

## Important Virulence Factors and Related Genes in Uropathogenic *E. coli* and their Relation to Fluoroquinolone Resistance

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*Escherichia coli* (*E. coli*) is the predominant pathogen causing urinary tract infection. *E. coli* expresses a wide spectrum of virulence factors that enable it to cause the disease. The present study was conducted on 50 *E. coli* isolates to assess some important phenotypic virulence factors of *E. coli*: haemolytic activity, cytotoxicity and haemagglutination and their relations to the following virulence genes: *hlyA*, *cnf-1* and *papC*. Fluoroquinolones susceptibility was also assessed. Fifty *E. coli* isolates were isolated from non-hospitalized patients with UTIs and identified by colony morphology and standard biochemical reactions. The isolates were examined phenotypically for haemolytic activity using 5% sheep blood agar, cytotoxicity by their cultivation on HEP-2 cell line and haemagglutination of group O human RBCs. Virulence genes were detected by multiplex PCR. Fluoroquinolones susceptibility was done using disk diffusion method. Phenotypic methods showed that 30% of the isolates were  $\beta$ - haemolytic, 18% of the isolates showed haemagglutination with human RBCs, with higher prevalence for MRHA (12%) than MSHA (6%), and 92% of the isolates showed morphological changes, detachment and lysis in HEP-2 cells. Multiplex PCR showed that the most prevalent gene was *cnf-1* (30%), while 26% of isolates were positive for *hlyA* and 26% of isolates were positive for *papC*. Only one UPEC isolate showed the simultaneous presence of *papC*, *cnf-1* and *hlyA*, while 10 isolates were harbouring *hlyA* and *cnf-1*, 2 isolates were harbouring *cnf-1* and *papC* genes and 2 isolates were harbouring *hlyA* and *papC* simultaneously. Fluoroquinolones susceptibility showed that 60% of the isolates were resistant to the used fluoroquinolones.

**Keywords:** *Escherichia coli*, virulence, multiplex PCR, fluoroquinolones susceptibility.

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Urinary tract infection (UTI) is among the most common bacterial infectious diseases encountered at all ages. *Escherichia coli* is being the etiologic agent in 50-80%. (Farell *et al.*, 2003; Matute *et al.*, 2004; Zhanel *et al.*, 2006). Certain strains of *E. coli* are consistently associated with uropathogenicity and are designated as uropathogenic *E. coli* (UPEC) (Connell *et al.*, 1996). UPEC strains encode widespread virulence factors closely related to colonization, persistence, and pathogenesis of bacteria in the urinary tract; the

most important of these factors include adhesins or fimbriae, biofilm formation, siderophores and toxins such as haemolysins (Hly) (Dhakal *et al.*, 2008) and cytotoxic necrotizing factor-1 (CNF-1) (Wiles *et al.*, 2008<sup>a</sup>). The virulence factors among UTI agents are really different and clinical isolates could not be detected as UPEC by using traditional methods. Therefore, using molecular detection methods is critical for identification of UPEC strains (Saraylu *et al.*, 2012). In clinical management, it is usually accepted that resistance, especially multidrug resistance, equates to the virulence of the strain. However, molecular studies have indicated an inverse relationship between the distribution of virulence factors and antimicrobial

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resistance, particularly to quinolones as well as fluoroquinolones (Vila *et al.*, 2002; Johnson *et al.*, 2003; Moreno *et al.*, 2006; Piatti *et al.*, 2008).

#### **Aim of Work**

The present study was conducted to detect some important virulence factors of *E. coli* phenotypically: haemolytic activity, cytotoxic activity and haemagglutination and related virulence genes: *hlyA*, *cnf-1* and *papC*, by using multiplex PCR. Study the relation between the bacterial resistance to fluoroquinolones and the virulence potential of *E. coli*.

### **MATERIALS AND METHODS**

The study has been approved by the Research and Ethical Committee of Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University.

