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Serotyping of Syrian Uropathogenic *Escherichia coli* with Adhesion Genes *FimH* & *PapGII*

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Abstract

Urinary tract infections (UTIs) are the most common infections caused mainly by *Escherichia coli* and the O-antigen and *FimH* & *PapG*II adhesion genes are among the most important virulence factors. The aim of this study was to investigate the prevalence of some O-antigen in uropathogenic *E. coli* (UPEC) and molecular characterization of the adhesion genes *FimH* & *PapG*II63. *E. coli* isolates (50.4% from total number of samples) were collected from urinary tract infection patients from July to October in 2021 and identified using molecular and biochemical tests. Sevenantigenic types of uropathogenic *E. coli* were investigated, as well as the presence of *FimH* & *PapG*II genes in uropathogenic *E. coli* strains. The results indicated that the majority of UPEC belonged to serotypes O1, O2, O4, O6, O7, O18ac and O75 in varying proportions, where the O1 serotype was the most predominant, followed by O2, O6, O75, O7, O4 and O18ac respectively. The results of molecular tests showed the presence of the *FimH* gene in 52 isolates with a percentage of 82.50% and the presence of the *PapG*II gene in 19 isolates with a percentage of 30.15%. From the results we can conclude that the serotypes O1 and O2 had the most virulence role due to presence of the adhesion genes *FimH* and *PapG*II.

Keywords: Uropathogenic *Escherichia coli, fimH*&*papG* II, O Antigen, Urinary tract infections.

1- Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans that occur in both community and health care establishments. UTIs are caused by contamination with many bacterial species that are able to get into the urinary tract, the most important of which is uropathogenic *Escherichia coli* (UPEC) [1]. *E. coli* possesses many virulence factors that enable it to colonize the host's cells, such as the ability to attach by fimbriae which helps the bacteria to adhere and colonize in living membranes and form biofilms. Also, the production of many toxins and enzymes such as hemolytic enzyme are important virulence factors [2]. *Escherichia coli* is characterized by the presence of antigens, specially surface O-antigens which are found in smooth colonies (S) strains. And as a consequence, the classification of these antigens is based on the antigenic structure of the lipopolysaccharide (LPS) which ranges from O1-O180 [3] [4]. The most common O-antigen serogroups are: O1, O2, O4, O6, O7, O15, O18ac and O75 which cause about 75% of urinary tract infections. It is worth noting that it is very hard to determine pathogenic serotypes from non-pathogenic *E. coli* according to biochemical criteria alone which are available in most laboratories. Hence, serological tests are mainly used to determine specific serotypes like

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using Kauffman scheme for serotyping of *E. coli* [5]. Studies have demonstrated that lipopolysaccharides play a major role in the adhesion with the help of O-antigen, as it has been proven that the mutation in the genes is responsible for the synthesis of this layer that affects the adhesion ability of *E.coli* and, as a consequence, the inability to form a biofilm [6]. More than 700 serotypes of *E. coli* have been distinguished which include more than 180 O-antigen groups, 53 known H antigen types and 80 K-antigen types [7]. The diversity of O-antigen is the current standard in the classification of enteropathogenic strains as well as uropathogenic strains [8]. Since some serotypes of *E. coli* play a role in pathogenicity and adhesion, it is believed that they synergize with other virulence factors such as the fimbriae which play an important role in the process of adhesion to the urethral epithelium and is a prerequisite for the formation of the biofilm and the transmission of quorum-sensing signals (QS) from one cell to another when controlling the levels of division [9]. *E. coli* possesses several types of fimbriae, the most important of which are type I and type III (type P) which contribute to the binding on the surface receptors of the host cells. Johnson *et al.* demonstrated the role of operon *pap* in adhesion and virulence especially the *papG* gene that codes the tip of this type of fimbriae which is considered an important point of adhesion, [10]. Whereas the *fimH* operon encodes the first type of fimbriae which is characterized by a high quality of the receptors on the urinary epithelium. Hojati *et al.* demonstrated the role of the *fimH* gene in the pathogenicity of UPEC bacteria and therefore the *fimH* gene encodes the tip of this fimbriae and is necessary for the colonization of *E. coli* to the urinary tract [11]. Due to the wide spread of uropathogenic *E. coli* that is able to infect and colonize the urinary epithelium and considering the great diversity of *E. coli* distributing through the world in general and in Syria in particular, our research concentrated on serological investigation of the surface O-antigens and identification of the most important serotypes prevalent in Syria, and the detection of some fimbriae encoding genes. Finally, studying the relationship between the presence of surface O-antigen and the genes *fimH* and *papG*II.

