

## Research Article

# Determination of phenotypic expression of the fimbriae and hemolysin of uropathogenic *Escherichia coli* (UPEC)

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### ABSTRACT

**Background:** The bacterial strains that cause symptomatic urinary tract infections possess diverse distinctive properties that enable them to overcome the local host defences. In *Escherichia coli*, virulence results from the cumulative impact of several virulence factors, which can vary according to the patient populations. The study was undertaken to assess the prevalence of the virulence factors by phenotypic assays in the *E. coli* isolates which were isolated from patients with UTI from a tertiary care hospital in Kerala.

**Methods:** A total of 300 *E. coli* isolates were obtained from symptomatic cases of urinary tract infections and 30 *E. coli* faecal isolates were obtained from apparently healthy individuals and they were tested for phenotypic properties like haemolysin production, mannose resistant hemagglutination to indicate P fimbriae, cell surface hydrophobicity.

**Results:** Among the 300 *E. coli* isolates from the cases group, 135 (43.5%) were hemolytic, 106 (35.5%) were MRHA positive, 123 (41%) were hydrophobic. Among the 30 controls, 2 (6%) were hemolytic, 02 (6%) were MRHA positive and 04 (10%) were hydrophobic. The difference between the cases and the control group was significant ( $P < 0.001$ ). Multiple virulence factors were observed in 51% of the isolates.

**Conclusion:** The present study showed varied phenotypic expression of the virulence factors in the urinary isolates as compared to the fecal isolates.

**Keywords:** UTI, *Escherichia coli*, Virulence factors, Phenotypic, Hemolysin, Hemagglutination, Serum resistance, Hydrophobicity, Adhesions

### INTRODUCTION

The Urinary Tract Infections (UTIs) are one of the most prevalent bacterial infections.<sup>1</sup> *E. coli* accounts for 50% - 90% of all the uncomplicated urinary tract infections.<sup>2</sup> These *E. coli* are primarily derived from the faecal flora, which can colonize the periurethral area, overcome the local host defences and enter and multiply within the urinary tract. These *E. coli* strains are designed as Uropathogenic *E. Coli* (UPEC) which possess distinctive traits that confer an enhanced extraintestinal virulence potential.<sup>4,6</sup>

UTI is predominantly a disease of the females, because of the anatomy of the female urethra. The incidence of bacteriuria increases during pregnancy, due to the anatomical and the hormonal changes. In most of the hospitalized patients, nearly all the UTIs are preceded by the instrumentation of the urinary tract, mainly urinary catheterization and it is a frequent cause of significant morbidity, sepsis and death.<sup>4</sup>

Most of the strains of *E. coli* which cause UTIs belong to a restricted range of serotypes which are different from the distribution in the faecal isolates. However, the serotypes alone cannot explain the uropathogenicity of *E.*

*coli* and various other factors are necessary for its virulence.<sup>6,7</sup>

The Virulence Factors (VFs) serve in distinguishing the potential pathogens from the harmless intestinal strains.<sup>8</sup> The VFs like toxins, adhesions and the surface hydrophobicity and the serum resistance have an influence on the pathogenicity of the organisms.<sup>9,10</sup> The virulence of the individual strains in a given infection is determined by the presence and the actual expression of the virulence genes which are present in them and also by the environmental conditions in the host.<sup>10</sup> These VFs are responsible initially for the colonization of the organisms and subsequently for the tissue damage. The pathogenic strains adhere to the urinary tract epithelial cells and the survival of the complement action appears to contribute to the urinary tract virulence, which are not as frequently observed among the normal faecal flora. These markers of (UPEC) are expressed with different frequencies in different disease states, which range from asymptomatic bacteriuria to chronic pyelonephritis.<sup>11</sup> Most of the UPEC strains with virulence factors, belong to the phylogenetic group B2 and to a lesser extent to group D, while the commensal strains belong mainly to the groups A and B1.<sup>12</sup>

## METHODS

This study was conducted in the Karuna Institute of Medical Sciences, Chittur. A total number of 300 *E. coli* isolates were obtained from 300 urine samples (cases) and 30 stool samples (controls).