#### **Isolation and Identification of *E. coli***

Urine samples from non-hospitalized cases of urinary tract infections were collected from clinical laboratories of Kasr El Ainy hospital. Microscopic examination of a wet film of urine was carried out. Samples with pus cells  $\geq 10$ /HPF were quantitatively inoculated on MacConkey's agar (Oxoid, UK) and then incubated at 37 °C for 24 hrs. Culture plates with bacterial count  $\geq 10^5$  CFU/ml and growth of single morphotype of lactose fermenting colonies were selected for identification of *E. coli* by standard biochemical methods.

#### **Phenotypic Detection of UPEC Virulence Factors**

The *E. coli* isolates were tested for expression of haemolytic, cytotoxic activity and haemagglutination as described below:

##### **1. Assessment of haemolytic activity**

The cytolytic toxin secreted by most haemolytic *E. coli* isolates is known as alpha haemolysin. *E. coli* isolates were subcultured onto 5% sheep blood agar (Oxoid, UK) and incubated overnight at 37°C. Haemolysin production was detected by presence of a zone of complete haemolysis around the inoculation site (Raksha *et al.*, 2003; EL-Mosallamy *et al.*, 2015; Tabasi *et al.*, 2015).

##### **2. Assessment of cytotoxic activity**

The cytotoxic effect of *E. coli* isolates was detected according to Ghadir *et al.* (2010), however, HEp-2 cells was used instead of Vero

cells according to Mills *et al.* (2000).

#### **Preparation of bacterial isolates**

*E. coli* isolates were obtained from frozen stock and subcultured on blood agar (Oxoid, UK) then on MacConkey's agar (Oxoid, UK). Colonies were then inoculated in test tubes containing 10 ml of tryptic soy broth (TSB) (Oxoid, UK) and incubated for 18-20 h at 37°C and kept at 4°C and used for inoculum preparation. Bacteria were harvested by centrifugation at 3000 rpm (MSE Centaur 2, UK). The supernatant was discarded and the bacterial pellets were washed once with phosphate buffered saline (PBS), then resuspended in Eagle's minimum essential medium with Earl's balanced salts (EMEM) (Lonza, Belgium) and adjusted to a count  $1 \times 10^8$  CFU/ml.

#### **Cell line and growth conditions**

HEp-2 cells were maintained in EMEM (Lonza, Belgium) supplemented with 2% fetal bovine serum (FBS) (Lonza, Belgium), 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in 5 % CO<sub>2</sub> and subcultured every 3-4 days. HEp-2 cells suspended in MEM Earl's and supplemented with 10 % FBS were seeded in 24-well tissue culture plates and kept at 37°C with 5 % CO<sub>2</sub> for 24 hours to form a confluent monolayer. 100 µl of the bacterial cell suspension prepared above was added to each well and incubated at 37°C in 5 % CO<sub>2</sub> for 3 hours. HEp-2 cell morphology was assessed by examination using an inverted microscope (Olympus bx 50, Japan) 1, 2 and 3 hours post inoculation.

#### **3. Assessment of haemagglutination**

*E. coli* isolates were inoculated into nutrient broth and incubated at 37 °C for 48 hours for production of fimbriae. Five milliliter of group O venous blood was collected then washed three times in normal saline and a suspension of 3% RBCs in phosphate buffered saline (pH 7.4) was prepared (Lonza, Belgium). They were used immediately or within a week when stored at 3-5 °C. The slide haemagglutination test was carried out on a multiple-concavity slide by adding 50 µl of the RBC suspension to 50 µl of the broth culture and the slide was rocked at room temperature for 5 minutes. Presence of clumping was taken as positive for haemagglutination. At the same time, 50 µl of 3% D-mannose (Fluka AG, Switzerland) was added to the red cells and broth culture in another set and rocked as above. Haemagglutination inhibited in

presence of D-mannose was labeled as mannose sensitive haemagglutination (MSHA); indicating type1 fimbriae, if agglutination occurred even in presence of D-mannose, it was called mannose resistant haemagglutination (MRHA); indicating presence of P fimbriae (Uma *et al.*, 2013).