2-Materials:

2-1 Immunological study:

1- Strains serotyping was conducted by using 96-wells sterile microtitration plates.
2- The determination of O serotype was conducted by kit (ExPECStatens Serum Institut SSI Diagnostica, Denmark) [12].
3- The following culture media were used: MacConkey agar as a medium for the cultivation of UPEC strains to confirm the purity of the isolate for serotyping. Tryptic soy broth/agar (TSB/TSA) as a rich and complex medium for culturing strains to be serotyped. Luria-Bertani broth/agar was used to enrich and activate isolates for DNA extraction.

2-2 Molecular Study:

In this research, specific primers supplied by Invitrogen-Thermo Fisher Scientific Japan Ltd., were used for detection of *fimH* & *papG* (Table (1.

Table 1: Specific primers sequences for the *fimH* and *papG* II genes

Gene	Primer	Sequence (5→3)	Size bp	Reference
fimH	F	GTGCCAATTCCTCTTACCGTT	508	[11]
	R	TGGAATAATCGTACCGTTGCG		
*papG*II	F	GCAACAGCAACGCTGGTTGCATCAT	190	[10]
	R	AGAGAGAGCCACTCTTATACGGACA		

3- Methods

3-1-Bacterial Strains:

Uropathogenic *E. coli* were collected from patients with UTI's from Aleppo University Hospital. Urine samples were collected by clean catch method from midstream urine specimen using sterile closed single-use containers. Each sample was attached to an information form that included the patient's name, sex, age, marital status, diseases, whether he was taking antibiotics or not and the frequency of their urinary tract infection [13].

3-2-Bacterial Identification:

The identification of the *E. coli* isolates was carried out using microscopic examination, physiological characteristics and colonies morphology on different culture media and biochemical tests according to Bergey's manual of determinative bacteriology [14].

3-3- Detection of O-antigen Types:

According to the instruction from ExPECStatens Serum Institut SSI Diagnostica, Denmark to detect O1, O2, O4, O6, O7, O18ac and O75 *E. coli* serotypes, which is in brief purification of the previously isolated strains, was done on a selective medium (MacConky Agar, Himedia, USA) and then the purity of the species was confirmed by cultivating them on a non-selective medium like TSA, and finally a bacterial suspension was prepared in a liquid medium (TSB). This medium was heated at a temperature greater than 90°C for 1 hour, in order to get rid of the phenomenon of cross-reaction with the rest of the antigens (K-H-F) that were destroyed by heat, except for the heat-stable O-antigen. After completion of boiling, an equal amount of antibody serum was mixed with the boiled bacterial suspension in the wells of the microtitration plates, and incubated for 24 hours at 50°C, and the results were then recorded [15].

