and then the disks were placed on the agar. The plates were incubated at 35°C for 18 h. An increase of more than 5 mm in the inhibition zone diameter detected for the disks containing ESBLs inhibitor (clavulanate) in compression to the disk without inhibitor was considered as ESBLs production. *E. coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 70063 were used for quality control.

2.2.3 | Modified carbapenem inactivation method

Carbapenemase-production was detected using Modified Carbapenem Inactivation Method (mCIM) according to CLSI guidelines.[11] Briefly, a loop of tested bacteria and a meropenem disk (10 µg) were immersed in 2 mL TSB and incubated for 4 h at 37°C. Then, this disk was placed on the MHA, which was previously inoculated with the 0.5 McFarland suspension of *E. coli* ATCC 25922. After 24 h of incubation at 37°C, inhibition zone of 6–15 mm and 16–18 mm was considered as a positive and intermediate results of tests, respectively. Inhibition zone with a diameter more than 19 mm was considered as a negative test result (inability to produce carbapenemase by the bacteria).

2.2.4 | Detection of biofilm formation ability

The microtiter plate test (MPT) was performed to semiquantitatively assay of biofilm formation according to the previously described method.[13] Bacterial colonies were inoculated in tube containing 4 mL of TSB and incubated for 20 h at 37°C. Then, this suspension was diluted 1:100 in TSB supplemented with 1% glucose and 200 µL of this was transferred to well of 96 well plates. After incubation overnight at 37°C, TSB with suspended bacteria was removed from wells. The wells were carefully washed three times with PBS and air dried. The attached biofilms to wells were stained using 200 µL of 0.9% crystal violet solution for 15 min. After removing the crystal violet, wells were washed with PBS and the attached dye was solubilized with 95% ethanol. Optical density (OD) of the adherent biofilm was determined by a microtiter plate OD reader at wavelength of 450–630 nm. Wells containing TSB and 1% glucose was considered as a negative control and biofilm formation ability was interpreted according to the Table 1.

2.2.5 | DNA extraction

DNA extraction was performed by boiling method. First, a fresh colony of bacteria was dissolved in 20 µL lysate buffer and was placed at 95°C for 10 min. Then, this suspension was centrifuged for 1 min at 12,000 rpm and 180 µL of deionized water was added to it. Extracted DNA was stored at −20°C.

TABLE 1 Interpretation of OD obtained from the microtiter plate assay.

Biofilm formation ability	OD
Negative	mean $OD_t \geq ODc$
Weak	$ODc < \text{mean } OD_t \leq 2 \times ODc$
Moderate	$2 \times ODc < \text{mean } OD_t \leq 4 \times ODc$
Strong	$4 \times ODc < \text{mean } OD_t$

Abbreviations: C, control; t, test.

2.2.6 | Detection of virulence genes

PCR was used to detect of virulence genes *cnf1*،*hlyA*، *hlyF*، *iroN*، *papA papC*, and *fimH* using specific primers (Table 2). Each PCR reaction was performed in 25 µL of reaction mixture containing 2 µL of DNA sample, 2 µL of each primer, 1.25 µL of MgCl2 (50 mM), 0.5 µL d NTP (10 mM), 2.5 µL of PCR Buffer (10X), 1 µL of Taq polymerase (2.5 u/µL), and 13.25 µL of distilled water. The PCR products were analyzed by electrophoresis on 1% agarose gels in TBE buffer (89 mM Tris base, 89 mM boronic acid, 2 mM Na2, EDTA, pH 8.25). The agarose gel was stained with DNA safe stain and visualized under UV light in the Prescence of A 100 bp ladder as a DNA molecular size marker.

2.2.7 | Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 20. $p \leq 0.05$ were considered to be statistically significant.

3 | RESULTS

3.1 | Patients

In the present study, 100 UPECs were isolated from nosocomial UTIs in 57 female and 43 male patients hospitalized at Imam Reza Hospitals of Tabriz, Iran. The isolates were collected with the highest frequency from patients in the ICU (37%), followed by surgery (19%), urology (18%), infectious diseases (14%), transplant, lung (5%), and endocrinology (2%) wards.

