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Original Article

Evaluation of adhesin genes and risk factors associated with urinary tract infections by drug-resistant uropathogenic *Escherichia coli* in North of Iran

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Abstract

Background: Uropathogenic *Escherichia coli* (UPEC) isolates, have a wide variety of virulence factors to promote colonization and survival in the urinary tract. This study aimed to evaluate adhesin genes, biofilm formation ability, antibiotic resistance profiles of UPEC strains, and the related risk factors in patients with UTIs caused by drug-resistant UPEC.

Methodology: A total of 105 UPEC isolates were evaluated for biofilm formation using 96-well microtiter plates, the presence of adhesin genes by PCR assay and the antimicrobial susceptibility pattern using the disk diffusion method. Demographic and clinical characteristics of patients were investigated to identify predisposing factors for drug-resistant isolates.

Results: Out of 105 UPEC isolates, 84.8% were positive for biofilm formation. Biofilm-producing isolates exhibited a significantly higher prevalence of *fimH*, *kpsMTII*, *csgA*, *afa/draBC*, and *pap* adhesin genes compared to non-biofilm-producing strains ($p < 0.05$). The results also revealed that 52.4% of the isolates were ESBL-producing, and 84.8% were multidrug-resistant (MDR). Further analysis of antibiotic susceptibility among ESBL-producing strains showed the highest resistance rates to ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole. Conversely, the highest susceptibility, in addition to carbapenems, was observed for fosfomycin, amikacin, cefoxitin, and nitrofurantoin. We identified hypertension as a potential risk factor for infection with ESBL-producing UPEC strains.

Conclusions: Our results revealed a significant rate of drug resistance among UPEC isolates obtained from UTIs in our region. This underscores the importance of monitoring the empirical use of antibiotics and identifying specific risk factors in our geographical area to guide the selection of appropriate empirical treatment for UTIs.

Key words: Uropathogenic *Escherichia coli*; adhesin genes; biofilm formation; risk factor; multidrug-resistant; urinary tract infection.

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Introduction

Urinary tract infections (UTIs), as a global health problem, are one of the most common bacterial infections, affecting a wide range of hospitalized and outpatients with high mortality and morbidity rates worldwide [1-3]. Uropathogenic *Escherichia coli* (UPEC) is the major cause of UTIs in 70-95% of community-acquired cases and 50% of all nosocomial infections [4,5]. UPEC encodes various virulence factors, such as toxins, capsules, invasins, and adhesins, which contribute to attaching to uroepithelial cells, and development and severity of UTIs [6,7]. Surface virulence factors (adhesins) such as P fimbriae (*pap*), Type1 fimbriae (*fim*), afimbrial adhesins (*afa*), and polysaccharide surface structures (capsule) are the main attachment factors increase the ability of bacteria to colonize and formation of biofilm and consequently persist in the urinary tract, which is associated with recurrence of urinary tract infections [2,8,9]. In addition, *pga* genes are required to synthesize related polysaccharide adhesins and optimal biofilm formation under various growth conditions [10,11]. *Csg* operons as regulators affect the expression of the curli genes [12,13]. Antigen 43 (*flu*), as a prominent surface protein in *E. coli*, with auto-aggregation and microcolony formation through cell-to-cell interactions, promotes biofilm formation [14,15]. Also, biofilm, as a sticky exopolysaccharide matrix, can cause of restricting the binding of antimicrobials and the exchange of plasmids between bacteria, which develops resistance against a broad spectrum of antibiotics [11,16,17]. Moreover, excessive and inappropriate use of antibiotics contributes significantly to the increasing rate of multiple-drug resistant (MDR) strains commonly

related to increased extended-spectrum beta-lactamases (ESBLs)-producing bacterial strains. The prevalence of ESBLs-producing UPECs is raising in urinary tract infections [11]. In addition, ESBLs-producing UPEC harbors resistance to a broad spectrum of β-lactam antibiotics, cephalosporins, monobactams, aminoglycosides, quinolones, tetracyclines, nitrofurantoin and trimethoprim-sulfamethoxazole [6].

Currently, the emergence of MDR and ESBLs-producing strains among UPEC isolates has increased as a major problem in treating urinary tract infections, leading to limited therapeutic choices and a serious threat to global health. Therefore, identifying risk factors for antimicrobial resistance may improve the empirical treatment of UTIs caused by ESBLs-producing and MDR UPEC strains [18].

The multidrug resistance observed among UPEC strains significantly restricts therapeutic options and poses a life-threatening risk, with a profound impact on human health worldwide. The aim of the present study was to assess colonization-associated genes, biofilm formation ability, antibiotic resistance profiles of ESBLs-producing UPEC strains isolated from patients with UTIs, along with their associated risk factors. The findings of this study will offer vital epidemiological insights into UPEC strains, which will be instrumental in devising more effective strategies for the prevention, control, and treatment of UTIs in the investigated region.