#### Molecular Detection of UPEC Virulence Genes

*E. coli* isolates were tested for the presence of virulence genes including: *hlyA*, *cnf-1* and *papC* using multiplex PCR.

#### 1. DNA Extraction

DNA extraction was performed for all the clinical isolates using CinnaPure DNA extraction kit (Tehran, Iran) according to manufacturer's instructions.

#### 2. Amplification

Amplification was done according to Bashir *et al.* (2012) using 50 µl reaction mixture for each isolate. Primers sequences are illustrated in Table (1).

**Table 1.** Primers' Sequences

Primer	Sequence	Reference
<i>hly A</i>	F: 5'AACAAGGATAAGCACTGTTCTGGCT3' R: 5'ACCATATAAGCGGTCATTCCCCTCA3'	Yamamoto <i>et al.</i> (1995)
<i>cnf-1</i>	F: 5'AAGATGGAGTTTCTATGCAGGAG3' R: 5'CATTTCAGAGTCCTGCCCTCATTAT3'	Arisoy <i>et al.</i> (2006)
<i>papC</i>	F: 5'GACGGCTGTACTGCAGGGTGTGGCG3' R: 5'ATATCCTTCTGCAGGGATGCAATA3'	Daigle <i>et al.</i> (1994)

The thermal cycler (Biometra T 3000) conditions were as follows: an initial denaturation step at 94°C for 5 min followed by 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min) and final extension of 5 min at 72°C.

#### 3. Agarose Gel Electrophoresis

Amplified PCR products were visualized by 2% (w/v) agarose gel electrophoresis (stained with ethidium bromide; 0.5 µg/ml) at 120 volts for 45 minutes. The bands were visualized under UV transilluminator (Biometra Ti 3) and photographed. 100 bp DNA ladder H3 RTU (GeneDire, Taiwan) (which gave 12 discrete bands at 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 3000 bp) was used.

#### Fluoroquinolones susceptibility using Kirby Bauer Disk Diffusion method

Disk diffusion method was done using fluoroquinolones: ciprofloxacin (CIP 5µg),

levofloxacin (LEV 5µg), norfloxacin (NOR 10µg), and ofloxacin (OFX 5µg) (Oxoid, UK).

The *E. coli* colonies were suspended in sterile normal saline and adjusted to 0.5 MacFarland standard, then inoculated on Muller-Hinton agar plates and incubated with the previously mentioned antimicrobial disks at 35°C ±2 for 18 hours. The interpretation of the diameters of the inhibition zones were done according to CLSI, (2014) (Table 3).

#### Statistical Analysis

Data were statistically described in terms of frequencies (number of cases) and percentages and compared using Chi-square ( $\chi^2$ ) test. Exact test was used instead when the expected frequency is less than 5, *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science;

**Table 2.** Expected PCR product size

Genes	Amplicon size (bp)	Reference
<i>hly A</i>	1177	Yamamoto <i>et al.</i> (1995)
<i>cnf-1</i>	498	Arisoy <i>et al.</i> (2006)
<i>papC</i>	328	Daigle <i>et al.</i> (1994)

**Table 3.** Interpretation of sensitivity testing of *E. coli* with fluoroquinolones disks

Strain	Antimicrobial agent	Disk content	Zone diameter		
			R	I	S
<i>E. coli</i>	Ciprofloxacin	5 µg	≤15	16-20	≥21
	Levofloxacin	5 µg	≤13	14-16	≥17
	Norfloxacin	10 µg	≤12	13-16	≥17
	Ofloxacin	5 µg	≤12	13-15	≥16

SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows.

## RESULTS

The present study was performed on 50 *E. coli* isolates isolated from urine samples of patients with UTI. *E. coli* isolates were identified by their colony morphology on MacConkey's agar and standard biochemical reactions.

### Phenotypic Detection of Virulence Factors of *E. coli* Isolates

#### 1. Haemolytic activity

In the current study 15 (30%) isolates showed  $\beta$ -haemolysis, while the remaining 35 (70%) isolates were non-haemolytic.