The inclusion criteria were *E. coli* which was isolated from the urine who had significant bacteriuria and the stool samples were of healthy individuals.

The patients who were on a current antibiotic therapy were excluded from study.

### Sample collection

Mid-stream clean catch urine, catheterized urine and stool samples were collected in sterile containers which were labelled with the patients' details. The specimens were transported to the laboratory in leak proof boxes and they were processed as soon as possible. When the containers processing was delayed, it was stored at 4°C.

### Specimen processing

The urine samples were observed macroscopically for their colour and turbidity. Wet mounts of the samples were prepared and examined for the presence of pus cells and organisms.

Semiquantitative cultures were done by inoculating thoroughly the mixed urine onto a 5% sheep blood agar plate and on a Mac Conkey's agar plate with a calibrated

loop. The inoculated plates were incubated at 37°C overnight.

The identification of the isolates was done on the basis of the colony morphology, gram staining and the standard biochemical tests. All the *E. coli* isolates which were obtained, were screened for the presence of virulence markers through phenotypic assays.

### Hemolysin

The bacteria was inoculated onto 5% sheep blood agar and incubated overnight at 37°C. Hemolysin production was detected by the presence of lysis. A zone of complete lysis of the erythrocytes around the colony and clearing of the medium.

### Hemagglutination

#### Mannose resistant fimbrial hemagglutination (MRHA)

The *E. coli* were inoculated into 5ml of Muller Hinton broth to give a turbid suspension [ $2.4 \times 10^9$  CFU/ml] The tubes were incubated at 37°C for 5 days to get fimbriae enriched *E. coli*. The pellicle which was formed was subcultured onto Muller Hinton agar and incubated overnight at 37°C. The group A positive venous blood was added to equal amounts of the Alsever's solution, this was washed three times and a 3% erythrocyte suspension was made in PBS, pH 7.4

40µl of this erythrocyte suspension was added to 40µl of PBS 7.4. In a different circle of the tile 40 µl of 3% D-mannose was added to erythrocyte suspension. The colonies from the Muller Hinton agar plates were mixed in both the wells. The slides were rotated for 4 minutes and the hemagglutination reactions were recorded.

The hemagglutination was considered to be mannose resistant when it occurred in the presence of D-mannose and it was considered to be mannose sensitive when it was inhibited by D-mannose.

### Cell surface hydrophobicity (CSH)

Salt Aggregation Tests (SAT): The *E. coli* which was grown on the Mac Conkey's agar plates were inoculated into 1 ml of phosphate buffer, pH 6.8 and the turbidity was matched with the Mcfarland's standard 6 to get a colony count of  $5 \times 10^9$  colonies/ml.

Ammonium sulphate solutions of molar concentrations 1M, 1.4M, 2M were prepared. 40 µl of the *E. coli* suspension was mixed with an equal volume of the ammonium sulphate solution of different molarities on a glass slide. The slides were placed on a VDRL rotator for 4 minutes and the clumps which were formed in different concentrations of the ammonium sulphate were observed.

The *E. coli* strains were considered as hydrophobic if they aggregated in the ammonium sulphate solution of concentration,  $\leq 1.4M$ .

**Statistical methods**

The data which was collected was analyzed by computing the descriptive statistics, namely the mean, standard deviation and the range. Any significant differences between the mean values of the study groups and the control groups were tested. The Chi-square test was used to find the significance of the study parameters on a categorical scale between two or more groups.

**RESULTS**

A total of 300 *E. coli* isolates were obtained from the symptomatic cases of UTI, 30 *E. coli* isolates were obtained from the stool samples of the apparently healthy individuals for the control group. A majority of the patients in the case group were in the age group of the 20-40 years, followed by those who were in the age group of 61-80 years, with a female majority.

Hemagglutination was the most common virulence factors which was present. CSH was second most common virulence factor. There was a significant difference between the presence of the virulence factors in the cases and the control groups, as has been shown in Table 1 & Table 2.