Methodology

Study design and bacterial identification

This cross-sectional study was conducted over a ten-month period from January to October 2021 in the department of Microbiology, Virology and Microbial Toxins, faculty of medicine at Guilan University of Medical Sciences, Rasht, Iran. This study was approved by the Research Ethics Committee (IR.GUMS.REC.1399.603) at Guilan University of Medical Sciences in Rasht, Iran. UPEC isolates were obtained from urine samples collected from patients with UTIs who were referred to Razi Teaching Hospital. Then, the isolates were identified using standard bacteriological and biochemical tests. The pure colonies were stored in trypticase soy broth supplemented with 20% glycerol at -80°C for later use.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility of UPEC isolates was tested on Mueller-Hinton agar against ampicillin (AM; 10µg), ampicillin-sulbactam (SAM; 10/10µg), amoxicillin-clavulanate (AMC; 20/10µg), piperacillin-tazobactam (PTZ; 100/10µg), cefazolin (CZ; 30µg), cefuroxime (CXM; 30µg), ceftriaxone (CRO; 30µg), cefepime (CPM; 30µg), cefoxitin (FOX; 30µg), imipenem (IMP; 10µg), meropenem (MEM; 10µg), gentamicin (GM; 10µg) , amikacin (AK; 30µg), ciprofloxacin (CIP; 5µg), levofloxacin (LEV; 5µg), trimethoprim-sulfamethoxazole (TS; 1.25/23.75µg), nitrofurantoin (F/M; 300µg), fosfomycin (FOS; 200µg) and azithromycin (AZM; 15µg) using disk diffusion (Oxoid Limited, United Kingdom) method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI), 2021 [19]. Those isolates was considered as MDR if they were resistant to at least one agent of ≥ 3 classes of antibiotics [20]. Phenotypic identification of ESBLs-producing isolates was performed by double-disk synergy test using ceftazidime (30 µg) and cefotaxime (30 µg) disks and combination with clavulanic acid (10 µg) according to the guidelines of the CLSI, 2021. Strains were identified as ESBL producers, showing an increase of ≥ 5 mm in the diameter of the inhibition zones around the single antibiotic disk compared to the inhibition zones around the combination disk [19]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive quality control strains, respectively.

Phenotypic detection of biofilm production in UPEC

The biofilm formation assay of UPEC isolates was carried out using 96-well microtiter plates according to Stepanovic *et al.* with some modifications. Briefly, isolates were grown overnight in trypticase soy broth (TSB) at 37°C. The cultures were adjusted to 0.5 McFarland standard turbidity and diluted 1: 100 with Mueller-Hinton Broth (MHB). Then 200 µL of diluted suspension was added to each well of the microplate (Maxwell, China), supplemented with 2% glucose (Merck, Germany), and incubated at 37°C for 24 hours. MHB containing 2% glucose was considered as a negative control. After the incubation, the content of the wells was discarded and washed with distilled water three times to remove non-adherent bacteria. The adhered cells were stained with 125 µL of 0.1% (w/v) crystal violet for 15 minutes. Then, the dye was discarded, and the microplate was washed three times and dried at room temperature. Furthermore, 125 µL of 33% glacial acetic acid (Merck, Germany) was added to the wells to release the dye. Subsequently, samples were transferred to another microplate, and the absorbance was measured at 570 nm using an ELISA reader. UPEC strains were categorized according to the criteria of biofilm formation ability introduced by

Stepanovic *et al.* as a non-biofilm producer, weak biofilm producer, moderate biofilm producer, or strong biofilm producer [21]. All assays were performed in triplicate, and results are given as the mean of at least two independent experiments ± SD.

DNA extraction and detection of adhesin genes by PCR assay

Chromosomal DNA of all isolates of UPEC was extracted by boiling method. Briefly, 500µL aliquots of overnight Brain-Heart Infusion broth (Merck, Germany) cultures were centrifuged at 14,000 rpm for 10 minutes at room temperature. The cell pellets were resuspended in 400µL of 1 × Tris-EDTA buffer, heated at 95 °C for 10 minutes, and placed at room temperature for 5 minutes. Samples were placed at -20 °C for 10 minutes, and after centrifugation at 14,000 rpm for 10 minutes at 4 °C, the supernatant was used as the DNA template. The concentration of DNA was measured using Nano drop. Amplification of different adhesin genes was performed by conventional PCR using specific primers (TAG Copenhagen A/S, Denmark). Primer sequences, predicted sizes of the PCR products, and amplification conditions are shown in Table 1. PCR reactions were done in a final volume of 20 µL containing 10 µL master mix (2 × PCRBIO Taq Mix Red - PCR BIOSYSTEMS), 1 µL of each primer (10 pmol/µL), 1 µL of extracted DNA and sterile Double distilled water to reach to 20 µL. The PCR conditions consisted of an initial denaturation step at 95 °C for 5 mins, followed by 35 cycles of DNA denaturation at 95 °C for 30 seconds and primer annealing for 30 seconds. Temperature depended on the sequences of primers, and primer extension at 72°C for 1 minute, followed by a final extension at 72 °C for 7 minutes. Amplicons were analyzed by electrophoresis on a 1.5% agarose gel.

Data analysis

Statistical analysis was performed using SPSS™ software, version 21.0 (IBM Corp., Armonk, NY, USA). The results are presented as descriptive statistics in percentage base distribution. Statistical analysis was carried out using Chi-square (χ^2) test to evaluate the relationship between the variables. A p value < 0.05 was considered statistically significant.

Results

A total number of 105 UPEC isolates were collected from patients with UTIs, predominantly from females (84.8%) compared to male patients 16 (15.2%). The majority of the patients belonged to the age group 61–80 years (54.3%), followed by age group 41-60 years (28.6%), age group 20-40 years (9.5%), and age group > 81 years (7.6%).