3.2 | Antibiotic susceptibility patterns

The highest frequency of resistance was observed to piperacillin (82%), followed by aztreonam and ciprofloxacin (81%), cefepime

TABLE 2 The primer sequences used in the present study.

Gene	Primer sequence (5′—3′)	Size of product (bp)	References
cnf1	F: TTA-TAT-AGT-CGT-CAA-GAT-GGA R: CAC-TAA-GCT-TTA-CAA-TAT-TGA	760	Van Bost et al.[14]
hlyA	F: AGCTGCAAGTGCGGGTCTG R: TACGGGTTATGCCTGCAAGTTCAC	569	Awad et al.[15]
hlyF	TCGTTTAGGGTGCTTACCTTCAAC TTTGGCGGTTTAGGCATTCC	444	Deku et al.[16]
iroN	F: AAGTCAAAGCAGGGGTTGCCCG R: GACGCCGACATTAAGACGCAG	665	Khasheii et al.[17]
papA	F: ATGGCAGTGGTGTTTTGGTG R: CGTCCCACCATACGTGCTCTTC	717	Rodriguez-Siek et al.[18]
papC	F: GTGGCAGTATGAGTAATGACCGTTA R: ATATCCTTTCTGCAGGGATGCAATA	205	Rodriguez-Siek et al.[18]
fimH	F: GAGAAGAGGTTTGATTTAACTTATTG R: AGAGCCGCTGTAGAACTGAGG	559	Tajbakhsh et al.[19]

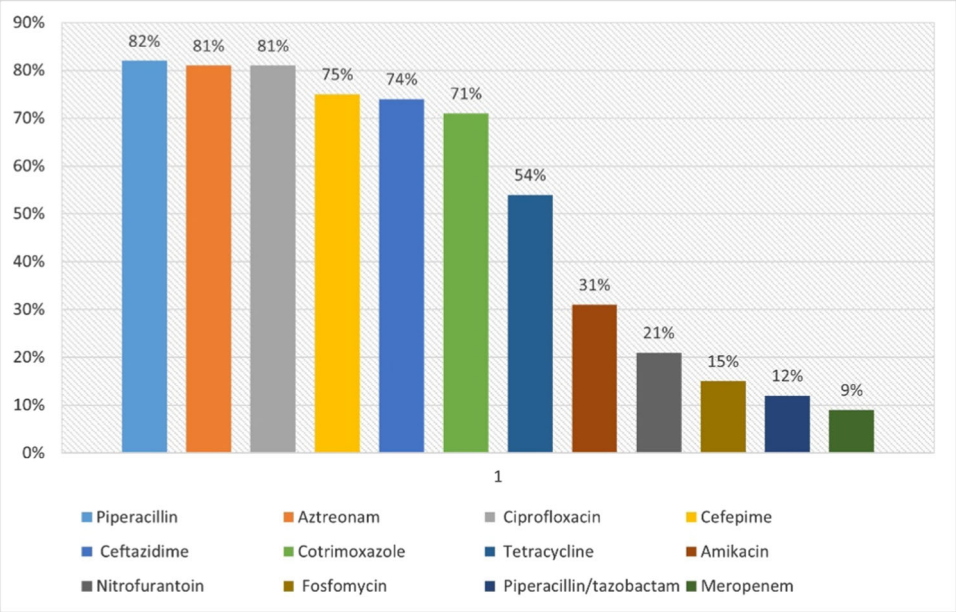

FIGURE 1 Antibiotics susceptibility patterns of bacterial isolates studied in the present study.