**Table 1: Comparison of virulence factors in cases and control groups.**

Virulence factors	300 <i>E. coli</i> isolates from UTI	30 <i>E. coli</i> isolates from stool sample
Haemolysin	43.5%	6%
Haemagglutination	48%	16%
MRHA*	35.5%	6%
MSHA**	12.5%	10%
No hemagglutination	52%	-
Surface hydrophobicity	41%	10%

MSHA\*: Mannose sensitive hemagglutination  
MRHA\*: Mannose resistant hemagglutination

**Table 2: SAT value of *E. coli* isolates.**

	UPEC hydrophobicity (SAT value) 41%
$\leq 1.4M$ Ammonium sulphate	123
$< 1M$	108

M: molar

Coexistence of two virulence factors: Table 3 & Table 4.

**Table 3: Hemolysin and fimbriae (P fimbriae & type1 fimbriae).**

UPEC hemolysis on blood agar 43.5%		
58 isolates having hemolysin showed MRHA	24 isolates having hemolysin showed MSHA	
43% of isolates with hemolysin exhibited MRHA	17.7% of isolates with hemolysin exhibited MRHA	39% isolates did not exhibit hemagglutination

**Table 4: Cell surface hydrophobicity and fimbriae.**

UPEC hydrophobicity is 41%	
16 isolates having MRHA showed hydrophobicity	6 isolates having MSHA showed hydrophobicity

**DISCUSSION**

*E. coli* accounts for 45- 90% of all the uncomplicated urinary tract infections.<sup>2</sup> The Uropathogenic *E. Coli* (UPEC) express several surface structures and they secrete a protein which is peculiar to the strains of *E. coli* which cause UTIs. Various chromosomally encoded factors have been identified and designated as the candidate virulence markers which are expressed in different frequencies in different disease states. The VFs serve in distinguishing the potential pathogens from the harmless intestinal strains.<sup>8</sup> Apart from the bacterial factors, the host defence must also be considered, since certain individuals contract UTIs more frequently than others and as certain individuals are predisposed to a particular type of condition.<sup>13</sup>

**Sex distribution**

In the present study, among the 300 *E. coli* urinary isolates, 234 were from females and 66 from males. This observation was comparable with that of the previous studies which were done by Anja Siitonen<sup>14</sup> as well as Risto et al.,<sup>15</sup> where UTIs were more common among females than among males. As per Foxman and Brown, women have a higher incidence of UTI than men, with an annual incidence of 12% as compared to the 3% incidence among men.<sup>16</sup> The higher incidence among the females is attributed to the difference in the anatomy, the moist periurethral area in women and the shorter distance between the anus and the urethral opening and between the urethral opening and the bladder.<sup>17</sup>

**Age distribution**

In the present study, UTIs were found to occur more commonly among all the age groups, with a peak incidence in the 20-40 years age group, This was in agreement with the findings of the research which was done by Foxman and Brown, where UTIs were found to occur more frequently in people of all age groups, with a peak incidence in women of the age group of 20-24

years. Among men, UTIs were found to occur in the oldest age group of 60-85 years with a 7.3% incidence.<sup>18</sup>

However, in a previous study which was done by Braumer et al, on 104 patients with UTIs, 54 were found to have a median age of 64 years and 50 were found to have a median age of 63 years.<sup>16</sup>

A study which was done by Anja Siitonen showed that the mean age of both the women and the men was 45 years (ranges of 15-81 and 16-70 years respectively).<sup>15</sup>

### **The virulence markers of *E. coli***

#### *Hemolysin*

The cell bound form of the cytolytic protein toxin is known as beta hemolysin and the cytolytic protein toxin which is secreted by most of the hemolytic *E. coli* strains is known as alpha haemolysin. The hemolysin, is strongly pro-inflammatory, leading to the secretion of IL- 6 and chemotaxins, which sets the pace for the pathogenesis of renal diseases, especially the more severe forms of the infection.<sup>20</sup>

In the present study, the difference between the cases and the controls for the production of hemolysin was highly significant (P <0.001). This observation was similar to the findings of the study which was conducted by Raksha et al., where hemolysin was found to be produced by 41% of the urinary and 12% of the faecal isolates.<sup>12</sup>

It has been suggested that the colonization with the hemolytic strains of *E. coli* is more likely to cause urinary tract infections. Hemolysis, though it is not essential for the establishment of acute pyelonephritis, may contribute to tissue injuries, to the survival of the organisms in the renal parenchyma and to their entry into the blood stream.

