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ORIGINAL RESEARCH

Characterization of antibiotic resistance and virulence factors of *Escherichia coli* strains isolated from Iranian inpatients with urinary tract infections

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Background: Urinary tract infections (UTIs) are one of the most frequent human infectious diseases causing considerable amount of morbidity and mortality. The present study aimed to investigate the occurrence of antibiotics resistance and virulence genes among *Escherichia coli* strains isolated from UTIs in the north of Iran.

Methods: This cross-sectional study was performed at 5 teaching hospitals in Rasht in the north of Iran. Totally, 129 *E. coli* isolates were identified by standard microbiologic tests. Antimicrobial susceptibility pattern was determined using disk diffusion method. The presence of virulence genes was detected by PCR method.

Results: The results of antibiotic susceptibility showed that the highest resistance rates were to ampicillin (78.3%) followed by nalidixic acid (74.4%) and trimethoprim/sulfamethoxazole (69.8%). On the other hand, the highest susceptibility was toward nitrofurantoin (96.1%) and imipenem (92.2%). Further analysis revealed that the rate of ESBL-producing and multiple-drug resistant isolates was 51.2% and 84.5%, respectively. Molecular analysis revealed that *traaT* (87.6%) gene was the most prevalent virulence factors followed by *fyuA* (86%) and *kpsmt* (76%) genes. Also, *fimH* gene was the most frequently detected adhesion-associated gene with 74.4%.

Conclusion: In summary, our results showed a remarkable rate of drug resistance and heterogeneity for virulence factors among *E. coli* strains isolated from UTIs in the north of Iran. The emergence of such strains can be a predictive marker for their persistence in the hospital and consequently a significant threat for hospitalized patients.

Keywords: urinary tract infections, *Escherichia coli*, antibiotics resistance, ESBL, virulence factors

Introduction

Urinary tract infections (UTIs) are one of the most frequently occurring infectious diseases in both hospital and community settings which cause considerable amount of morbidity and mortality.[1] Although the UTIs caused by a wide array of pathogens, *Escherichia coli* is responsible for the majority of infections.[2] UTIs nearly recognized to occur in all age groups, but some groups such as neonates, pregnant women or the elderly patients are more vulnerable.[3,4]

Acquisition of potential virulence markers by *E. coli* strains might increase their ability to resist and overcome the host immune defenses and subsequently a severe

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infection.[5] These virulence factors are usually carried on the large number of pathogenicity-associated islands which can be easily disseminated by microorganisms through different horizontal gene transfer mechanisms.[6] The characterization of virulence factors such as adhesions, toxins and iron uptake systems can be useful to improve our understanding the pathogenicity of symptomatic or complicated UTIs.[7]

On the other hand, the emergence of antimicrobial-resistant strains has become a serious public health concern and leading to increased mortality and morbidity.[8] Excessive and inappropriate use of antibiotics is the main factor for the increasing rate of multiple-drug resistant (MDR) strains which are commonly related to the increasing trend of extended-spectrum beta-lactamase (ESBL)-producing bacteria.[8,9] The recent emergence of multi-resistant extraintestinal pathogenic *Escherichia coli* (ExPEC) strains linked to increasing prevalence of ESBLs in both hospital and community settings. *E. coli* sequence type 131 is an international high-risk clone, which well known for high ability of horizontal gene transfer that could confer resistance to most of the critically important antimicrobial classes, including fluoroquinolones and third- and fourth-generation cephalosporins.

Due to diversity of virulence patterns among *E. coli* strains causing UTIs and also the limited therapeutic options for management of infections caused by MDR strains,[5] the knowledge about their pathogenicity and antibiotic resistance trends and is a rational way to overcome the risk of treatment failure. Therefore, the present study aimed to investigate the occurrence of antibiotics resistance and virulence genes among *E. coli* strains isolated from UTIs in the north of Iran.