Antibiotic resistance of the UPEC isolates was investigated using ten antibiotics classes. Out of 105 UPEC strains, 55 (52.4%) were ESBLs-producing. The resistance pattern was compared between ESBLs and non-ESBLs-producing strains and are shown in Table 2. The ESBLs-producing strains showed significantly (p < 0.05) a higher degree of resistance to ampicillin, cefazolin, cefuroxime, cefepime, ceftriaxone, piperacillin- tazobactam, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, levofloxacin and azithromycin as compared to non-ESBLs-producing strains. Among the ESBLs-producing isolates, the highest resistance rate was observed for cefuroxime (100%), followed by ampicillin (96.4%) and ciprofloxacin (94.5%). However, the highest susceptibility was toward imipenem and meropenem (96.4%), amikacin (87.3%), and cefoxitin (83.6%). Also, the frequency of MDR isolates was recorded 84.8%. The rate of MDR isolates was significantly

Table 1. List of primers used in this study.

Genes	Function	Primer (5'-3')	Amplicon size (bp)	Tm (°C)	Ref.
fimH	Type 1 fimbrial	F-TGCAGAACGGATAAGCCGTGG R-GCAGTCACCTGCCCTCCGGTA	508 bp	67.6	**[28]**
papC	P fimbrial	F-GTGGCAGTATGAGTAATGACCGTTA R-ATATCCTTTCTGCAGGGATGCAATA	200 bp	60	
papGII		F-GGGATGAGCGGGCCTTTGAT R-CGGGCCCCCAAGTAACTCG	190 bp	64	
afa/draBC	Mannose-resistant hemagglutination	F-GGCAGAGGGCCGGCAACAGGC R-CCCGTAACGCGCCAGCATCTC	559 bp	67	
kpsMTII	Polysaccharide capsule	F-GCGCATTTGCTGATACTGTTG R-CATCCAGACGATAAGCATGAGCA	272 bp	59	
flu (agn43)	cell-to-cell aggregation	F-ACGCACAACCATCAATAAAA R-CCGCCTCCGATACTGAATGC	600 bp	52	**[15]**
pgaC	Responsible for production of β-1,6-N-acetyl-D-glucosamine	F-ATGATTAATCGCATCGTATCG R-CATCGGTTCCACAATATATGC	540 bp	60	**[11]**
csgA	Regulators the expression of the curli genes	F-ATCTGACCCAACGTGGCTTCG R-GATGAGCGGTCGCGTTGTTACC	178 bp	61	**[13]**

Table 2. Antibiotic resistance pattern of UPEC isolates among ESBLs-producing strains.

Antibiotics	ESBLs producers (55) Number (%)		No-ESBLs producers (50) Number (%)		*p* value
	Resistant	Susceptible	Resistant	Susceptible	
AM	53 (96.4)	2 (3.6)	41 (82)	9 (18)	0.01
SAM	35 (63.6)	20 (36.4)	23 (46)	27 (54)	0.07
AMC	35 (63.6)	20 (36.4)	24 (48)	26 (52)	0.1
PTZ	22 (40)	33 (60)	6 (12)	44 (88)	0.001
CZ	47 (85.5)	8 (14.5)	9 (18)	41 (82)	0.001
CXM	55 (100)	0 (0)	12 (24)	38 (76)	0.001
CPM	51 (92.7)	4 (7.3)	7 (14)	43 (86)	0.001
CRO	43 (78.2)	12 (21.8)	3 (6)	47 (94)	0.001
FOX	9 (16.4)	46 (83.6)	7 (14)	43 (86)	0.73
GM	22 (40)	33 (60)	6 (12)	44 (88)	0.001
AK	7 (12.7)	48 (87.3)	4 (8)	46 (92)	0.43
CIP	52 (94.5)	3 (5.5)	27 (54)	23 (46)	0.001
LEV	48 (87.3)	7 (12.7)	22 (44)	28 (56)	0.001
T/S	45 (81.8)	10 (18.2)	27 (54)	23 (46)	0.002
IMP	2 (3.6)	53 (96.4)	1 (2)	49 (98)	0.61
MEM	2 (3.6)	53 (96.4)	3 (6)	47 (94)	0.57
F/M	13 (23.6)	42 (76.4)	9 (18)	41 (82)	0.47
FOS	6 (10.9)	49 (89.1)	3 (6)	47 (94)	0.36
AZM	36 (65.5)	19 (34.5)	16 (32)	34 (68)	0.001

AM: Ampicillin; SAM: Ampicillin-sulbactam; AMC: Amoxicillin-clavulanate; PTZ: Piperacillin- tazobactam; CZ: Cefazolin; CXM: Cefuroxime; CPM: Cefepime; CRO: Ceftriaxone; FOX: Cefoxitin; IMP: Imipenem; MEM: Meropenem; GM: Gentamicin; AK: Amikacin; CIP: Ciprofloxacin; LEV: Levofloxacin; TS: Trimethoprim-sulfamethoxazole; F/M: Nitrofurantoin; FOS: Fosfomycin; AZM: Azithromycin.

higher among ESBLs-producers than non-ESBL producers (100% vs 68%, $p < 0.001$).