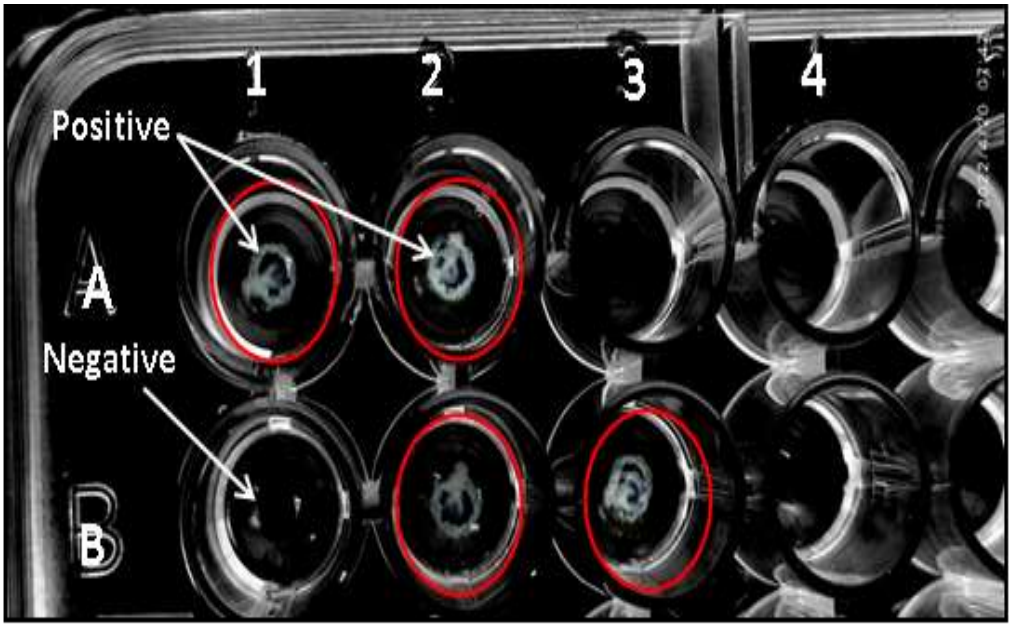

Figure 1: Titration plates for the detection of serotypes containing 96 wells. Where: A, 1-4\ B, 13-14 tested isolates.

3-4- Detection of *fimH* & *papG* Genes:

Total DNA was extracted from 63 isolates using the optimized heat shock method where the isolates were cultured in LB broth for 18 hours at 37°C. After the incubation period, 1.5 mL of the broth was centrifuged, and the pellet was suspended in 200 µL of sterile deionized water and incubated at 100°C for 10 minutes. The supernatants were used as a template DNA

after centrifugation of the lysate [16]. Next, the electrophoresis process was performed using Consort E452 electrophoresis–Belgium in order to ensure the quality of the extracted DNA. The PCR reaction for the *fimH* and *papG*II genes was carried out in 25 µL containing: 2.5 µL of ×5 PCR reaction buffer (Green GoTaq®, Promega, USA) with $MgCl_2$ (1.6 mm), 0.5 µL (200 µM) of deoxynucleoside triphosphates mixture (dNTPs, 10 mm), 0.5µL of each primer (10 pm/µL), 2 µL of the DNA template (50 ng) with 0.5 µL (3 U/µL) Taq DNA polymerase. The amplification conditions for the *fimH* gene included an initial denaturation at 94°C for three minutes, 37 cycles at 96°C for 30 seconds, 64°C for five minutes, 72°C for 60 seconds and a final extension at 72°C for five minutes [11]. And for the *papG*II gene, it included an initial denaturation at 95°C for 12 min, 25 cycles at 94 °C for 30 seconds, 63°C for 30 seconds, 68 °C for 3 min and a final extension at 72°C for 10 minutes [10]. The PCR amplifications were performed on a thermal cycler, Peqlab , Germany. The PCR products were analyzed by agarose gels (2%) electrophoresis and then stained with ethidium bromide [10] [11].

Gel electrophoresis of the amplified products was run in order to detect the targeted gene on 1.5% agarose gel, with 0.5 μg/ml ethidium bromide along with 3 μl from100bp DNA Ladder RTU for an hour at 100 volts. The gel was examined after the electrophoresis by UV Transilluminator (Herolab,Germany) and the molecular sizes of the bands were estimated according to the marker [17].

Reference strains were used for comparison containing the two target genes that were kindly provided by the laboratory of Molecular Microbiology in Center for Southeast Asian Studies (CSEAS) Kyoto University/Japan.

4-Results and Discussion:

4-1-Bacterial Identification:

Sixty-three bacterial strains of *E. coli* were isolated and studied. All of which were motile, lactose fermenting, gram-negative bacilli, also they were catalase, indole and methyl red (MR) positive and oxidase, Voges-Proskauer (VP), citrate utilization negative [14].

4-2-Molecular Investigation of the *fimH* Gene:

The results of the electrophoresis showed that 52 out of 63 strains of *E. coli* contained the *fimH* gene with a percentage of 82.50%. While 11 strains (17.50%) of *E. coli* did not contain the gene *fimH* (Table 2 and Figure 1

Table 2: Presence of the *fimH* gene in UPEC

Gene Name	The Total Number of Isolates	Bacterial Strains Containing the *fimH*	Bacterial Strains that Do Not Contain the *fimH*
fimH	63	(82.50%) 52	(17.50%) 11

The above table indicates the wide spread of *fimH* gene in UPEC strains of and its role in the adhesion of fimbria to the urinary epithelium, in comparison with various global research (Table 3).