Materials and methods

Study population and bacterial isolates

This cross-sectional study was performed at five teaching and remedial hospitals in Rasht in the north of Iran. The study design was in accordance with the declaration of Helsinki and approved by the institutional ethics committee of Guilan University of Medical Sciences (Approval No. IR.SUMS. REC.1395.S747). However, because only leftovers from clinical specimens were used, the local ethics committee waived the need for informed consent. Midstream voided urine specimens were collected using the clean-catch method in sterile disposable tube for adult, sterile urine bags for children and catheter or suprapubic needle aspiration for neonates and analyzed immediately in the laboratory. After microscopic examination, urine samples were cultured on 5% blood agar and MacConkey agar using standard quantitative 10 µL loops and incubated aerobically for 24 hrs at 37°C. UTI is defined as the presence of single organism in the urine in quantities of 10^3–10^5 colony forming unit (CFU) per milliliter (cfu/mL). The mixed growth of bacteria was considered as contamination. A total of 129 non-duplicate *E. coli* isolates were isolated from clean-catch midstream urine of studied subjects. The bacteria were identified and confirmed using standard microbiologic biochemical tests including Gram staining, cultivation, and reactions on MacConkey agar, triple sugar iron agar, SIM medium, Simmons' citrate agar, MR-VP medium.

Antimicrobial susceptibility testing

Antibiotic susceptibility of all isolates to ampicillin, amoxicillin-clavulanate, cefoxitin, cefixime, ceftazidime, ceftriaxone, cefotaxime, cefepime, ciprofloxacin, ofloxacin, nalidixic acid, aztreonam, tetracycline, gentamicin, trimethoprim/sulfamethoxazole, nitrofurantoin, and imipenem (Mast Co., UK) was carried out on Muller-Hinton agar (Oxoid Co., UK) using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI).[10] *E. coli* ATCC 25,922 was used as the quality control strain for antibacterial susceptibility testing. The isolates non-susceptible to ≥1 agent at least three of antibiotic categories were defined as MDR.[11]

Phenotypic detection of ESBL

All isolates were tested for ESBL production by double-disk synergy test using ceftazidime (30 µg) and cefotaxime (30 µg) disks, and combination with clavulanic acid (10 µg) disk as described by CLSI procedure. According to the CLSI guidelines, an increase of ≥5 mm in the diameter of the inhibition zones around the combination disk as compared to the inhibition zones around the single antibiotic disk indicated as ESBL producers.[10] *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively.

DNA extraction and virulence genotyping

Genomic DNA was extracted from all *E. coli* isolates using High Pure DNA Template preparation kit (Roshe, Germany) according to the manufacturer's instructions. The targeted genes and nucleotide sequences of the oligonucleotide primers used in this study were chosen as previously described (Table 1).[12–14] PCR amplification for detection of virulence

Table 1 The sequences of used primers

Target	Direction	Sequences (5′ to 3′)	Function	Role	Ref.
iroN	F R	AAGTCAAAGCAGGGGTTGCCCG GACGCCGACATTAAGACGCAG	Siderophore receptor	Iron chelator	13
fyuA	F R	TGATTAACCCCGCGACGGGAA TGATTAACCCCGCGACGGGAA	Ferric yersiniabactin uptake receptor		12
iutA	F R	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	Ferric aerobactin receptor		
bmaE	F R	ATGGCGCTAACTTGCCATGCTG AGGGGGACATATAGCCCCCTTC	M blood group antigen-specific M fimbriae	Adhesin	
sfa/focDE	F R	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	S fimbrial adhesins and F1C fimbriae		
sfaS	F R	GTGGATACGACGATTACTGTG CCGCCAGCATTCCCTGTATTC	S fimbrial adhesins		
focG	F R	CAGCACAGGCAGTGGATACGA GAATGTCGCCTGCCCATTGCT	F1C fimbriae		
afa/draBC	F R	GGCAGAGGGCCGGCAACAGGC CCCGTAACGCGCCAGCATCTC	Mannose-resistant hemagglutination		
papG II	F R	GGGATGAGCGGGCCTTTGAT CGGGCCCCCAAGTAACTCG	Gal (alpha 1–4) Gal-binding adhesin molecules of P fimbriae		
papG III	F R	GGCCTGCAATGGATTTACCTGG CCACCAAATGACCATGCCAGAC			
fimH	F R	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	Type 1 fimbrial adhesin		
papAH	F R	ATGGCAGTGGTGTCTTTTGGTG CGTCCCACCATACGTGCTCTTC	P fimbrial		
papC	F R	GTGGCAGTATGAGTAATGACCGTTA ATATCCTTTCTGCAGGGATGCAATA			
papEF	F R	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA			
kpsMT II	F R	GCGCATTTGCTGATACTGTTG CATCCAGACGATAAGCATGAGCA	Polysaccharide capsule	Protective	
rfc	F R	ATCCATCAGGAGGGGACTGGA AACCATACCAACCAATGCGAG	O antigen polymerase		
traT	F R	GGTGTGGTGCGATGAGCACAG CACGGTTCAGCCATCCCTGAG	Anti-complementary protein of TraT		
usp	F R	ACATTCACGGCAAGCCTCAG AGCGAGTTCCTGGTGAAAGC	Uropathogenic-specific protein (genotoxin)	Invasion	14
ibeA	F R	AGGCAGGTGTGCGCCGCGTAC TGGTGCTCCGGCAAACCATGC	Invasion of the brain endothelium protein A		12
cnf1	F R	AAGATGGAGTTTCCTATGCAGGAG CATTCAGAGTCCTGCCCTCATTATT	Cytotoxic necrotizing factor-1		
hlyA	F R	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	Hemolysin A		