Biofilm production assay showed that 89 (84.8%) and 16 (15.2%) of UPEC isolates were biofilm producers and non-biofilm producers, respectively (Table 3). In this study, we considered strong and moderate biofilm producers as biofilm producer, weak and no biofilm producers as no biofilm producer. In the present study, there was a prevalence of 53.9% and 85.4% ESBL-producing and MDR in biofilm-producing isolates, respectively. Among the 83 patients with recurrent UTI, 70 (84.3%) UPEC isolates were biofilm producers and of the 22 patients with no-recurrent UTI, 19 (86.4%) UPEC isolates were biofilm producers. According to our results, there was no

Table 3. Correlation between adhesin genes among biofilm producer and non-biofilm producers of UPEC isolates.

	Biofilm producer (89) n (%)	No-biofilm producer (16) n (%)	*p* value
ESBLs producers (55)	48 (53.9)	7 (43.8)	0. 45
No-ESBLs producers (50)	41 (46.1)	9 (56.2)	
MDR (89)	76 (85.4)	13 (81.2)	0.6
No-MDR (16)	13 (14.6)	3 (18.8)	
Adhesin genes			
fimH			
Positive	89 (100)	13 (81.2)	< 0.001
Negative	0 (0)	3 (18.8)	
papC			
Positive	47 (52.8)	1 (6.2)	< 0.001
Negative	42 (47.2)	15 (93.8)	
papGII			
Positive	54 (60.7)	0 (0)	< 0.001
Negative	35 (39.3)	16 (100)	
flu (agn43)			
Positive	43 (48.3)	6 (37.5)	0.42
Negative	46 (51.7)	10 (62.5)	
pgaC			
Positive	58 (65.2)	10 (62.5)	0.83
Negative	31 (34.8)	6 (37.5)	
afa/draBC			
Positive	52 (58.4)	5 (31.2)	0.04
Negative	37 (41.6)	11 (68.8)	
kpsMTII			
Positive	70 (78.7)	2 (12.5)	< 0.001
Negative	19 (21.3)	14 (87.5)	
csgA			
Positive	61 (68.5)	6 (37.5)	0.01
Negative	28 (31.5)	10 (62.5)	

significant correlation between biofilm production and recurrent UTI.

The results of the prevalence of adhesin genes among biofilm producers and non-biofilm producers of UPEC isolates are shown in Table 3. Among adhesins, the most prevalent genes among all the biofilm producer isolates were *fimH* (100%) followed by *kpsMTII* (78.7%) and *csgA* (68.5%). Also, the frequency of 4 adhesin genes (*papC, papGII*, *afa/draBC* and *pgaC)* was more than 50% among the biofilm producer isolates.

In our study, analysis of the risk factors for ESBLs-producing isolates showed that hypertension could predispose patients to infection by an ESBLs-producing UPEC strains (Table 4).

Discussion

The increasing resistance to the broad spectrum of antibiotics among ESBLs-producing and MDR UPEC strains is a global challenge for appropriate treatment of UTIs [18,22]. As an essential virulence factor, biofilm formation enhances antibiotic resistance and results in bacterial persistence and chronic infections [7,23-25]. According to the recommendation of the America Infectious Diseases Society, monitoring the empirical use of antibiotics at a community level or geographic region is vital to select a proper treatment for UTIs [26]. Therefore, we report biofilm formation's ability, the antibiotic resistance pattern of ESBLs-producing and MDR UPEC isolates, and the related risk factors for UTIs in the north of Iran.

Consistent with the previous studies in Iran and other countries, in our results, 52.4 and 84.8% of UPEC isolates belonged to ESBLs-producing and MDR strains, respectively [27-31]. The prevalence of MDR isolates was higher than the pooled prevalence of MDR UPEC with 49.4% [32] and 65.8% [33] in Iran. Also, according to previous reports, the relative frequency of ESBLs-producing isolates varied from 24% to 72.9% in different regions of Iran [34]. Our findings showed a high antibiotic resistance rate among both ESBLs-producing and MDR isolates compared to non ESBLs-producing and non-MDR isolates. In agreement with some results from different parts, most ESBLs-producing and MDR isolates were resistant to ampicillin, ciprofloxacin and trimethoprim-sulfamethoxazole, which preclude their use as the first-line empirical therapy of UTIs [31,35,36].

Increased antibiotic resistance during recent years in different geographical areas of Iran has created challenges in the empirical treatment of UTIs due to differences in infection control policy and inappropriate and overuse of antibiotics [37].

On the other hand, our results revealed that not only were the majority of ESBLs-producing and MDR isolates susceptible to imipenem, meropenem, and fosfomycin but susceptibility to amikacin, cefoxitin, and nitrofurantoin was also considerable. This finding is consistent with some previous reports from Iran and

Table 4. Risk factors and demographic characteristics of patients with infection with ESBL-producing UPEC strains.