#### *Hemagglutination*

The hemagglutination and the adherence are mediated by fimbriae.<sup>21</sup> The MRHA can be mediated by P fimbriae and also by X, FIC and Dr fimbriae. These adhere to the fibronectin on the uroepithelial cells, thus contributing to the persistence. In the present study, MRHA was positive in 106 (35.5%) isolates in the cases group with the group A+ve erythrocytes and in 2 (6%) isolates in the control group. Hence, there was a significant difference in the MRHA positivity between the cases and the controls (P <0.001). In the findings of the study carried out by Siegfried et al, 42% *E. coli* isolates were MRHA positive with the group A+ve erythrocytes.<sup>20</sup>

The expression of the type 1 fimbriae is indicated by MSHA. The MSHA were 12.5% isolates in this study.

A good proportion of the *E. coli* isolates which cause UTIs in pregnancy are P fimbriated. These UTIs, if not treated, can progress to pyelonephritis in about 30-50 per

cent of the cases. Therefore, the *E. coli* which are isolated from asymptomatic bacteriuria in pregnant women should be tested for its virulence factors to identify the risk of developing pyelonephritis.<sup>1</sup> The presence of both P fimbriae and hemolysin which was reported in other studies was 15%.<sup>21</sup>

### **Cell surface hydrophobicity**

The surface hydrophobicity is another important virulence factor of *E. coli* that causes extraintestinal infections. Hydrophobicity was a recently described as a virulence mechanism of *E. coli*. The bacterial surface structures are of considerable interest, because they have a key role to play in the interaction with the surrounding cell surface. The crystalline surface layers (S layers) which are present on both the gram positive and the gram negative organisms play an important role in this interaction. The high hydrophobicity of the bacterial cell surface promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells.

In the present study, there was a significant difference for CSH between the cases and the controls (P <0.001). This was consistent with the results of previous studies, where 26.36%,<sup>12</sup> 33.4%,<sup>10</sup> and 46%<sup>20</sup> of the isolates were hydrophobic respectively.

### **Multiple virulence factors**

The results showed that 51% of urinary isolates had more than one virulence factor. In the present study, 43% of the haemolysin producing isolates were MRHA positive. In the findings of a previous study, where a combination of all the three virulence factors, such as haemolysin, surface hydrophobicity and MRHA positivity, was present in 11.2% of the isolates.<sup>10,12,22</sup>

Expression of multiple virulence factors contribute synergistically in overcoming normal host defence mechanism.<sup>23</sup>

A previous study indicated that although the virulence of an organism cannot be accurately predicted on the basis of its measurable virulence factor phenotype, the presence of multiple virulence factors increases the virulence of the organisms,<sup>23</sup> and the compromising host conditions decrease the need for multiple virulence factors in the strains which cause serious infections.<sup>24</sup>

In this study the *E. coli* strains are definitely associated with the aetio-pathogenesis of UTIs. The *E. coli* strains with virulence factors were significantly more in the urinary isolates than in the controls.

### **CONCLUSION**

*E. coli* has the capacity to adapt and survive at extra intestinal sites like the urinary tract, by producing various virulence factors. Thus, the observations which we made

in this study indicate that the pathogenic *E. coli* express more MRHA, they are more hemolytic and that they have a higher cell surface hydrophobicity which may help in the initiation of infections. Hemolysin and adherence through P fimbriae are important properties of the uropathogenic *E. coli*.

Genotypic assays of the isolates shall help to characterize the prevalence of specific genes among UPEC isolates and to correlate with phenotypic characters.

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