Table 3: Comparison of the presence of the *fimH* gene with some international studies

The Current 2022Study	Mexico 2021 [18]	Malaysia 2015 [19]	Iran 2012 [20]	Tunisia 2013 [21]	Japan 2017 [22]	Saudi Arabia 2019 [23]	India 2017 [24]	Brazil 2008 [25]
82.5%	(95.23%)	%100	79.67%	68%	%89.9	66.99	%90	%97.6

We noted the convergence of the results of the current study with most international studies, which have confirmed that the *fimH* gene is a major virulence factor in UPEC, regardless of its geographical distribution.

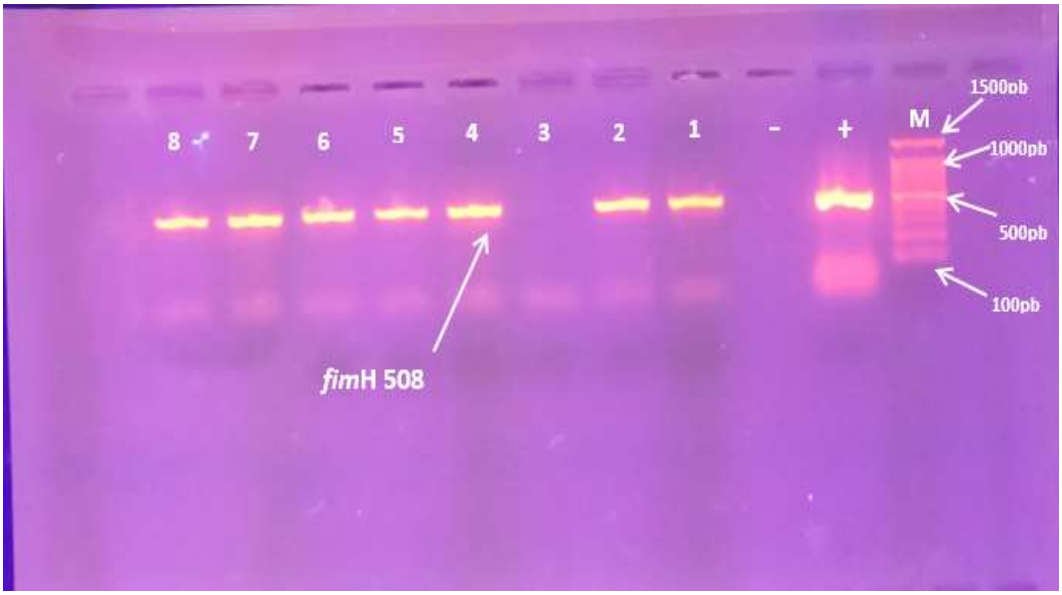

Figure 2: *fimH* gene bands are 508bp in size compared to the 100bp marker. Where: M marker 100bp, 1-8 tested isolates.

4-3-Molecular Investigation of the *papG* II Gene:

The results of the electrophoresis showed that out of 63 strains only 19 strains of *E. coli* contained the gene *papGll*. Whereas 44 strains (69.84%) did not contain the gene *papG*II which indicated the lower prevalence of the second allele of the *papG* gene at UPEC that is often associated with pyelonephritis by adhesion to the kidney epithelium (Table 4 and Figure 2.

Table 4: Presence of *papG* II gene in UPEC

Gene	The Total Number of Isolates	Bacterial Strains Containing the *papG*II Gene	Bacterial Strains that Do Not Contain the *papG*II Gene
papG II	63	(30.15%) 19	(69.84%) 44

By comparing the current study results with some international research, we noted the convergence of the current study with those studies such as the Iraqi study (32.7%), the Pakistani study (45%), the Taiwanese study (42%) and the Japanese study (34%) (Table 5).

Table 5: Comparing the percentage of *papG* gene presence with some international studies.

