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First report for the Distributions of the most common serogroups of uropathogenic *Escherichia coli* and trimethoprim resistant dfrA genes from Damascus – Syria

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Abstract: Urinary tract infections (UTIs) are one of the most common bacterial infections with global expansion. These infections are predominantly caused by uropathogenic *Escherichia coli* (UPEC). The aims of our work were to determine the prevalence of O serogroups of *Escherichia coli* (*E. coli)* strains that cause UTI and to find out the genes which are responsible of trimethoprime antibiotic resistance. In this study, 75 patients from different age groups of females and males with UTI referred to two general Hospitals in Damascus were enrolled, during the period from 7/2012 to 8/2013. Multiplex PCR methods was used to screen a collection of several O-serogroups, including O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83, which are preferentially associated with UPEC strains. Furthermore, five common integron carried trimethoprime resistant genes (*dfrA1, dfrA5, dfrA7, dfrA12, dfrA17*) were selected as multiplex PCR targets to determine the most common resistant gene. Our results shown that O8 (20%), and O25 (24.61%) were the most two common types. There was no significant correlation between the presence of O antigens and age of patients ($P > 0.05$). *dfrA17* (47.69%) gene was the most common resistant gene. There was significant correlation between the presence of the O antigens and the five trimethoprim resistance *dfrA* genes ($P<0.05$). This is the first report of *E. coli* serotyping in patients with UTI from Damascus in Syria and their relation to trimethoprime resistance.
Keywords: Multiplex PCR, O-antigen, Urinary tract infection, trimethoprim resistance, *dfr* genes.

Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases encountered in the clinical practice, mainly being associated with different members of the *Enterobacteriaceae* family [1, 2]. It is estimated that between 40 and 50% of women experience a UTIs at least once during their lifetime, and that 33% of women in the United States suffering from a UTIs require antimicrobial therapy by the age of 24 [3, 4]. In general, rates of UTIs are higher among women than men [5, 6].

Escherichia coli (*E. coli*) -the most prominent member of the family of *Enterobacteriaceae*- is the number one cause of UTIs [7]. *E. coli*, the most prevalent facultative gram-negative bacillus in the human fecal flora, usually inhabits the colon as an innocuous commensal [8]. Extraintestinal pathogenic *E. coli* (ExPEC) strains that cause UTIs are called uropathogenic *E. coli* (UPEC) [9]. *E. coli* is a clonal species that includes both commensal and pathogenic clones of strains, which are normally identified by serological typing of their O (lipopolysaccharide), H (flagellar), and, in some cases, K (capsular) surface antigens. Recently, 174 O-serogroups had been described for *E. coli* [10]. Thus far, the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* based at the Statens Serum Institut (SSI) in Denmark

(http://www.ssi.dk/English.aspx) has recognized 184 *E. coli* O serogroups. It is generally believed that the O serogrouping of *E. coli* strains provides valuable information for identifying pathogenic clonal groups, especially for public health surveillance [11].

For UPEC, the virulence factor (VF) profile of each strain is related with their O-serogroups. It has been widely accepted that serotyping can be used as an epidemiological marker for pathogenicity for *E. coli* [12]. Several O-serogroups, including O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83, are preferentially associated with UPEC strains [1, 13-19]. Certain O-serogroups were found to occur with a variable frequency in the individual patient groups with UTI [20].

In *E. coli*, the genes required for O-antigen biosynthesis are clustered at a chromosomal locus flanked by the colanic acid biosynthesis gene cluster (wca genes) and the histidine biosynthesis (his) operon. Generally, the O-antigen biosynthesis genes fall into three classes: (i) the nucleotide sugar biosynthesis genes, (ii) the sugar transferase genes, and (iii) those for O-unit translocation and chain synthesis (wzx/wzy in the Wzx/Wzy-dependent pathway and wzm/wzt in the Wzm/Wzt-dependent ABC transporter pathway) [21]. To date, >90 types of O-antigen biosynthesis gene cluster (O-AGC) sequences have been determined, with the majority derived from major human and animal pathogens[22].

The treatment of UTIs caused by UPEC often requires antimicrobial therapy. Trimethoprim is commonly used in the treatment of UTIs in all parts of the world [23]. However, already soon after the introduction of the drug, trimethoprim resistance was reported in several species [24] and are now in unselected UTIs materials at levels of 15–65% in *E. coli* [25-27].