(75%), ceftazidime (74%), cotrimoxazole (71%), tetracycline (54%), amikacin (31%), nitrofurantoin (21%), fosfomycin (15%), piperacillin/tazobactam (12%), and meropenem (9%) (Figure 1). The antibiotic susceptibility patterns of isolates from different hospital wards are shown in Table 3. According to the antibiotic susceptibility patterns, 87% of isolates were MDR. According to the χ^2, no significant association was observed between resistance to antibiotics and MDR phenotype with the hospital wards where the bacteria were isolated (Table 3).

3.3 | ESBLs production

Based on the results of the ESBLs detection by phenotypic method, 62% of the isolates were ESBLs producers. Among the ESBLs-producing isolates, 79% were resistant to at least three groups of antibiotics and were reported as MDR. The highest antibiotic sensitivities of ESBLs-producing isolates were observed for meropenem (93.5%), fosfomycin (87.1%), nitrofurantoin (79%), amikacin (64.5%), tetracycline (64.5%), cotrimoxazole (27%), and ciprofloxacin (17.7%).

TABLE 3 The frequency of resistant bacteria isolated from different hospital wards.

	Antibiotic susceptibility patterns (%)											
Wards	**Piperacillin**	**Aztreonam**	**Ciprofloxacin**	**Cefepime**	**Ceftazidime**	**Cotrimoxazole**	**Tetracycline**	**Amikacin**	**Nitrofurantoin**	**Fosfomycin**	**Piperacillin/tazobactam**	**Meropenem**
ICU	83.3	73	89.2	78.4	78.4	62.2	56.8	37.8	21.6	16.2	13.5	13.5
Surgery	78.9	84.2	84.2	68.4	63.2	78.9	68.4	15.8	21.1	15.8	10.5	10.5
Urology	72.2	77.8	83.3	72.2	77.8	72.2	44.4	38.9	11.1	16.7	0	0
Infectious	92.9	92.9	64.3	85.7	85.7	78.6	57.1	21.4	28.6	7.1	28.6	14.3
Transplant	60	80	40	60	60	80	60	20	20	20	0	0
Lung	100	100	80	60	60	60	20	40	20	20	0	0
Endocrinology	100	100	100	100	50	50	0	50	50	0	50	0
p Value	0. 44	0.57	0.11	0.73	0.68	0.98	0.31	0.58	0.90	0.97	0.10	0.59

TABLE 4 Biofilm formation ability of bacterial isolates from different hospital wards.

	Biofilm formation ability (%)			
Wards	**Strong**	**Moderate**	**Weak**	**Negative**
ICU	32.4	24.3	29.7	12.5
Surgery	36.8	47.4	5.3	5.3
Urology	27.8	27.8	27.8	11.1
Infectious	21.4	42.9	14.3	14.3
Transplant	0	0	40	0
Lung	60	20	0	20
Endocrinology	0	50	50	0
p Values	0.40	0.31	0.20	0.82

According to the χ^2 test, there was a significant association between ESBLs- producing isolates and hospital wards ($p < 0.05$).

3.4 | Carbapenemase production

In the present study, 9 isolates were resistant to meropenem disk (10 μg) and considered as carbapenem resistant. Based on the mCIM results, 8 out of 9 carbapenem-resistant isolates were carbapenemase producer. Except for the isolate without carbapenemase enzyme, other carbapenem resistant isolates were nonsusceptible to at least three class of antibiotics and MDR. Among carbapenem-resistant isolates, higher sensitivity was observed to nitrofurantoin (88.9%), followed by amikacin (77.8%), fosfomycin (66.7%), cotrimoxazole (44%), ciprofloxacin (33.3%), and ciprofloxacin (11.1%). Based on the χ^2 test, there was not a significant association between ESBLs-producing isolates and hospital wards ($p > 0.05$).

3.5 | Biofilm formation ability

According to the results of MTP method, 32% were strong biofilm formers, 33% were moderate biofilm formers, and 24% were weak biofilm formers, while 11% of isolates did not show the biofilm formation ability (Table 4). Based on the χ^2 test, no significant association was observed between biofilm formation ability and the hospital wards from which the bacteria were isolated (Table 4).