#### 2. Cytotoxic assay

Upon visual assessment of the HEp-2 cell line one hour after infection with  $1 \times 10^8$  CFU/ml of the tested isolates, no effect was seen on HEp-2 cells. Two hours post infection; it was found that out of 50 UPEC isolates, 46 (92%) isolates showed cytotoxic effect with mild detachment and lysis of HEp-2 cell line and almost complete detachment of the cell line 3 hours post infection, while only 4 (8%) isolates showed no effect on the cell line (Figures 1, 2 and 3).

#### 3. Haemagglutination assay

Out of the 50 *E. coli* isolates, 9 (18%) isolates showed haemagglutination (HA) of group O RBCs, while 41 (82%) were non-haemagglutinating. Out of the 9 isolates positive



Fig. 1. HEp-2 cell line, control cell line

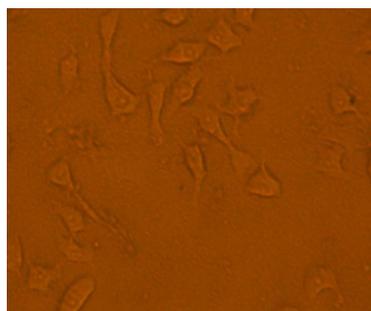


Fig. 2. The cytotoxic effect of the tested *E. coli* isolate on HEp-2 cells with mild detachment 2 h post infection

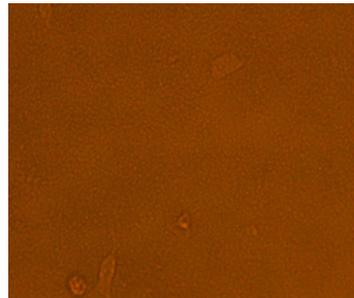


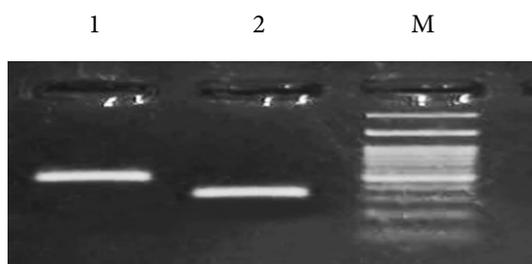
Fig. 3. The cytotoxic effect of the tested *E. coli* isolate on HEp-2 cells with almost complete detachment 3 h post infection

for HA, 6 isolates were MRHA (6/50, 12%) and 3 isolates were MSHA (3/50, 6%).

#### Molecular Detection of *E. coli* Virulence Genes

Virulence genes including *hlyA*, *cnf-1* and *papC* of 50 UPEC were detected by the multiplex PCR. Amplicon sizes of *hlyA*, *cnf-1* and *papC* were 1177, 498, 328 (bp), respectively (Figure 4 and 5).

Twenty five isolates were positive for one or more of the targeted 3 genes, while the remaining isolates were negative for the targeted genes. It was found that one isolate was positive for the three targeted virulence genes, 10 isolates showed the presence of both *cnf-1* and *hlyA*, 2 isolates showed the presence of both *cnf-1* and



**Fig. 4.** Multiplex PCR for virulence genes in *E. coli* isolates. Lane (1): *cnf-1* (band at 498 bp), Lane (2): *papC* (band at 328 bp), Lane (M): 100 bp DNA ladder

*papC*, 2 isolates showed the presence of both *hlyA* and *papC*, while 8 isolates showed the presence of *papC* and 2 isolates showed the presence of *cnf-1* gene (Table 4).

It was found that the most prevalent gene was *cnf-1* which was detected in 15 (30%) isolates, while 13 (26%) isolates were positive for *hlyA* and 13 (26%) isolates were positive for *papC*.