genes was carried out on a Veriti 96-well thermal cycler instrument (Applied Biosystems at Life Technologies, Foster City, CA, USA). The PCR program consisted of an initial denaturation step at 95°C for 5 mins, followed by 30 cycles of DNA denaturation at 95°C for 30 s, primer annealing for 30 s. Temperature was depending on the sequences of primers, and primer extension at 72°C for 1 min, followed by a final extension at 72°C for 7 mins. The amplifications were separated on 1.5% agarose gel prepared in 1X Tris/Boric acid/EDTA (TBE) buffer and visualized using ultraviolet light after staining with safe stain dye (CinnaGen Co., Iran).

Data analysis

The analysis of results was performed by using SPSS™ software, version 21.0 (IBM Corp., Armonk, NY, USA). The results are presented as descriptive statistics in terms of relative frequency. The Chi-square (χ^2) or Fisher's exact tests was performed to analyze significant differences. A P-<0.05 was considered to be significant.

Results

Of 129 *E. coli* isolates included in our study, 90 (69.8%) and 39 (30.2%) were isolated from femaleand male patients, respectively. The mean age ± SD of the patients was 50.1 ±27.7 years, ranging from 1 to 90 years old. Also, 17.8% of patients were aged 1–18 years, 37.2% aged 19–60 years, and 45% aged more than 60 years old.

The results of antibiotic susceptibility showed that the highest resistance rate was against ampicillin (78.3%) followed by nalidixic acid (74.4%) and trimethoprim/sulfamethoxazole (69.8%). On the other hand, the highest susceptibility was toward nitrofurantoin (96.1%) followed by imipenem (92.2%) and cefoxitin (74.4%). Further analysis revealed that the rate of ESBL-producing isolates was 51.2% (66/129). Moreover, there was a significant correlation between ESBL-producing isolates and their antibiotic resistance to all of the antibiotics, except for nitrofurantoin and imipenem. The full results of antibiotic resistance patterns of isolates are presented in Table 2. Also, the frequency of MDR isolates was estimated at 84.5%. The rate of MDR isolates was significantly higher among ESBLs-producers than non-ESBL producers (100% vs 68.3%, P<0.001).

Molecular analysis revealed that the *traaT* (87.6%) was the most prevalent virulence factors followed by *fyuA* (86%) and *kpsmt* (76%) genes. Also, *fimH* gene was the most frequently detected adhesion-associated gene with 74.4%. The full results of the investigated virulence factors distribution in *E. coli* isolates are presented in Table 3. According to the results, the frequency of 9 genes (*fyuA, papC, papG* II, *fimH, kpsMT, traT, iroN, usp* and *afa/draBC*) was more than 50% among the isolates. In addition, the frequency of *bmaE* gene was significantly higher among the ESBLs-producing isolates compared to non-ESBL-producing isolates (P<0.001).

Analyzing drug-resistance and gene patterns showed a high heterogeneity among isolates (Table S1). The most prevalent drug-resistance pattern was AZT-GEN-SXT-TET-CIP-OFX-NAL-CAZ-CRO-FEP-CFM-CTX-FOX-CEP-AUG-AMP with 8.5%, while none of the gene pattern exceeded 2%.

Discussion

Characterization of the drug resistance and virulence determinants of *E. coli* strains, particularly in hospitalized patients allows the physicians to reduce the risk of complications and also optimizing available infection control policies.[5] Here, we report the prevalence of antibiotic resistance and virulence factors of ESBL-producing *E. coli* isolates causing UTIs in the north of Iran.