The current study 2022	Iraq 2013 [26]	Pakistan 2009 [27]	Taeyeon 2013 [28]	Japan 1998 [29]	American 2000 [30]	Slovakia 2004 [31]
30.15%	32.7%	45 %	42%	34%	71%	86%

It contrasts with the American (71%) and the Slovakian studies (86%) indicated that the prevalence of this gene is related to the type of strain and the geographical area in which it has inhabited.

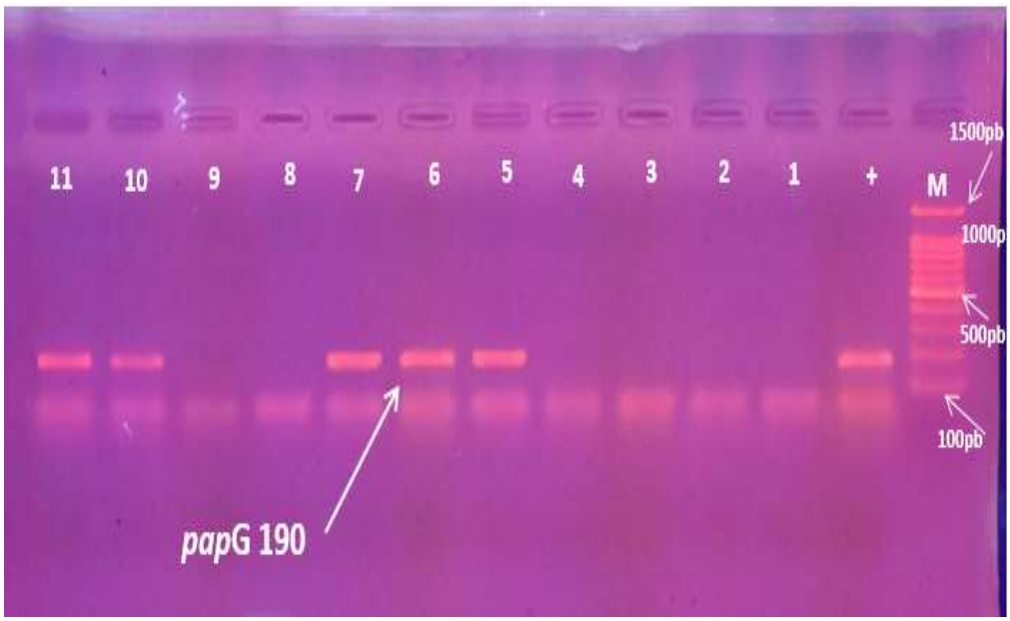

Figure 3: *papG* II gene bands are 190bp in size compared to the 100bp marker. Where: M marker 100bp, 1-11 tested isolates.

Whereas, after performing the crossover, it was found that 13 strains (20.63%) contained both *fimH* and *papG* II genes

4-4-Prevalence of UPEC O-Serotypes:

Agglutination tests revealed that the majority of studied UPEC strains,)57 out of 63 90.47%), belonged to common O-serotypes, whereas 6 strains %%9.63) were not recognized by the dominant O-antigen in UPEC which are believed to be belonging to some other O-serotypes (Table 6).

4-4-1. O-serotypes prevalent in Syrian UPEC:

Prevalence of O-antigen serotypes in UPEC were detected in approximately equal proportions. The most common serotypes detected in UPEC strains were serotype O1 (19%), followed by serotypes O2 (17.4%), O6 (12.7), O75 (12.7), O7 (11.11), O4 (11%), and O18ac (6.4) respectively.

Table 6: Percentage of presence of serotypes

UPEC Total Number	O1	O2	O4	O6	O7	O18ac	O75	Percentage of Strains Containing O-antigen
63	12 19%	11 %17.4	7 %11	8 %12.7	7 %11.11	4 %6.4	8 %12.7	%90.47

It is noticeable that these percentages conformed many international studies, like [6] where all UPEC serotypes belonged to most common serogroups as follows: O1 (19.33%), O2 (13.33%), O6 (13.33%), O4 (11.66%) and O18 (11.66%) [32].