3.6 | Presence of virulence genes

In this study, the most common virulence genes were *fimA* (74%), followed by *hlyF* (68%), *papA* (44%), *papC* (32%), *iroN* (26%), *hlyA* (25%), and *cnf* (20%). The presence of virulence genes in isolates collected from different hospital wards is shown in Table 5. According to the χ^2 test, no significant association was observed between the

presence of virulence factors with the hospital wards which the bacteria were isolated (Table 5).

The co-presence virulence gene patterns of bacterial isolates are shown in Table 6. At least one virulence gene was present in 99% of the isolates. The most frequent pattern of virulence genes was *fimA+hlyF +papA* and *fimA+hlyF*, which were detected in 8% and 7% of isolates, respectively. According to the Kruskal–Wallis test, no significant association was observed between the co-presence virulence gene patterns and the hospital wards from which the bacteria were isolated.

4 | DISCUSSION

UPEC strains are the most common cause of nosocomial UTIs, which are a considerable concern for public health, resulting in increased costs and mortality rates worldwide. Drug resistance and the presence of virulence factors such as the capacity for attachment to different surfaces and biofilm formation, are two important characteristics of UPEC strains that contribute to their ability to cause nosocomial UTIs. In this study, the highest frequency of resistance was observed for piperacillin (82%), followed by aztreonam and ciprofloxacin (81%). Based on the Infectious Diseases Society of America (IDSA) principle, cotrimoxazole is the drug of choice for antibiotic therapy of UTI patients in settings where more than 80% of isolates be nonresistance to this drug.[20] If the frequency of cotrimoxazole resistance exceeds 20%, quinolones may be considered as the alternative choice for UTIs patients. Therefore, these antibiotics do not seem to be an appropriate choice for UTIs in our health centers.

Several studies from Iran have reported a high frequency of resistance to cotrimoxazole, fluoroquinolone, aminoglycosides and β-lactams among *Enterobacteriaceae* and *E. coli* isolated from UTIs. Malekzadegan and colleagues reported the highest and lowest frequencies of resistance to ampicillin (88.9%), and imipenem (0.8%), respectively, among *E. coli* isolated from UTIs patients. Additionally, the resistance to ciprofloxacin was 55.6%. They reported that 77.8% of isolates were MDR and 54.8% were considered ESBLs producers.[21] In another study, high resistance rates were observed to nalidixic acid (82%), ciprofloxacin (78%), cephalothin (62%), and cotrimoxazole (59%).[22] Fatima and colleagues reported the high resistance of UPEC strains to ampicillin, cefixime, ceftriaxone, nalidixic acid, ciprofloxacin, and ofloxacin.[23] The high frequency of resistance to cephalosporins, cotrimoxazole, and ciprofloxacin has been reported among UPEC isolates from Egypt.[24] Ashika and colleagues from India, reported UPECs with resistance to several antibiotics and highest sensitivity to imipenem (91%) and amikacin (91%).[25]

In the present study, ESBLs producing isolates were isolated from 62% of the patients. Several studies reported more than 50%

TABLE 5 The frequency of virulence genes in different hospital wards.

Frequency of virulence genes (%)							
Wards	***fimA***	***hlyF***	***papA***	***papC***	***iroN***	***hlyA***	***cnf***
ICU	73	64.9	32.4	37.8	29.7	24.3	24.3
Surgery	89.5	78.9	42.1	26.3	31.6	21.1	15.8
Urology	66.7	61.1	61.1	38.9	11.1	27.8	11.1
Infectious diseases	64.3	64.3	50	35.7	35.7	28.6	21.4
Transplant	80	80	40	0	0	20	40
Lung	80	80	60	20	20	40	0
Endocrinology	50	50	50	0	0	0	50
p-Value	0.65	0.97	0.57	0.56	0.45	0.97	0.50

TABLE 6 Various co-presence of virulence genes detected in bacterial isolates.