Trimethoprim resistance in clinically significant Gram-negative bacteria is usually caused by horizontally transferable resistance genes (*dfr* genes) coding for alternative resistant dihydrofolate reductases. Most such genes can be found as gene cassettes carried by integrons forming parts of transposons, which mediate widespread dissemination of trimethoprim resistance [23, 28]. Of 31 known *dfr* genes, a few types seem to predominate in most parts of the world. In a study of 90 *dfr* gene cassettes carried by class 1 integrons in uropathogenic *E. coli* isolates from 16 European countries and Canada, 36 were *dfrA1*, eight were *dfrA5*, four were *dfrA7*, four were *dfrA12* and 28 were *dfrA17*. In our country Syria this type of studies is most needed as in developing countries where antibiotics misuse is commonplace.

With all the data we collected, and view of the situation in our region, the objective was to investigate the distribution of the most common serogroups O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83 which are preferentially associated with UPEC strains isolated from patients with UTIs in Damascus, and the frequency of the common integron carried genes (*dfrA1, dfrA5, dfrA7*, *dfrA12* and *dfrA17*).

Methods

Bacterial strains

A total of 75 strains isolated from patients with UTIs in deferent age groups, males and females were enrolled in the current investigation. Isolates were collected in the form of bacterial colonies on the EMB agar. These Samples were taken from tow general hospitals in Damascus and during a period of time ranging between 7/2012 and 8/2013. All 75 isolates were purified on EMB agar plates (Titan Biotech- India) incubation was at 37□C for 24 hours. The colonies were identified based on biochemical characteristics using API20E kit. Further confirmation was performed by PCR amplifying a target fragment (919 bp) of the *E. coli* 16S rRNA gene. The strains which were confirmed as *E. coli*- positive were kept in Luria-Bertani /glycerol at −80°C.

DNA purification

Each strains of *E. coli* was cultured in 3 ml LB broth medium (Luria-Bertani medium), (Merck, Germany) overnight at 37°C with aeration. Genomic DNA was isolated from the bacterial pellet after centrifugation of 1 ml of medium using Wizard DNA purification kit (Promega USA) according to the manufacturer's instructions then stored at −20°C and used as template in multiplex PCR for detection of uropathogenic *E. coli* seogroups.

While the template DNA used in multiplex PCR for detection of class 1 integrons - trimethoprim resistance *dfrA* genes was extracted from a single *E. coli* colony using QIAprep Spin Miniprep Kit (QIAGEN GmbH, 27104) according to manufacturer's instructions, then stored at −20°C as a template DNA stock.

Detection of uropathogenic *E. coli* serogroups, trimethoprim resistance *dfrA* genes

In our study various multiplex PCR assay were applied for detection of UPEC serogroups (O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83), and the five common integron carried genes (*dfrA1, dfrA5, dfrA7, dfrA12* and *dfrA17*). Table 1 shows the primers used for detection of UPEC serogroups. While table 2 shows the primers used for detection of the five *dfrA* genes.

In order to reduce non-specific amplification, the thermal cycler was pre-warmed to 80 °C before the reaction tubes were inserted. PCR amplification of the DNA was confirmed by running 10 µl of the PCR product on a 1.5% agarose gel (í-Mupid, Mini Agarose Gel Electrophoresis USA). Gel images have been captured digitally and analyzed using the UV transilluminator (cleaver scientific Ltd UK).

Antibiotic Susceptibility Testing using E-test

E. coli isolates were screened for susceptibility to trimethoprim with the use of E-test strips (bioMérieux S.A.69280 Marcy l'Etoile France). A bacterial suspension using 0.5 MCfarland methods was prepared and bacterial isolate transferred to Muller Hinton agar plates. E. test strip of trimethoprim antibiotic was placed on the surface of the plate. After 24 hours incubation in 37°C, the triangle inhabited growth areas were studied the sensitivity was determined using the reference table provided by the procedure of E. test attached by the manufacture.

Table 1. Primers used for detection of uropathogenic *E. coli* serogroups [29].