Risk factors	ESBL (+) (n = 55) n (%)	ESBL (-) (n = 50) n (%)	*p* value
Male gender	9 (16.4)	7 (14)	0.7
Female gender	46 (83.6)	43 (86)	
Age > 60 years	35 (63.6)	30 (60)	0.7
Age < 60 years	20 (36.4)	20 (40)	
Recurrent UTI			
Yes	47 (85.5)	36 (72)	0.09
No	8 (14.5)	14 (28)	
History of antibiotic use in last 3 months			
Yes	41 (74.5)	37 (74)	0.9
No	14 (25.5)	13 (26)	
History of hospitalization in last 3 months			
Yes	30 (54.5)	22 (44)	0.2
No	25 (45.5)	28 (56)	
History of urinary catheterization			
Yes	13 (23.6)	12 (24)	0.9
No	42 (76.4)	38 (76)	
Hypertension			
Yes	31 (56.4)	37 (74)	0.04
No	24 (43.6)	13 (26)	
Urinary tract stone			
Yes	7 (12.7)	8 (16)	0.6
No	48 (87.3)	42 (84)	
Diabetes mellitus			
Yes	21 (38.2)	17 (34)	0.6
No	34 (61.8)	33 (66)	

ESBL: Extended spectrum β-lactamases; MDR: multi-drug resistant.

other countries [29,31]. As carbapenems are β-lactam antibiotics with high antibacterial activity, they are commonly used as first-line treatments for infections caused by ESBLs-producing Enterobacterales. However, to prevent the emergence of carbapenem-resistant strains, it is essential to consider the use of alternative drugs [38,39]. Hence, to preserve carbapenems as valuable antibiotics, and based on our findings, oral options for treating UTIs caused by drug-resistant UPEC strains in our study region include nitrofurantoin and fosfomycin [36].

Most of our UPEC isolates showed the ability of biofilm formation (84.8%), in similar to other published reports [7,30]. In agreement with previous studies, our results according to Table 3, showed no significant correlation between biofilm formation and ESBLs production and MDR [11,30]. On the contrary, some reports found a positive correlation between biofilm formation and drug resistant [40,41]. These results reveal that although antibiotic resistance mechanisms may be associated with the formation of biofilm among UPEC strains, the development of antibiotic resistance in UPEC strains may be due to natural selection pressure caused by excessive and inappropriate use of antibiotics, exchange of plasmid-mediated genes, etc. [42-44].

Pathogenic UPEC strains encode a variety of adhesin genes, including fimbrial adhesins (such as Type 1 and P), afimbrial adhesins (*afa*), and polysaccharide surface structures (*kps*), which are among the most common virulence factors. These genes enable the bacteria to colonize and survive in the urinary tract [8,9].

In agreement with some previous studies, our results demonstrated a significant association ($p < 0.05$) between biofilm production and the presence of *fimH*, *kpsMTII, pap* genes, *afa/draBC,* and *csgA* adhesin genes [17,44,45]. All 54 *papGII*-positive isolates, were biofilm producers. These results show a possible relationship between the presence of this gene and biofilm formation in UPEC isolates. Nonetheless, the results of some studies similar to this study showed no significant correlation between biofilm production and frequency of *flu* and *pgaC* genes [11,24,46]. It may be due to the involvement of various adhesin genes and their expression in different types of UPEC strains, i.e., *csg*, *fimH*, *afa*, *pap* locus, *kps*, etc., responsible for the production of biofilm [47,48]. In addition, a mutation in the target gene could be an important reason for the lack of correlation between the presence of these genes and biofilm formation [49].

In our study, most of the patients were categorized as community-acquired urinary tract infections (CA-UTIs) and we identified that hypertension might be a risk factor for infection with ESBLs-producing UPEC strains. Previous studies have evaluated risk factors for UTIs caused by multidrug-resistant UPEC strains. A large study from Scotland involving 40,984 isolates demonstrated that increasing age is the main risk factor for multidrug resistance (MDR) due to the weakening of the immune system with aging, making the treatment of UTIs more difficult and recurrence more frequent [50]. Other studies have also shown male gender [51-53], older age [22,26,52,53], urinary catheterization [18,26], history of antibiotic use in the last three months [22,52], previous hospitalization [22,54-56], diabetes mellitus [54-57], and recurrent UTI [52] as predisposing factors that increase the risk of urinary tract infections by multidrug-resistant bacteria.

There are several limitations to the present study. First, this is a study of 105 UPEC strains over a 10-month period in a single hospital, limiting the scope of the study. Second, survey to the *papG* alleles and all of the effective genes in adhesion and the expression of them. Also, to evaluate risk factors, similar groups of patients in a large population size could be considered.

Conclusions

This study highlights the analysis of antimicrobial resistance, showing a high level of resistance to the first-line empiric treatments for UTIs caused by UPEC isolates in our studied region. Our finding revealed that, in addition to carbapenems, most ESBLs-producing strains were sensitive to fosfomycin, amikacin, cefoxitin and nitrofurantoin. Although the majority of isolates were biofilm producers, no significant association was found between biofilm formation and drug resistance. The presence of *fimH*, *pap* genes, *kpsMTII*, *afa/draBC*, and *csgA* adhesin genes appears to be associated with a high potential for biofilm formation. We identified that hypertension might be a risk factor for infection with ESBLs-producing strains in our studied region. Finally, our findings demonstrate the importance of monitoring empirical use of antibiotics and noticing certain risk factors in our geographical area to select an appropriate empirical treatment for UTIs.