The presence of virulence genes patterns	Frequency (%)
fimA+hlyF+papA	8
fimA+ hlyF	7
fimA+hlyA+cnf+papC+iroN+hlyF+papA, fimA+papC+iroN+hlyF	5
fimA+hlyA, fimA+papC+iroN+hlyF, fimA+papC+hlyF+papA, fimA+iroN+hlyF+papA and hlyF	4
fimA+hlyA+hlyF	3
fimA+hlyA +papC+ hlyF, fimA+hlyA+ papC+papA, fimA+hlyA+hlyF+papA, fimA+cnf+papC+hlyF+papA, fimA+papC+iroN +hlyF+papA, fimA+papA, cnf+hlyF+papA, fimA+hlyA+papA, cnf, papC+papA, fimA+hlyA+cnf, fimA+hlyA+papC+hlyF +papA, fimA+hlyA+papC and fimA+hlyA+iroN+hlyF+papA	2
fimA+hlyA+iroN+hlyF, fimA+cnf+papC+iroN+hlyF, fimA+cnf+papC+hlyF, fimA+cnf+papC, fimA+cnf+iroN+hlyF+papA, fimA+cnf+iroN+hlyF, fimA+cnf+iroN, fimA+cnf+hlyF+papA, fimA+cnf+hlyF, fimA+papC+hlyF, fimA+papC+papA, fimA +iroN, hlyA+cnf+iroN+hlyF, hlyA+cnf+hlyF+papA, hlyA+papC+papA, hlyA+hlyF, hlyA, cnf+papC+hlyF+papA, cnf+papC +papA, cnf+iroN+hlyF+papA, cnf+iroN+papA, papC+iroN+hlyF, papC+hlyF, papC, iroN, hlyF+papA, and *papA*	1

frequency of ESBLs producing UPEC isolates from different countries.[21,24,25]

The ESBLs expression is associated with resistance to various β-lactamases except for cephamycins or carbapenems. There is an increasing concern for ESBLs- producing MDR UPECs, which show commonly cross-resistance to several classes of antimicrobial agents. Therefore, there is limited options for effective antibiotic therapy of UTIs caused by ESBLs-producing MDR UPECs.[26] Carbapenems are the most effective drug for treatment of UTIs caused by these isolates. However, the development of carbapenem resistant isolates may decrease their efficiency. Similar to others reports, in our study, while the lowest resistance was found to meropenem (9%). Ashika and colleagues reported resistance to imipenem in 9% of isolates, -while Yekani and colleagues reported it in 3.2% of isolates.[25,27] Therefore, carbapenems can be used to treat UTIs caused by ESBLs-producing and MDR UPECs isolates. However, the develop of carbapenem-resistant UPEC strains highlights the importance of performing an antibiogram before beginning the antibiotics therapy of UTIs patients, as well as the necessity of controlling the spread of carbapenem- resistant strains.

Several virulence factors contribute to the attachment and colonization of UPEC in the epithelium urinary tract. Biofilm formation ability is the most essential feature of UPEC strains for the colonization, persistence, and recurrence of UTIs. Biofilms protect UPEC strains against harsh environmental circumstances, antimicrobial drugs, and the host's immune defense.[28] The MTP results revealed strong, moderate, and weak biofilm formation abilities in 32%, 33%, and 24% of the isolates, respectively. Our observations were similar to the findings of Alshaikh and colleagues, which demonstrated that 31%, 29%, 32%, and 8% of the UPEC isolates were strong, moderate, weak, and nonbiofilm producers, respectively.[24] Zhao and colleagues showed that more than 84% of UPEC isolates presented high capability to form biofilm in Iranian patients.[29] It has been proposed that isolates with lower rate of antimicrobial resistance mostly depend on biofilm formation to maintain their survival.[24] Consequently, during treatments of UPEC infections, the biofilm formation capability of strains should be taken into account to prevent therapeutic failure and/or recurrence of infections.[24]