***E. coli* serogroup**	**Specific gene**	**Gene bank accession no or reference**	**Primer name**	**Primer sequence (5′–3′)**	**Amplicon size (bp)**
EC O1	wzx	GU299791	wl-14632 wl-14633	(F)GTGAGCAAAAGTGAAATAAGGAACG (R) CGCTGATACGAATACCATCCTAC	1098
EC O6	wzy	AJ426423	wl-14646 wl-14647	(F)GGATGACGATGTGATTTTGGCTAAC (R) TCTGGGTTTGCTGTGTATGAGGC	783
EC O7	wzx	AF125322	wl-14648 wl-14649	(F) CTATCAAAATACCTCTGCTGGAATC (R) TGGCTTCGAGATTAAACCTATTCCT	610
EC O8	orf469	AB010150	wl-14652 wl-14653	(F)CCAGAGGCATAATCAGAAATAACAG (R)GCAGAGTTAGTCAACAAAAGGTCAG	448
EC O16	wzx	AAC31631	wl-14654 wl-14655	(F) GGTTTCAATCTCACAGCAACTCAG (R) GTTAGAGGGATAATAGCCAAGCGG	302
EC O21	wzx	EU694098	wl-14676 wl-14677	(F) CTGCTGATGTCGCTATTATTGCTG (R) TGAAAAAAAGGGAAACAGAAGAGC	209
EC O75	wzy	GU299795	wl-17413 wl-17414	(F) GAGATATACATGGGGAGGTAGGCT (R) ACCCGATAATCATATTCTTCCCAAC	511
EC O 2	wzy	GU299792	wl-14636 wl-14637	(F) AGTGAGTTACTTTTTAGCGATGGAC (R) AGTTTAGTATGCCCCTGACTTTGAA	770
EC O 4	wzx	AY568960	wl-14642 wl-14643	(F) TTGTTGCGATAATGTGCATGTTCC (R) AATAATTTGCTATACCCACACCCTC	664
EC O 15	wzy	AY647261	wl-14672 wl-14673	(F) TCTTGTTAGAGTCATTGGTGTATCG (R) ATAAAACGAGCAAGCACCACACC	183
EC O 18	wzx	GU299793	wl-14656 wl-14657	(F) GTTCGGTGGTTGGATTACAGTTAG (R) CTACTATCATCCTCACTGACCACG	551
EC O 22	wzx	DQ851855	wl-14660 wl-14661	(F) TTCATTGTCGCCACTACTTTCCG (R) GAAACAGCCCATGACATTACTACG	468
EC O 25	wzy	GU299796	wl-14666 wl-14667	(F) AGAGATCCGTCTTTTATTTGTTCGC (R) GTTCTGGATACCTAACGCAATACCC	230
EC O 83	wzx	GU299797	wl-14668 wl-14669	(F) GTACACCAGGCAAACCTCGAAAG (R) TTCTGTAAGCTAATGAATAGGCACC	362

Table 2. Primers used for detection of the five trimethoprim resistance dfrA genes [30].

Primer	Locus	Primer sequence (5′–3′)	Amplicon size (bp)
dfr1-f *dfr1-r*	*5′- dfr1* *3′- dfr1*	TGGTAGCTATATCGAAGAATGGAGT TATGTTAGAGGCGAAGTCTTGGGTA	425
dfr5-f *dfr5-r*	*5′- dfr5* *3′- dfr5*	AGCTACTCTTTAAAGCCTTGACGTA GTGTTGCTCAAAAACAACTTCG	341
dfr7&17-f *dfr7&17-r*	*5′- dfr7 and 5′- dfr17* *dfr17 and- 3′ dfr7- 3′*	ACATTTGACTCTATGGGTGTTCTTC AAAACTGTTCAAAAACCAAATTGAA	280
dfr7-r	*3′- dfr7*	ACCTCAACGTGAACAGTAGACAAAT	227 with *dfr7&17*-f
dfr17-r	*3′- dfr17*	TCTCTGGCGGGGGTCAAATCTAT	171 with *dfr7&17*-f
dfr12-f *dfr12-r*	*5′- dfr1* *3′- dfr1*	GAGCTGAGATATACACTCTGGCACT GTACGGAATTACAGCTTGAATGGT	155

Statistical analysis

The data were analyzed using SPSS software (Version 17. SPSS Inc, United States) Descriptive analyses were done for parametric and non-parametric variables. Chi-square test was used for evaluation of correlation between uropathogenic *E. coli* serotypes and age. And to find any significant correlation between uropathogenic *E. coli* serotypes and the five trimethoprim resistance *dfrA* genes in isolates taken from patients with UTI. Statistical significance was regarded at a P value<0.05.