**Table 4.** Distribution of Virulence genes among *E. coli* isolates

No. of Virulence genes	Virulence genes	No. of isolates
3	<i>cnf-1, hlyA, papC</i>	1
2	<i>cnf-1, hlyA</i>	10
2	<i>cnf-1, papC</i>	2
2	<i>hlyA, papC</i>	2
1	<i>cnf-1</i>	2
1	<i>papC</i>	8
Total		25

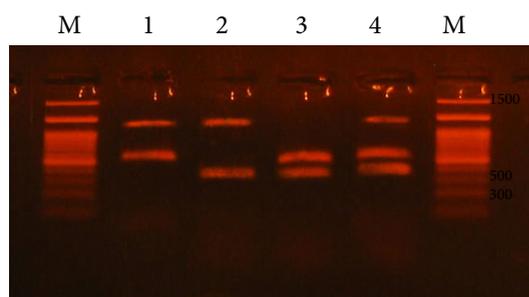
### Virulence Factors in Relation to Virulence Genes

#### Haemolytic activity in relation to *hlyA* gene

It was found that *hlyA* gene was detected in higher percentage in  $\beta$ - haemolytic isolates (61.5%) than non- haemolytic isolates (38.5%) with statistically significant difference (P- value = 0.011).

#### Cytotoxicity in relation to *cnf-1* and *hlyA*

Out of 46 isolates showing cytotoxicity by phenotypic methods, 17 (37%) isolates were positive for the cytotoxic genes; 11 isolates were



**Fig. 5.** Multiplex PCR for virulence genes in *E. coli* isolates. Lane (1): *hlyA* and *cnf-1*, Lane (2): *hlyA* and *papC*, Lane (3): *cnf-1* and *papC*, Lane (4): *hlyA, cnf-1* and *papC*, Lane (M): 100 bp DNA ladder

positive for both *cnf-1* and *hlyA* gene, while 4 isolates were only positive for *cnf-1* gene and 2 isolates were only positive for *hlyA* gene. The 4 cytotoxicity negative isolates expressed neither of the 2 genes.

#### Haemagglutination (HA) in relation to *papC* gene

Out of the 9 isolates showing haemagglutination by phenotypic methods; only 3 (33%) isolates were positive for *papC* gene (2 isolates were MRHA and one isolate was MSHA). On the other hand, 10 (24.4%) out of the 41 non-haemagglutinating isolates were positive for *papC* gene. Although, haemagglutination positive isolates showed a higher percentage of *papC* gene than the non- haemagglutinating isolates there was no statistically significant difference between them (P- value = 0.679).

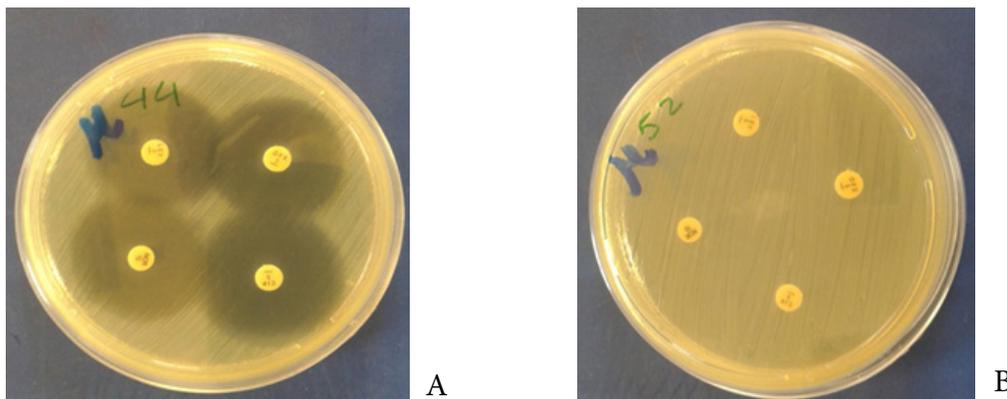
#### Results of Fluoroquinolones Sensitivity of the *E. coli* Isolates

*In vitro* susceptibility pattern of *E. coli* isolates showed that 30 (60%) isolates of *E. coli* were resistant to all fluoroquinolones, while 20 (40%) isolates were sensitive (Figure 6).