UPEC is the main cause of UTIs in both community and hospital settings.[7] It possesses various virulence factors such as adhesins (P fimbriae, type 1 fimbriae, curli fibers, S fimbriae, F1C fimbriae, Dr fimbriae, afimbrial adhesins, and PapC), toxins (as α-hemolysin, cytotoxic necrotizing factor, and serine protease autotransporter), and siderophore-iron transporter proteins that enable the bacterium to colonize urinary tract, evade host defense mechanisms, and ultimately damage the uroepithelium.[5,30] The presence of some virulence factors may contribute to biofilm formation. Furthermore, factors like cellular hydrophobicity, surface electric charge, outer membrane proteins, and adhesion material properties contribute to biofilm formation on abiotic surfaces.[26]

The most common virulence genes were *fimA* (74%,) followed by *hlyF* (68%), *papA* (44%), *papC* (32%), *iroN* (26%), and *cnf* (20%). Farajzadeh sheikh and colleagues reported that among 232 UPEC strains, the common virulence factors were *kpsM* (23%), *neuA* (76.3%, capsule), *cnf* (29.6%, toxin), and *Pap* (54.8%, adhesin).[3] Dadi and colleagues reported that the most frequent UPEC virulence genes were *fimH*, *aer*, *hly*, *pap*, *cnf*, *sfa*, and *afa*, respectively.[6] Another research performed by Landraud and colleagues indicated that the *cnf1* gene was detected in 30% of the strains.[31] The most commonly detected virulence factors in Mashayekhi and colleagues study were *fim* (71.2%), *set-1* (66.6%), *iha* (62.1), *papGI* (59%), *usp* (56%), and *sen* (22.7%).[32] Moreover, it has been shown that the *fim* gene was highly detected in 92.5% of UPEC strains. Additionally,the *sfa*, *pap*, and *hly* genes were observed in 53.8%, 38.7%, and 18.5% of isolates, respectively. Further, the *cnf* gene was detected in 12.1% of strains.[10] The difference in the frequency of virulence factors among different studies can be due to difference in sample size and methodology. In addition, there is a considerable correlation between UPEC phylogroup and some virulence factors.[6] These diverse profiles of virulence factors among UPEC phylogroup may be due to the presence of chromosomal or plasmid encoded-genes for virulence factors, as well as the potential for gene transfer between strains.[33]

5 | CONCLUSION

The results of the present study show the high frequency of MDR *E. coli* isolates causing nosocomial UTIs, which leads to considerable limitations in the treatment of these infections. Therefore, effective antibiotic therapy for UTIs caused by these strains should be based on the results of antibiotic sensitivity assay, which requires collaboration between clinicians and the laboratory. The presence of different virulence factors and the ability to form biofilms, on the one hand, and the high level of resistance to various antibiotics, on the other, provide the potential of the *E. coli* isolates to cause chronic and difficult-to-treat UTIs. Therefore, a change in patterns is critical for epidemiological surveillance and control plans for UTI infections in our hospitals.

AUTHOR CONTRIBUTIONS

Mohammad Yousef Memar: Methodology; conceptualization; formal analysis; writing—review and editing. **Masoud Vosughi**: Methodology; software; writing—review and editing; writing—original draft. **Yalda Rahbar Saadat**: Methodology; writing—review and editing. **Mohammadreza Ardalan**: Writing—review and editing; conceptualization; methodology; writing—original draft. **Mina Yekani**: Writing—original draft; writing—review and editing; investigation. **Bahram Niknafs**: Writing—review and editing; writing—original draft; validation; project administration. **Sepideh Zununi Vahed**: Conceptualization; methodology; software; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

This article was written based on a data set from a project registered at Tabriz University of Medical Sciences (number 68198). The Vice Chancellor for Kidney Research Center, Tabriz University of Medical

Sciences provided financial support for this research that is greatly acknowledged.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

TRANSPARENCY STATEMENT

The lead author Bahram Niknafs, Sepideh Zununi Vahed affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

Corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. All relevant data and materials are provided within manuscript.