Results

Detection of O serogroups

Our results showed that 86.66% (n=65) of collected isolates diagnosed as *E. coli* according to biochemical characteristic using API 20E and with further confirmation applying PCR depends on *E. coli* 16S rRNA gene. Identified isolates showed high distribution of UPEC serogroups (Table 3). O8 (20%), O25 (24.61%), O15 (6.15%), had the highest percentage while O 21 (6.15%), O6 (6.15%), O18 (3.07%), O16 (3.07%), O1 (3.05%), O4 (4.61%), O22 (3.07%), O83 (4.61%) had the lowest percentage. Besides, the serogroups of 17.85% (n=10) UPEC strains isolated from patients could not be detected and were diagnosed as non-detected serogroups (table 3).

Table 3. Frequency of *E. coli* serogroups in the patients with urinary tract infection.

***E. coli* serogroups**	**number**	**percentage**
EC O 1	2	3.05%
EC O 6	4	6.15%
EC O 7	0	0
EC O 8	13	20%
EC O 16	2	3.07%
EC O 21	4	6.15%
EC O 75	0	0
EC O 2	0	0
EC O 4	3	4.61%
EC O 15	4	6.15%
EC O 18	2	3.07%
EC O 22	2	3.07%
EC O 25	16	24.61%
EC O 83	3	4.61%
non-detected serogroups	10	17.85%
Total	65	100

The Relationship between age of the patients and serotypes shown in (table 4). There was no significant correlation between the presence of the O antigens and the age of patients (P=0.317>0.05).

Table 4. The Relationship between age of the patients with urinary tract infections and uropathogenic *E. coli* serotypes.

	O1	O6	O7	O8	O16	O21	O75	O2	O4	O15	O18	O22	O25	O83
Children	2	3	0	2	1	3	0	0	1	1	1	0	7	2
adults	0	1	0	11	1	1	0	0	2	3	1	2	9	1
P value	0.137													

P = 0.137, there was no significant between the presence of the O antigens and the age of patients.

Detection of trimethoprim resistance *dfrA* genes

E. test results indicated that the UPEC serogroups had high resistance to trimethoprim. 81.53% (n=53) of isolates were resistant, and 18.46% (n=12) were susceptible which agree with multiplex PCR results (table 5).

According to multiplex PCR results, *dfrA17* (47.69%), *dfrA5* (13.84%) had the highest distributions of the five trimethoprim resistance *dfrA* genes. *dfrA7* (4.61%), *dfrA12* (9.23%), *dfrA1* (6.15%) had the lowest. While the *dfrA* genes were not detected in 18.46% (n=12) of isolates.

Table 5 shows the distributions of trimethoprim resistant *dfrA* genes of UPES isolated from patients with UTIs. And Table 6 explained the relation between distribution of trimethoprim resistant genes and uropathogenic *E. coli* serogroups isolated from UTI in Damascus-Syria.

According to Chi-square test, There was significant correlation between the presence of the O antigens and the five trimethoprim resistance *dfrA* genes (P = 0.0.038<0.05).

Depending on Cramer's test, the degree of correlation is 48% which means that we have medium correlation. This result depends on the volume of the sample input.

Table 5. Distribution of trimethoprim resistant genes in uropathogenic *E. coli* isolated from urinary tract infections.

***dfrA* genes**	**number**	**percentage**
dfrA7	3	4.61%
dfrA17	31	47.69%
dfrA1	4	6.15%
dfrA5	9	13.84%
dfrA12	6	9.23%

Table 6. Distribution of trimethoprim resistant genes in uropathogenic *E. coli* serogroups isolated from urinary tract infections in Damascus-Syria.

Gene	**O1 (2)**	**O6 (4)**	**O8 (13)**	**O16 (2)**	**O21 (4)**	**O15 (4)**	**O18 (2)**	**O22 (2)**	**O25 (16)**	**O83 (3)**	**O4 (3)**	**Non detect (10)**
dfrA7	-	-	1	-	-	-	-	-	1	-	-	1
dfrA17	1	-	3	1	4	2	-	-	10	1	1	5
dfrA1	-	-	2	-	-	-	1	-	1	-	-	-
dfrA5	-	2	1	-	-	2	-	1	-	1	-	2
dfrA12	-	2	-	-	-	-	-	-	-	-	2	1
P value	0.038											

P = 0.038, There was significant correlation between the presence of the O antigens and the five trimethoprim resistance *dfrA* genes