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References

1. Rubini D, Varthan PV, Jayasankari S, Vedahari BN, Nithyanand P (2020) Suppressing the phenotypic virulence factors of uropathogenic *Escherichia coli* using marine polysaccharide. Microb Pathog 141: 103973. doi: 10.1016/j.micpath.2020.103973.
2. Shah C, Baral R, Bartaula B, Shrestha LB (2019) Virulence factors of uropathogenic *Escherichia coli* (UPEC) and correlation with antimicrobial resistance. BMC Microbiol 19: 204. doi: 10.1186/s12866-019-1587-3.
3. Mukherjee SK, Mukherjee M (2019) Characterization and biotyping of multidrug resistance plasmids from uropathogenic *Escherichia coli* isolated from clinical setting. Front Microbiol 10: 2913. doi: 10.3389/fmicb.2019.02913.
4. Hozzari A, Behzadi P, Kerishchi Khiabani P, Sholeh M, Sabokroo N (2020) Clinical cases, drug resistance, and virulence genes profiling in Uropathogenic *Escherichia coli.* Appl Genet 61: 265-273. doi: 10.1007/s13353-020-00542-y.
5. Hojabri Z, Mirmohammadkhani M, Darabi N, Arab M, Pajand O (2019) Characterization of antibiotic-susceptibility patterns and virulence genes of five major sequence types of *Escherichia coli* isolates cultured from extraintestinal specimens: a 1-year surveillance study from Iran. Infect Drug Resist 12: 893-903. doi: 10.2147/idr.s199759.
6. Cristea VC, Gheorghe I, Czobor Barbu I, Popa LI, Ispas B, Grigore GA, Bucatariu I, Popa GL, Angelescu MC, Velican A, Marutescu L, Popa M, Chifiriuc MC, Popa IM (2019) Snapshot of phylogenetic groups, virulence, and resistance markers in *Escherichia coli* uropathogenic strains isolated from outpatients with urinary tract infections in Bucharest, Romania. Biomed Res Int 2019: 5712371. doi: 10.1155/2019/5712371.
7. Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori R, Aghazadeh M (2015) Relationship of biofilm formation and different virulence genes in uropathogenic *Escherichia coli* isolates from Northwest Iran. GMS Hyg Infect Control 10: Doc11. doi: 10.3205/dgkh000254.
8. Lo AW, Moriel DG, Phan MD, Schulz BL, Kidd TJ, Beatson SA, Schembri MA (2017) 'Omic' approaches to study uropathogenic *Escherichia coli* virulence. Trends Microbiol 25: 729-740. doi: 10.1016/j.tim.2017.04.006.
9. Khairy RM, Mohamed ES, Abdel Ghany HM, Abdelrahim SS (2019) Phylogenic classification and virulence genes profiles of uropathogenic *E. coli* and diarrhegenic *E. coli* strains isolated from community acquired infections. PLoS One 14: e0222441. doi: 10.1371/journal.pone.0222441.
10. Wang X, Preston JP, Romeo T (2004) The pgaABCD locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. J Bacteriol 186: 2724-34. doi: 10.1128/jb.186.9.2724-2734.2004.
11. Shrestha R, Khanal S, Poudel P, Khadayat K, Ghaju S, Bhandari A, Lekhak S, Pant ND, Sharma M, Marasini BP (2019) Extended spectrum β-lactamase producing uropathogenic *Escherichia coli* and the correlation of biofilm with antibiotics resistance in Nepal. Ann Clin Microbiol Antimicrob 18: 42. doi: 10.1186/s12941-019-0340-y.
12. Hammar M, Arnqvist A, Bian Z, Olsén A, Normark S (1995) Expression of two *csg* operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. Mol Microbiol 18: 661-70. doi: 10.1111/j.1365-2958.1995.mmi_18040661.x.
13. Moori Bakhtiari N, Javadmakoei S (2017) Survey on biofilm production and presence of attachment factors in human uropathogenic strains of *Escherichia coli*. Jundishapur J Microbiol 10: e13108. doi: 10.5812/jjm-13108.
14. Arjmand M, Ghaemi E, Jamalli A (2017) Impact of various environmental and growth conditions on antigen 43 gene expression and biofilm formation by uropathogenic *Echserchia coli*. Med Lab J 11: 16-22. doi: 10.18869/acadpub.mlj.11.1.16.
15. Riveros M, García W, García C, Durand D, Mercado E, Ruiz J, Ochoa TJ (2017) Molecular and phenotypic characterization of diarrheagenic *Escherichia coli* strains isolated from bacteremic children. Am J Trop Med Hyg 97: 1329-1336. doi: 10.4269/ajtmh.17-0066.
16. Karigoudar RM, Karigoudar MH, Wavare SM, Mangalgi SS (2019) Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. J Lab Physicians 11: 17-22. doi: 10.4103/jlp.jlp_98_18
17. Boroumand M, Sharifi A, Manzouri L, Khoramrooz SS, Khosravani SA (2019) Evaluation of pap and sfa genes relative frequency P and S fimbriae encoding of uropathogenic *Escherichia coli* isolated from hospitals and medical laboratories; Yasuj City, Southwest Iran. Iran Red Crescent Med J 21: e89499. doi: 10.5812/ircmj.89499
18. Koksal E, Tulek N, Sonmezer MC, Temocin F (2019) Investigation of risk factors for community-acquired urinary tract infections caused by extended-spectrum beta-lactamase *Escherichia coli* and *Klebsiella* species. Investig Clin Urol 60: 46-53. doi: 10.4111/icu.2019.60.1.46
19. Clinical and Laboratory Standards Institute (2021) Performance standards for antimicrobial susceptibility testing, thirty firth informational supplement. CLSI Document M100-S31, Wayne, Pennsylvania, USA.
20. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-81. doi: 10.1111/j.1469-0691.2011.03570.x
21. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, Ruzicka F (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis 115: 891-9. doi: 10.1111/j.1600-0463.2007.apm_630.x.
22. Tüzün T, Sayın Kutlu S, Kutlu M, Kaleli İ (2019) Risk factors for community-onset urinary tract infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. Turk J Med Sci 49: 1206-1211. doi: 10.3906/sag-1902-24.
23. Kot B, Wicha J, Grużewska A, Piechota M, Wolska K, Obrębska M (2016) Virulence factors, biofilm-forming ability, and antimicrobial resistance of urinary *Escherichia coli* strains isolated from hospitalized patients. Turk J Med Sci 46: 1908-1914. doi: 10.3906/sag-1508-105.
24. Hancock V, Ferrières L, Klemm P (2007) Biofilm formation by asymptomatic and virulent urinary tract infectious *Escherichia coli* strains. FEMS Microbiol Lett 267: 30-7. doi: 10.1111/j.1574-6968.2006.00507.x.
25. Sabir N, Ikram A, Zaman G, Satti L, Gardezi A, Ahmed A, Ahmed P (2017) Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. Am J Infect Control 45: 1101-1105. doi: 10.1016/j.ajic.2017.05.009.