#### Association of Virulence Factors Expression with Resistance to Fluoroquinolones among *E. coli* Isolates

#### Haemolytic activity in relation to fluoroquinolones resistance

Phenotypic analysis showed that out of the 15 isolates with  $\beta$ - haemolysis; 10 (33.3%) were fluoroquinolone resistant and 5 (25%) were fluoroquinolones sensitive isolates with no statistically significant difference between the two groups (P- value = 0.380).



**Fig. 6.** Fluoroquinolones susceptibility of isolated *E. coli* to different fluoroquinolones using disk diffusion method (A): An *E. coli* isolate showing sensitivity to all used fluoroquinolones (B): An *E. coli* isolate showing resistance to all used fluoroquinolones

#### Cytotoxicity in relation to fluoroquinolones resistance

Out of the 46 isolates showing cytotoxicity to HEp-2 cells; 27 (90%) isolates were fluoroquinolone resistant and 19 (95%) isolates were fluoroquinolones sensitive with no statistically significant difference between the two groups (P- value = 0.641).

#### Haemagglutination in relation to fluoroquinolones resistance

Out of the 9 haemagglutinating isolates; 5 (16.6%) isolates were fluoroquinolones resistant, (3 MRHA and 2 MSHA), while 4 (20%) isolates were fluoroquinolones sensitive, (3 MRHA and 1 MSHA), with no statistically significant difference between the two groups (P- value = 0.456).

#### Association of Virulence Genes with Resistance to Fluoroquinolones among *E. coli* Isolates

Out of the 30 fluoroquinolones resistant isolates; 16 (53.3%) isolates harboured the virulence genes with variable distribution. It was found that *hlyA*, *cnf-1* and *papC* genes were detected in the following percentages: 27%, 30% and 30%, respectively. On the other hand, in the susceptible group 8 (40%) isolates harboured the virulence genes with variable distribution. It was found that *hlyA*, *cnf-1* and *papC* genes were detected in the following percentages respectively 25%, 30% and 20%. There was no statistically significant difference between the resistant and susceptible groups regarding the 3 virulence genes.

#### DISCUSSION

UPEC is the most frequent cause of community and hospital-acquired urinary tract infections (Calhau *et al.*, 2015). These infections can vary from a simple cystitis to a serious parenchymatous attack. The degree of severity depends on the virulence of the responsible isolates (Srinivasan *et al.*, 2003; Soto *et al.*, 2011).

Several virulence factors such as fimbriae, toxins, and siderophores contribute to the colonization and pathogenicity of UPEC (Qin *et al.*, 2013). Measuring a phenotype *in vitro* does not always correlate with *in vivo* expression and very often underestimates the presence of a virulence factor *in vivo* (Kaper *et al.*, 2004). The distribution of virulence properties can vary depending upon host characters, type of infection and predisposing factors which determine the host parasite interaction *in vivo* which can culminate in an active infection (Vijayalakshmi *et al.*, 2015). In addition there is always the possibility of mutation at the level of the corresponding gene, leading to the absence of its detection. Therefore, a positive PCR shows the presence of the virulence gene, but a negative PCR does not point to the absence of the corresponding operon (Tarchouna *et al.*, 2013).

This study was designed to assess some important phenotypic virulence factors of *E. coli*: haemolytic activity, cytotoxic activity and haemagglutination and their relations to the following genes: *hlyA*, *cnf-1* and *papC*.

We found that 30 % of *E. coli* isolates showed  $\beta$ - haemolysis on sheep blood agar. Similar results were obtained by other studies done by Fatima *et al.* (2012), EL-Mosallamy *et al.* (2015), Tabasi *et al.* (2015) and Vijayalakshmi *et al.* (2015) who reported that 30%, 24%, 34% and 25% of the isolates were  $\beta$ - haemolytic, respectively.