Discussion

E. coli strains were classified based on various types of O antigen for the first time by Kauffmann; [35]. 164 types of O antigen have been detected till now[36].Systematic O-serogrouping of *E. coli* began in the early 1930s [31], and it became an important tool for the classification of *E. coli* strains in clinical settings. Moreover, O-serogrouping of *E. coli* has been an invaluable typing method for epidemiological investigations [12]. It has been reported that O16 strains are specifically associated with febrile UTI in infants [32] and asymptomatic bacteriuria (ABU) in girls [16], O18 strains are associated with ABU in infants [32], O8, O25, and O83 strains are associated with cystitis in girls [33], whereas O4 occurs with relatively greater frequency in males [34].

In our study, we studied limited types of O antigen in UTI isolated *E. coli* strains.

The uropathogenic strains of *E. coli* characterized belonged to 11 different O serogroups (O1, O6, O8, O16, O21, O4, O15, O18, O25, O22, and O83). O8 and O25 were the most common types found in Damascus-Syria (table 3). We have noticed the absence of *E. coli* serogroups (O75, O2, and O7). In our present study, there was no significant correlation between the presence of the O antigens and the age of patients witch agree with the results of Emamghorashi, F.*, et al* 2011 in Iran [40]. In other countries, such as in Rio de Janeiro, 40 *E. coli* isolates were tested between 1999 and 2004, and O6 was the most common type found [37]. In a study by Blanco and co-workers, most uropathogenic *E. coli* belonged to 10 (O1, O2, O4, O6, O7, O14, O18, O22, O75 and O83) of the 12 serogroups [38]. Serotype O101 was found to be the commonest serotype (7/26) of uropathogenic *E. coli* causing UTI in two studies from India [21, 22].

In the study of Fatemeh Emamghorashi, and co-workers in Iran, O1 was the most common type in UTI-isolated *E. coli* strains [40], while O6 was a common sero-type in *E. coli* strains isolated from children with UTI in Slovakia [39].

In a study of community-acquired UTI in Santiago, the uropathogenic strains of *E. coli* characterized belonged to 27 different O serogroups; 68% were from one of ten serogroups (O1, O2, O4, O6, O9, O18, O27, O73, O75 and O77) and 36% were from one of three serogroups (O2, O4 and O6) [38].

Our results indicated that *dfrA17* (47.69%), *dfrA5* (13.84%) had the highest distributions of the five trimethoprim resistance dfrA genes studied in Damascus – Syria. In a study of Aleppo-Syria The frequencies of *dfrA1* and *dfrA7,17* were found to be 16% and 70.66% respectively, *dfrA1*and *dfrA7,17* were the most common *dfrA* genes [43], which agree with our results with a part of it.

The results of Aleppo regarding the higher prevalence of *dfrA7, 17* over dfrA1 echoed many findings in a number of similar studies from Lebanon [44], Denmark, The Netherlands [45], Korea [46] and Australia [47] Conversely, *dfrA1* appeared to be more prevalent than *dfrA7, 17* in Spain, Portugal, France, Belgium and Turkey[48].

These differences can be attributed to a number of factors ranging from diverse sampling schemes to the genetic drift affecting horizontally transferred resistance genes.

Our findings showed that there was significant correlation between type of O antigen and trimethoprim resistant genes in uropathogenic *E. coli* isolated from urinary tract infections patients. It can be noted from the results of Fatemeh Emamghorashi, that the majority of seropositive cases were sensitive to all antibiotics except ampicilin [40]. In our study we founded that the serotype O25 carrying a high frequency of *dfrA17* (table 6). Few other studies have been conducted for evaluation of the correlation between antibiotic resistance and O antigen expression [41, 42]

Therefore, it was interesting according to Hartley, C.L., et al., to determine whether R plasmids were prevalent in certain of the chemotypes, or whether the chemical nature of the lipopolysaccharide layers had little to do with the carriage of resistant plasmids.

In general, therefore, there is little indication at present that there is a positive relationship between chemotype and the carriage of R plasmids [41].

In conclusion, our first report of *E. coli* serotyping in Patients with UTI from Damascus of Syria and their relation to trimethoprim resistant genes reveled that

O8 (20%), O25 (24.61%) were two common types that correlated with trimethoprim resistance. *dfrA17* (47.69%) gene was the most common resistant gene in *E. coli* strains isolated from children and adults with UTI in Damascus.

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