UTIs are generally treated empirically by physicians, therefore aware of the local epidemiological data for an efficient therapy is necessary and decrease the further uncomfortable outcome.[1] In our results, the majority of isolates were resistant to most of tested antibiotics and 84.5% of isolates were also MDR. Although our estimate of MDR isolates were higher than the pooled prevalence of MDR uropathogenic *E. coli* (UPEC) with 49.4% in Iran, it was also showed that the relative frequency of MDR isolates is varied from 10.5% to 79.2% in different regions.[9] Moreover, comparing to others, it seems that Asian and African countries experiencing the highest rates of MDR-UPEC while North America and Europe have the lowest rates. The high rate of resistance to these antibiotics may be because of the inappropriate and overuse of antibiotics in the last decade.[15] The majority of our isolates were susceptible to nitrofurantoin (96.1%) and imipenem (92.2%), which was closest with reports from Iran and other countries.[5,16–19] However, carbapenems remain as the last-line treatment option against infections caused by drug-resistant strains, since numerous side effects of nitrofurantoin limited its application.[5] As a consequence of increased prescription of fluoroquinolones, resistance to these antibiotics in uropathogens has been increasing globally.[20] Regarding this phenomenon, the majority of our isolates similar to recent fluoroquinolones

Table 2 Distribution of antibiotic resistance pattern according to ESBL production

Antibiotic	Total no. 129			ESBLs-positive no. 66			*P*-value
	Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)	Susceptible no. (%)	Intermediate no. (%)	Resistant no. (%)	
Ampicillin	24 (18.6)	4 (3.1)	101 (78.3)	0	1 (1.5)	65 (98.5)	<0.001
Amoxicillin-clavulanate	59 (45.7)	43 (33.3)	27 (20.9)	15 (22.7)	31 (47)	20 (30.3)	<0.001
Cefoxitin	96 (74.4)	13 (10.1)	20 (15.5)	37 (56.1)	12 (18.2)	17 (25.8)	<0.001
Cefixime	49 (38)	9 (7)	71 (55)	1 (1.5)	0	65 (98.5)	<0.001
Ceftazidime	51 (39.5)	21 (16.3)	57 (44.2)	2 (3)	9 (13.6)	55 (83.3)	<0.001
Ceftriaxone	51 (39.5)	5 (3.9)	73 (56.6)	1 (1.5)	0	65 (98.5)	<0.001
Cefotaxime	43 (33.3)	11 (8.5)	75 (58.1)	1 (1.5)	0	65 (98.5)	<0.001
Cefepime	76 (58.9)	7 (5.4)	46 (35.7)	17 (25.8)	5 (7.6)	44 (66.7)	<0.001
Ciprofloxacin	50 (38.8)	10 (7.8)	69 (53.5)	7 (10.6)	4 (6.1)	55 (88.3)	<0.001
Ofloxacin	64 (49.6)	0	65 (50.4)	13 (19.7)	0	53 (80.3)	<0.001
Nalidixic acid	27 (20.9)	6 (4.7)	96 (74.4)	3 (4.5)	0	63 (95.5)	<0.001
Aztreonam	56 (43.4)	9 (7)	64 (49.6)	1 (1.5)	5 (7.6)	60 (90.9)	<0.001
Tetracycline	46 (35.7)	3 (2.3)	80 (62)	13 (19.7)	2 (3)	51 (77.3)	<0.001
Gentamicin	84 (65.1)	7 (5.4)	38 (29.5)	30 (45.5)	2 (3)	34 (51.5)	<0.001
Trimethoprim/ sulfamethoxazole	38 (29.5)	1 (0.8)	90 (69.8)	10 (15.2)	0	56 (84.8)	<0.001
Nitrofurantoin	124 (96.1)	3 (2.3)	2 (1.6)	63 (95.5)	1 (1.5)	2 (3)	0.522
Imipenem	119 (92.2)	10 (7.8)	0	60 (90.9)	6 (9.1)	0	0.402

resistance reports from central part (61.3%),[21] and south (55.6%) of Iran,[5] were resistant to fluoroquinolones including ciprofloxacin (53.5%) and ofloxacin (50.4%).

In our results, a remarkable proportion of *E. coli* isolates was ESBL producers (51.2%), but this rate is consistent with the median values reported from Iran varied form 24% to 72.9% among UPEC.[22] These findings indicate to a great discrepancy in the prevalence of ESBL producers which is mainly due to differences in geographical regions, infection control policy, and sample source. ESBLs are a group of plasmid-mediated beta-lactamases with inactivation capability of beta-lactams and no detectable activity against cephamycins and carbapenems.[22] Concerning this, the rates of antibiotic resistance among our ESBL producers were significantly higher than non-ESBL producers. Hopefully, based on our results, nitrofurantoin and imipenem still have a promising activity against ESBL-producing isolates of *E. coli*.