Higher rates of  $\beta$ - haemolytic *E. coli* were detected by Mandal *et al.* (2001), Raksha *et al.* (2003), Rebecca and Elizabeth (2005), Santo *et al.* (2006), Regua- Mangia *et al.* (2010) and Sai *et al.* (2013) who reported that 45.5%, 41.36%, 40.7%, 96%, 47 % and 40% of *E. coli* isolates were  $\beta$ - haemolytic respectively. Several reasons explain the differences in the percentage of haemolysin production by UPEC like: source of blood, type of haemolysin produced, source of bacteria and method to screen the production ability (Al-Chalabi *et al.*, 2010).

Regarding cytotoxic effects of *E. coli* in this study, it was found that no change was detected in the HEP-2 cell line during the first hour of its incubation with *E. coli* suspension. However, 2 hours post infection; 92% of the tested *E. coli* isolates showed morphological changes and almost complete detachment in HEP-2 cells was detected 3 hours post infection. Our results were in accordance with Guignot *et al.* (2000) and Ghadir *et al.* (2010).

Agglutination of human erythrocytes by *E. coli* strain is an indirect evidence of the presence of fimbriae on that strain. MRHA of human RBCs is the phenotypic expression of P fimbriae on *E. coli*, while MSHA is the phenotypic expression of type 1 fimbriae and both of them mediate adherence and they are important in bladder colonization (Vijayalakshmi *et al.*, 2015).

In the present study, it was found that 18% of the isolates showed haemagglutination. Similar results were obtained by Vidhya *et al.* (2016) who found that 20% of the isolates were positive for haemagglutination. Higher rates were reported by Fatima *et al.* (2012), Desai *et al.* (2013), Sai *et al.* (2013) and Vijayalakshmi *et al.* (2015) who found that 48%, 66%, 59% and 68% of the isolates were haemagglutinating, respectively.

In this study, higher prevalence for MRHA (12%) than MSHA (6%) was observed. Our results were in agreement with Fatima *et al.* (2012) and Desai *et al.* (2013). On the other hand, higher prevalence for MSHA than MRHA was recorded

by Sai *et al.* (2013) and Vijayalakshmi *et al.* (2015).

The genes coding for alpha-haemolysin (*hlyA*), cytotoxic necrotizing factor (*cnf-I*) and P fimbrial adhesin (*papC*) were detected by PCR in the current study. It was observed that 13 isolates (26%) of *E. coli* isolates were positive for *hlyA* gene which was in agreement with Bingen-Bidois *et al.* (2002), Tiba *et al.* (2008), Bashir *et al.* (2012) and Fatima *et al.* (2012). Lower rates were obtained by other studies done by Arisoy *et al.* (2006), Farshad and Emamghorashi (2009) and Tarchouna *et al.* (2013). Higher rates were obtained by Santo *et al.* (2006) and Karimian *et al.* (2012) who reported that 96% and 50.4% of the isolates were positive for the gene, respectively.

As regard the *cnf-I* gene, it was found that 15 isolates (30%) of *E. coli* isolates in this study were positive for the gene. Similar results were obtained by Adwan *et al.* (2015) and Calhau *et al.* (2015). Lower results were obtained by Arisoy *et al.* (2006), and Tarchouna *et al.* (2013). On the other hand, higher rates were obtained by Karimian *et al.* (2012) who reported that 50.4% of the isolates were positive for the gene.

In the present study, it was found that 13 isolates (26%) were positive for *papC* gene. This finding was in accordance with Farshad and Emamghorashi (2009), Qin *et al.* (2013) and Calhau *et al.* (2015). Higher *papC* gene carriage rates of 36%, 32%, 32.7% and 34.6% were reported by Usein *et al.* (2001), Santo *et al.* (2006), Tiba *et al.* (2008), Firoozeh *et al.* (2014), respectively. Lower *papC* gene carriage rates of 23% and 24% were obtained by Arisoy *et al.* (2006) and Bashir *et al.* (2012), respectively. The diversity in frequency of *pap* gene among different studies can be attributed to the fact that UPEC strains can utilize a variety of adhesins to bind to the urinary epithelial cells, and start the infection. Hence, the strains lacking the *pap* operon may use other adhesins encoding operons such as *afa*, and *sfa* for binding (Neamati *et al.*, 2015).