4-4-2-Percentage of serotypes containing the fimH gene:

By studying the relationship between strains possessing the *fimH* gene and their serotype, we noted that 47 strains contained the *fimH* gene out of 52 strains that belonged to several different serotypes (Table 7).

Table 7: Percentage of serotypes containing the *fimH* gene

fimH	O1	O2	O4	O6	O7	O18ac	O75
52	10 %19.23	9 %17.3	7 %13.46	8 %15.38	7 %13.46	1 %1.92	5 %9.61

The most dominant serotype was O1 with a percentage of 19.23% and O2 at 17.13%, while dominant serotypes O18ac at a rate of 1.92%. The results of this study converged with each of the Korean study which was serotype O1 (7%), serotype O2 (29.3%) and serotype O18 (3.44%) [33]. While the percentages of serotypes in Iranian study were O1(6%), O2(13%) and O18(9.4%) [34]. Also, five strains of UPEC did not belong to any studied serotype and, therefore, may belong to non-dominant serotypes with lower prevalence.

4-4-3-Percentage of O-serotypes containing the papG II gene:

Also by studying the relationship between the possession of the tested UPEC of the *papG* II gene and its O-serotype we note that only 18 strains contained the *papG* II gene that belonged to several O-serotypes, and the most dominant serotypes were O1 and O2 with a percentage of 31.57 while the least dominant serotype was O18ac by 5.26%. The results of this study that converged with the Iranian study were the two serotypes O1 & O2 (Table 8).

Table 8: Percentage of serotypes containing the *papG*II gene

Gene *papG* II	O1	O2	O4	O6	O7	O18ac	O75
19	6 %31.57	6 %31.57	1 %5.26	1 %5.26	3 %15.78	1 %5.26	-

(17.77%), while the serotype O18 (11%) . [35], while no relationship was detected between the *papG* gene and serotype O75, at least in the studied local UPEC strains

4-4-4-Percentage of serotypes containing the genes fimH & papG II:

An intersection was conducted between the studied serotypes and the presence of the *fimH* & *papG* II genes in order to study their possible relationship in the pathological severity of some serotypes (Table 9).

Table 9: Percentage of serotypes containing the two genes *fimH* & *papG* II

fimH &papG II	O1	O2	O4	O6	O7	O18ac	O75
13	4 %30.76	4 %30.76	1 %7.69	1 %7.69	3 %23.07	-	-

Our study revealed that the serotype O1, O2 (30.76%) were the most closely related in the presence of the two genes *fimH* & *papG* which indicates an increase in the pathogenicity of these two serotypes, which is considered as a risk indicator when isolating these two serotypes from urinary tract infections, followed by serotype O7.

4-4-5-Percentage of serotypes not containing the two genes fimH & papG II:

An intersection was also conducted between the percentage of serotypes that did not contain the *fimH* & *papG*II (Table 10).

Table 10: Percentage of serotypes not containing *fimH* &*papG* II genes

O75	O18ac	O7	O6	O4	O2	O1	*papG* II &*fimH* Gene
3 %60	2 %40	-	-	-	-	-	5

It was noted that the most serotypes that did not contain the virulence genes, were O18ac (40%) & O75 (60%),, which may indicate either a lower pathogenic ability or the possession of other virulence factors that enable them to infect the urinary tract. Some UPEC survives the extracellular immune responses and invades bladder epithelial cells (BECs), which then initiate the intracellular efflux immune responses. Once BECs are invaded, two waves of UPEC expulsion in an innate immune signaling-orchestrated process occur, and these serotypes are worth studying to investigate the source of their pathogenicity.

5-Conclusions:

The results showed the wide spread of *fimH* and *papG* II genes in most Syrian UPEC.
O1, O2 were found to be the most prevalent serotypes of UPEC in the study.
The results showed a relationship between the presence of *fimH* and *papG* II and the O1, O2, O7 serotypes which could increase the pathogenicity.
The results showed that each of the serotype O1, O2 of *E. coli* is considered as a risk indicator in patients with urinary tract infection.

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Ethical approval: The research was done after Ethical Approval from the Aleppo University Hospital administration, and the Scientific Research Committee which is dated 11/07/2021 with number 1466/14T.

Conflict of Interest:

We hereby declare that there was no conflict of interest in our paper with any other authors.

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