ETHICS STATEMENT

This study was confirmed by the Ethics and Human Rights Committee of Tabriz University of Medical Sciences, Tabriz, Iran (Ethical code: IR.TBZMED.REC.1401.264) and written informed consent was obtained from all UTI patients before their participation in the study.

ORCID

Mohammad Yousef Memar http://orcid.org/0000-0002-0328-4753

Sepideh Zununi Vahed https://orcid.org/0000-0003-0179-4562

REFERENCES

1. Bunduki GK, Heinz E, Phiri VS, Noah P, Feasey N, Musaya J. Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. *BMC Infect Dis*. 2021;21:753.
2. Toval F, Köhler C-D, Vogel U, et al. Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients with urinary tract infection. *J Clin Microbiol*. 2014;52:407-418.
3. Farajzadah Sheikh A, Goodarzi H, Yadyad MJ, et al. Virulence-associated genes and drug susceptibility patterns of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection. *Infect Drug Resist*. 2019;12:2039-2047.
4. Hreha TN, Gilbert NM, Hunstad DA. *Uropathogenic* Escherichia coli *in Urinary Tract Infections, Molecular Medical Microbiology*. Elsevier; 2024:1271-1297.
5. Whelan S, Lucey B, Finn K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. *Microorganisms*. 2023;11:2169.
6. Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa, Ethiopia. *BMC Infect Dis*. 2020;20:108.
7. Tabasi M, Asadi Karam MR, Habibi M, Yekaninejad MS, Bouzari S. Phenotypic assays to determine virulence factors of uropathogenic *Escherichia coli* (UPEC) isolates and their correlation with antibiotic resistance pattern. *Osong Public Health Res Perspect*. 2015;6:261-268.
8. Al-Sa'ady AT, Mohammad GJ, Hussen BM. Genetic relation and virulence factors of carbapenemase-producing uropathogenic *Escherichia coli* from urinary tract infections in Iraq. *Gene Rep*. 2020;21:100911.
9. Khalili Y, Yekani M, Goli HR, Memar MY. Characterization of carbapenem-resistant but cephalosporin-susceptible *Pseudomonas aeruginosa*. *Acta Microbiol Immunol Hung*. 2019;66:529-540.
10. Kot B, Wicha J, Grużewska A, Piechota M, Wolska K, Obrębska M. Virulence factors, biofilm-forming ability, and antimicrobial resistance of urinary *Escherichia coli* strains isolated from hospitalized patients. *Turk J Med Sci*. 2016;46:1908-1914.
11. Clinical and Institute L.S. *Performance Standards for Antimicrobial Susceptibility Testing*. Clinical and Laboratory Standards Institute; 2022:1-230.
12. Magiorakos A-P, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18:268-281.
13. Memar MY, Adibkia K, Farajnia S, et al. In-vitro effect of imipenem, fosfomycin, colistin, and gentamicin combination against carbapenem-resistant and biofilm-forming *Pseudomonas aeruginosa* isolated from burn patients. *Iran J Pharm Res*. 2021;20:286-296.
14. Van Bost S, Jacquemin E, Oswald E, Mainil J. Multiplex PCRs for identification of necrotoxigenic *Escherichia coli*. *J Clin Microbiol*. 2003;41:4480-4482.
15. Awad WS, El-Sayed AA, Mohammed FF, Bakry NM, Abdou NEMI, Kamel MS. Molecular characterization of pathogenic *Escherichia coli* isolated from diarrheic and in-contact cattle and buffalo calves. *Trop Anim Health Prod*. 2020;52:3173-3185.
16. Deku JG, Duedu KO, Kinanyok S, Kpene GE, Feglo PK. Phylogenicity and virulence profiles of clinical *Escherichia coli* isolates in the Ho teaching hospital of Ghana. *BioMed Res Int*. 2022;2022:1-8.
17. Khasheii B, Anvari S, Jamalli A. Frequency evaluation of genes encoding siderophores and the effects of different concentrations of Fe ions on growth rate of uropathogenic *Escherichia coli*. *Iran J Microbiol*. 2016;8:359-365.
18. Rodriguez-Siek K, Nolan L, Giddings C, Doetkott C, Johnson T. Characterizing the APEC pathotype. *Vet Res*. 2005;36:241-256.
19. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrob Resist Infect Control*. 2016;5:11.
20. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clin Infect Dis*. 1999;29:745-758.
21. Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Jahanabadi Z. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic *Escherichia coli* isolates from Iranian patients. *BMC Infect Dis*. 2018;18:572.
22. Stephenson S, Brown P. Distribution of virulence determinants among antimicrobial-resistant and antimicrobial-susceptible *Escherichia coli* implicated in urinary tract infections. *Indian J Med Microbiol*. 2016;34:448-456.
23. Fatima S, Akbar A, Irfan M, et al. Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* EQ. 101 UPEC isolated from UTI patient in Quetta, Balochistan, Pakistan. *BioMed Res Int*. 2023;2023:7278070.
24. Alshaikh SA, El-Banna T, Sonbol F, Farghali MH. Correlation between antimicrobial resistance, biofilm formation, and virulence determinants in uropathogenic *Escherichia coli* from Egyptian hospital. *Ann Clin Microbiol Antimicrob*. 2024;23:20.
25. Ashika S, Sujatha K, Lakshmipriya N, Venkatesh K. Antimicrobial resistance properties of uropathogenic *Escherichia coli* in adults with