In addition, the differences in prevalence of UPEC virulence genes showed that the virulence properties of UPEC strains is closely depending on geographic region, weather climate of each regions, diets, the levels of public health, hospital's health and even methods of sampling (Karimian *et al.*, 2012).

In the present study, the simultaneous presence of *papC*, *cnf-1* and *hlyA* was observed in only one UPEC isolate. Bashir *et al.* (2012) found that the three genes were found collectively in 4 isolates (out of a total of 59 isolates). The cumulative presence of *papC*, *cnf-1* and *hlyA* is the evidence of the presence of pathogenicity island  $\Pi_{396}$ , which is highly prevalent among UTI isolates (Piatti *et al.*, 2008).

Regarding the relation between the phenotypic virulence factors and the virulence genes, it was found that *hlyA* gene was detected in a higher percentage in  $\beta$ -haemolytic *E. coli* 53% (8/13) than non-haemolytic *E. coli* 14% (5/13) and this difference was statistically significant (P-value = 0.011). The presence of the gene in some non-haemolytic strains may be explained by the possibility of modification of the gene leading to inactive haemolysin (Landraud *et al.*, 2000). Also, since alpha haemolysin is a pore-forming toxin, at low concentration its binding does not always lead to lysis (Boehm *et al.*, 1990; Ostolaza and Goni, 1995). On the other hand, 7  $\beta$ -haemolytic *E. coli* isolates in this study were negative for *hlyA* gene which means that haemolysis on sheep blood agar might be due to haemolysins other than  $\alpha$ -haemolysin (Fatima *et al.*, 2012).

In our study, we found that all isolates positive for *hlyA* gene exhibited cytotoxic activity on HEp-2 cell line; 11 of which harboured *cnf-1* gene in addition. Out of the 13 isolates positive for *papC* gene, only 2 isolates were positive for MRHA. This can be explained by Wullt (2002) who found that some *papC* positive isolates, especially those isolated from asymptomatic infections, do not express P fimbriae. In addition, the expression of P fimbriae is subjected to phase variation so *in vitro* growth conditions differing in glucose concentration, temperature and pH may switch the fimbriae on or off. We found that the remaining 4 isolates positive for MRHA were negative for *papC* gene. According to Sabitha *et al.* (2014), MRHA can be mediated by P fimbriae and also by X, F1C and Dr fimbriae.

Regarding sensitivity of *E. coli* to fluoroquinolones, it was observed that 30 isolates (60%) were resistant to all tested fluoroquinolones. This result was in agreement with Lúpez-Banda *et al.* (2014), Calhau *et al.* (2015) and Gururaju *et al.* (2015).

In the current study, although there was a higher percentage of both virulence factors and virulence genes in the fluoroquinolones resistant group than in the fluoroquinolones sensitive group, this difference was not statistically significant. This was in accordance with Oliveira *et al.* (2011). According to previous studies, Johnson *et al.* (2003), Horcajada *et al.* (2005), Moreno *et al.* (2006), Takahashi *et al.* (2009), and Kawamura-Sato *et al.* (2010) found that quinolones and fluoroquinolones resistant UPEC strains display overall reduced virulence while, susceptible *E. coli* strains were more virulent. Piatti *et al.* (2008) found a lower incidence of *papC*, *hlyA*, and *cnf-1* among fluoroquinolones resistant group than among susceptible group, which seems to be due to a loss of the corresponding PAI, probably as a result of the mutation that causes resistance.

## CONCLUSION

Virulence determinants may help us to get insights into pathogenesis of UPEC isolates and management of UTIs. Phenotypic methods are simple but not very specific, while multiplex PCR is rapid, easy and more specific in detecting virulence genes. The tested *E. coli* isolates exhibited a high resistance to fluoroquinolones with no significant difference was found between fluoroquinolones resistant and sensitive *E. coli* isolates regarding the virulence genes as well as virulence factors.

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