Adhesins associated genes are the most frequently found virulence factors in *E. coli* strains isolated from UTIs.[7] The prevalence of adhesins among UTIs derived *E. coli* can be greatly heterogeneous; however, it is suggested that type 1 and P fimbriae are the most common type/s.[5] In our results, *fimH* gene encoding of the type 1fimbriae was found in 74.4% of isolates as the most prevalent adhesion. This finding was in agreement with the most previous studies in this field form Iran and other countries.[5,16,17,23–25] Moreover, 71.3% of our isolates had *papG* II which is responsible for encoding PapG adhesion on the tips of P fimbriae. In previous studies, it has been shown that different classes of PapG adhesion are predominant in those *E. coli* strains isolated from UTIs,[5,17,26] which support the results of the present study.

Scarcity of iron in the human urinary tract leading to up-regulation of different iron-transport systems such as aerobactin and yersiniabactin in uropathogens.[27] In our results, *fyuA* gene encoding yersiniabactin receptor with 86% was found as the most frequent iron chelator factor. Previously, consistent with our results, it was suggested that the presence of *fyuA* as one of the best predictor of UPEC predicts more efficient colonization of *E. coli* isolates in the bladder.[25,28–30]

Among the different tested toxins of *E. coli* in our survey, *usp* genes which designated for an uropathogenic specific protein was found in 68.2% of isolates, more than other toxins (*cnf1, hlyA* and *ibeA*). Previously, several authors showed that *usp* may contribute to the causation of UTIs and may be considered as a major virulence determinant of UPEC.[31–34]

Finally, among protective virulence factors, *traT* which encoding an anti-complementary protein and *kpsMT* II

Table 3 Distribution of virulence factors according to ESBL production

Type	Gene	Total no. 129		ESBLs-positive No. 66		*P*-value
		No.	%	No.	%	
Iron chelator	*fyuA*	111	86	57	86.4	0.915
	iutA	32	24.8	19	28.8	0.284
	iroN	75	58.1	38	57.6	0.894
Adhesin	*bmaE*	35	27.1	23	34.8	0.044
	sfa/focDE	27	20.9	13	19.7	0.725
	sfaS	12	9.3	6	9.1	0.933
	focG	12	9.3	6	9.1	0.933
	afa/draBC	68	52.7	37	56.1	0.436
	papAH	39	30.2	23	34.8	0.243
	papC	72	55.8	39	59.1	0.443
	papEF	21	16.3	12	18.2	0.549
	papG II	92	71.3	44	66.7	0.232
	papG III	62	48.1	35	53	0.248
	fimH	96	74.4	52	78.8	0.244
Protective	*kpsMT* II	98	76	50	75.8	0.954
	rfc	4	3.1	1	1.5	0.288
	traT	113	87.6	59	89.4	0.526
Invasion	*ibeA*	31	24	17	25.8	0.639
	hlyA	39	30.2	23	34.8	0.243
	usp	88	68.2	47	71.2	0.455
	cnf1	44	34.1	26	39.4	0.195

encoding polysaccharide capsule were found in 87.6% and 76% of isolates, respectively. Previously, in two Iranian studies, the prevalence of *kpsMT* II in UPEC isolates was reported 9% and 4.1%, respectively.[35,36] In Germany, Toval et al reported the prevalence of *traT* in 67.9% and *kpsMT* II in 39.3% of UPEC isolates.[37] In another study from Brazil, the prevalence of *traT* in UPEC isolates was estimated at 76%.[38] This observation indicating heterogeneity in the distribution of virulence genes in UPEC strains in different regions.

As the main limitations of the present study, the lack of data on community or hospital origin of UTI, and genes expression assay should be acknowledged.

In summary, our results showed a remarkable rate of drug resistance and heterogeneity for virulence factors among *E. coli* strains isolated from UTIs in the north of Iran. The emergence of such strains can be a predictive marker for their persistence in the hospital and consequently a significant threat for hospitalized patients. These findings provide experimental evidences for the prescription of more effective therapy based on antibiogram and optimizing infection control policies.

Author contributions

Both authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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