urinary tract infection and molecular characterisation of virulence factor genes in resistant strains. *Int J Acad Med Pharm*. 2023;5:1002-1007.
26. De Souza GM, Neto ERDS, da Silva AM, et al. Comparative study of genetic diversity, virulence genotype, biofilm formation and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from nosocomial and community acquired urinary tract infections. *Infect Drug Resist*. 2019;12:3595-3606.
27. Yekani M, Baghi HB, Sefidan FY, Azargun R, Memar MY, Ghotaslou R. The rates of quinolone, trimethoprim/sulfamethoxazole and aminoglycoside resistance among Enterobacteriaceae isolated from urinary tract infections in Azerbaijan, Iran. *GMS Hyg Infect Control*. 2018;13:Doc07.
28. Naziri Z, Kilegolan JA, Moezzi MS, Derakhshandeh A. Biofilm formation by uropathogenic *Escherichia coli*: a complicating factor for treatment and recurrence of urinary tract infections. *J Hosp Infect*. 2021;117:9-16.
29. Zhao F, Yang H, Bi D, Khaledi A, Qiao M. A systematic review and meta-analysis of antibiotic resistance patterns, and the correlation between biofilm formation with virulence factors in uropathogenic *E. coli* isolated from urinary tract infections. *Microb Pathog*. 2020;144:104196.
30. Shevade SU, Agrawal GN. Study of virulence factors of *E. coli* in community and nosocomial urinary tract infection. *Indian J Med Sci*. 2015;6:158-160.
31. Landraud L, Gauthier M, Fosse T, Boquet P. Frequency of *Escherichia coli* strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. *Lett Appl Microbiol*. 2000;30:213-216.
32. Mashayekhi F, Moghny M, Faramarzpoor M, et al. Molecular characterization and antimicrobial resistance of uropathogenic *Escherichia coli*. *Iran J Biotechnol*. 2014;12:32-40.
33. Rezatofighi SE, Mirzarazi M, Salehi M. Virulence genes and phylogenetic groups of uropathogenic *Escherichia coli* isolates from patients with urinary tract infection and uninfected control subjects: a case-control study. *BMC Infect Dis*. 2021;21:361.

How to cite this article: Memar MY, Vosughi M, Rahbar Saadat Y, et al. Virulence genes and antibiotic susceptibility patterns of *Escherichia coli* isolated from nosocomial urinary tract infections in the northwest of Iran during 2022-2023: a cross-sectional study. *Health Sci Rep*. 2024;7:e70149. doi:10.1002/hsr2.70149