26. Guzmán M, Salazar E, Cordero V, Castro A, Villanueva A, Rodulfo H, De Donato M (2019) Multidrug resistance and risk factors associated with community-acquired urinary tract infections caused by *Escherichia coli* in Venezuela. Biomedica 39: 96-107. doi: 10.7705/biomedica.v39i2.4030.
27. Kung CH, Ku WW, Lee CH, Fung CP, Kuo SC, Chen TL, Lee YT (2015) Epidemiology and risk factors of community-onset urinary tract infection caused by extended-spectrum β-lactamase-producing *Enterobacteriaceae* in a medical center in Taiwan: a prospective cohort study. J Microbiol Immunol Infect 48: 168-74. doi: 10.1016/j.jmii.2013.08.006.
28. Haghighatpanah M, Mojtahedi A (2019) Characterization of antibiotic resistance and virulence factors of *Escherichia coli* strains isolated from Iranian inpatients with urinary tract infections. Infect Drug Resist 12: 2747-2754. doi: 10.2147/idr.s219696.
29. Hagos DG, Mezgebo TA, Berhane S, Medhanyie AA (2019) Biofilm and hemagglutinin formation: a Hallmark for drug resistant uropathogenic *Escherichia coli.* BMC Res Notes 12: 358. doi: 10.1186/s13104-019-4382-1.
30. Poursina F, Sepehrpour S, Mobasherizadeh S (2018) biofilm formation in nonmultidrug-resistant *Escherichia coli* isolated from patients with urinary tract infection in Isfahan, Iran. Adv Biomed Res 7: 40. doi: 10.4103/abr.abr_116_17.
31. Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Jahanabadi Z (2018) Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic *Escherichia coli* isolates from Iranian patients. BMC Infect Dis 18: 572. doi: 10.1186/s12879-018-3467-0.
32. Hadifar S, Moghoofei M, Nematollahi S, Ramazanzadeh R, Sedighi M, Salehi-Abargouei A, Miri A (2017) Epidemiology of multidrug resistant uropathogenic *Escherichia coli* in Iran: a systematic review and meta-analysis. Jpn J Infect Dis 70: 19-25. doi: 10.7883/yoken.JJID.2015.652.
33. Garousi M, Monazami Tabar S, Mirazi H, Asgari P, Sabeghi P, Salehi A, Khaledi A, Ghenaat Pisheh Sanani M, Mirzahosseini HK (2022) A global systematic review and meta-analysis on correlation between biofilm producers and non-biofilm producers with antibiotic resistance in Uropathogenic *Escherichia coli*. Microb Pathog 164: 105412. doi: 10.1016/j.micpath.2022.105412.
34. Sedigh Ebrahim-Saraie H, Nezhad NZ, Heidari H, Motamedifar A, Motamedifar M (2018) Detection of antimicrobial susceptibility and integrons among extended-spectrum β-lactamase producing Uropathogenic *Escherichia coli* isolates in southwestern Iran. Oman Med J 33: 218-223. doi: 10.5001/omj.2018.40.
35. Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, Surek M, Picheth G, Fadel-Picheth CM (2011) Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. Genet Mol Res 10: 4114-25. doi: 10.4238/2011.October.31.5.
36. Bader MS, Loeb M, Leto D, Brooks AA (2020) Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. Postgrad Med 132: 234-250. doi: 10.1080/00325481.2019.1680052.
37. Lee Ventola C (2015) The antibiotic resistance crisis: part 1: causes and threats. P T 40: 277-83.
38. Chen M, Zhang M, Huang P, Lin Q, Sun C, Zeng H, Deng Y (2018) Novel β-lactam/β-lactamase inhibitors versus alternative antibiotics for the treatment of complicated intra-abdominal infection and complicated urinary tract infection: a meta-analysis of randomized controlled trials. Expert Rev Anti Infect Ther 16: 111-120. doi: 10.1080/14787210.2018.1429912.
39. Sheu CC, Lin SY, Chang YT, Lee CY, Chen YH, Hsueh PR (2018) Management of infections caused by extended-spectrum β-lactamase-producing *Enterobacteriaceae*: current evidence and future prospects. Expert Rev Anti Infect Ther 16: 205-218. doi: 10.1080/14787210.2018.1436966.
40. Neupane S, Dutt Pant N, Khatiwada S, Chaudhary R, Raj Banjara M (2016) Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control 5: 5. doi: 10.1186/s13756-016-0104-9.
41. Tabasi M, Asadi Karam MR, Habibi M, Yekaninejad MS, Bouzari S (2015) Phenotypic assays to determine virulence factors of Uropathogenic *Escherichia coli* (UPEC) isolates and their correlation with antibiotic resistance pattern. Osong Public Health Res Perspect 6: 261-8. doi: 10.1016/j.phrp.2015.08.002.
42. Delgado-Valverde M, Sojo-Dorado J, Pascual A, Rodríguez-Baño J (2013) Clinical management of infections caused by multidrug-resistant *Enterobacteriaceae*. Ther Adv Infect Dis 1: 49-69. doi: 10.1177/2049936113476284.
43. Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI, Zhang L (2015) Mechanisms of antibiotic resistance. Front Microbiol 6: 34. doi: 10.3389/fmicb.2015.00034.
44. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F (2016) Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob Resist Infect Control 5: 11. doi: 10.1186/s13756-016-0109-4.
45. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, Dahbi G, Blanco M, Ponte Mdel C, Soriano F (2008) Correlation between virulence factors and in vitro biofilm formation by *Escherichia coli* strains. Microb Pathog 45: 86-91. doi: 10.1016/j.micpath.2008.03.003.
46. Asadi Karam MR, Habibi M, Bouzari S (2018) Relationships between virulence factors and antimicrobial resistance among *Escherichia coli* isolated from urinary tract infections and commensal isolates in Tehran, Iran. Osong Public Health Res Perspect 9: 217. doi: 10.24171/j.phrp.2018.9.5.02.
47. Cergole-Novella, MC, Pignatari AC, Guth BE (2015) Adhesion, biofilm and genotypic characteristics of antimicrobial resistant *Escherichia coli* isolates. Braz J Microbiol 46: 167-71. doi: 10.1590/s1517-838246120140077.
48. Schembri MA, Kjaergaard K, Klemm P (2003) Global gene expression in *Escherichia coli* biofilms. Mol Microbiol 48: 253-67. doi: 10.1046/j.1365-2958.2003.03432.x.
49. Hancock V, Ferrières L, Klemm P (2008) The ferric yersiniabactin uptake receptor *FyuA* is required for efficient biofilm formation by urinary tract infectious *Escherichia coli* in human urine. Microbiology (Reading) 154: 167-175. doi: 10.1099/mic.0.2007/011981-0.
50. Malcolm W, Fletcher E, Kavanagh K, Deshpande A, Wiuff C, Marwick C, Bennie M (2018) Risk factors for resistance and MDR in community urine isolates: population-level analysis using the NHS Scotland Infection Intelligence Platform. J Antimicrob Chemother 73: 223-230. doi: 10.1093/jac/dkx363.
51. Ramírez-Castillo FY, Moreno-Flores AC, Avelar-González FJ, Márquez-Díaz F, Harel J, Guerrero-Barrera AL (2018) An

evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. Ann Clin Microbiol Antimicrob 17: 34. doi: 10.1186/s12941-018-0286-5.
52. Guclu E, Halis F, Kose E, Ogutlu A, Karabay O (2021) Risk factors of multidrug-resistant bacteria in community-acquired urinary tract infections. Afr Health Sci 21: 214-219. doi: 10.4314/ahs.v21i1.28.
53. Ben-Ami R, Rodríguez-Baño J, Arslan H, Pitout JD, Quentin C, Calbo ES, Azap OK, Arpin C, Pascual A, Livermore DM, Garau J, Carmeli Y (2009) A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis 49: 682-90. doi: 10.1086/604713.
54. Kuster SP, Hasse B, Huebner V, Bansal V, Zbinden R, Ruef C, Ledergerber B, Weber R (2010) Risks factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care university hospital in Switzerland. Infection 38: 33-40. doi: 10.1007/s15010-009-9207-z.
55. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, Fishman NO, Bilker WB, Mao X, Lautenbach E (2005) Risk factors for increasing multidrug resistance among extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species. Clin Infect Dis 40: 1317-24. doi: 10.1086/429239.
56. Calbo E, Romaní V, Xercavins M, Gómez L, Vidal CG, Quintana S, Vila J, Garau J (2006) Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. J Antimicrob Chemother 57: 780-3. doi: 10.1093/jac/dkl035.
57. Inns T, Millership S, Teare L, Rice W, Reacher M (2014) Service evaluation of selected risk factors for extended-spectrum beta-lactamase *Escherichia coli* urinary tract infections: a case-control study. J Hosp Infect 88: 116-9. doi: 10.1016/j.jhin.2014.07.009.

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