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Original Research Article

Study of uropathogenic *Escherichia coli* with special reference to its virulence factors

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most common nosocomial infections, caused by *Escherichia coli*. UPEC differ from non-pathogenic *E. coli* by the production of specific virulence factors which enable the bacteria to adhere to uroepithelial cells and to establish UTI. The aim of this study is to check the virulence factors of uropathogenic *E. coli*.
Methods: A prospective study conducted in the Department of Microbiology of a tertiary level hospital in Mumbai over a period of one year (February 2011 to February 2012). A total of 123 Urine samples received in the laboratory were processed as per standard microbiological procedures to look for virulence factors like hemolysin, hemagglutination, cell surface hydrophobicity and gelatinase production.
Results: Out of 123 patients 69 (56.09%) cases were from females and 54 (43.90%) were males. Thus female: male ratio was 1:3. Hemolysin production was seen in 27.64%, hemagglutination in 53%, cell surface hydrophobicity in 27.64%.
Conclusions: UTI is more common in middle aged females and in community set-up. The knowledge of virulence factors of *E. coli* will help in better understanding of the organism pathogenicity and guided empirical therapy can result in better treatment outcome.

Keywords: Uropathogenic *Escherichia coli*, Hemagglutination, Salt aggregation test

INTRODUCTION

Urinary tract infection (UTI) is the most repeatedly diagnosed kidney and urologic disease and *Escherichia coli* by far the most common etiologic agent.[1] UTI is the second most common cause of bacterial infection in humans and thus represents a major source of human discomfort.[2]It is now known that there are subgroups of faecal *E. coli*, that can colonize in the periurethral area, enter urinary tract causing symptomatic disease. It has been known that certain serotypes of *E. coli* are consistently associated with uropathogenicity and are designated as uropathogenic *E. coli* that expresses chromosomally encoded virulence markers.[2]

For the first time in the late 1970s it was recognized that *E. coli* strains causing urinary tract infections typically agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbriae.[3] The important virulence factors in the pathogenesis of urinary tract infection (UTI) include adhesions (P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae), the aerobactin system, hemolysin, K capsule, and resistance to serum killing, hemolysin production and siderophore production. The ability of *E. coli* to adhere to the uroepithelium is mediated by fimbriae, thereby resisting elimination by the flow of urine. Adhesion is therefore measured to be important step in the pathogenesis of UTI.[2] UPEC strains take advantage of

variety of virulence properties in order to colonize and establish an UTI. Bacterial adherence and colonization of the urinary tract by UPEC strains are mediated by the expression of several types of fimbrial and non-fimbrial adhesins. The most common fimbriae found in UPEC strains are type I and P fimbriae which enhance virulence and are involved in initial urethral colonization. Hemolysin is produce by many UPEC, which may be involved in kidney disease.[4] Considering the high degree of morbidity and mortality due to UTIs caused by uropathogenic *E. coli*, we conducted this study in a tertiary care hospital in Mumbai to look for the various virulence factor of this pathogen.

METHODS

The study was conducted in the Department of Microbiology of a tertiary care hospital in Mumbai over a period of one year (February 2011 to February 2012). One hundred twenty three samples were studied for the detection of virulence markers of *E. coli.* The samples were processed immediately using standard microbiological procedures. Wet mount microscopic examination of the urine sample was done followed by culture on Blood agar and MacConkey agar and incubated at 37^0C for 24 hours. Then the isolates were identified based on colony morphology on blood agar, MacConkey's agar, Gram staining and by standard biochemical tests.[5] The isolates were maintained by inoculating nutrient agar butts and stored at 4^0C temperature.

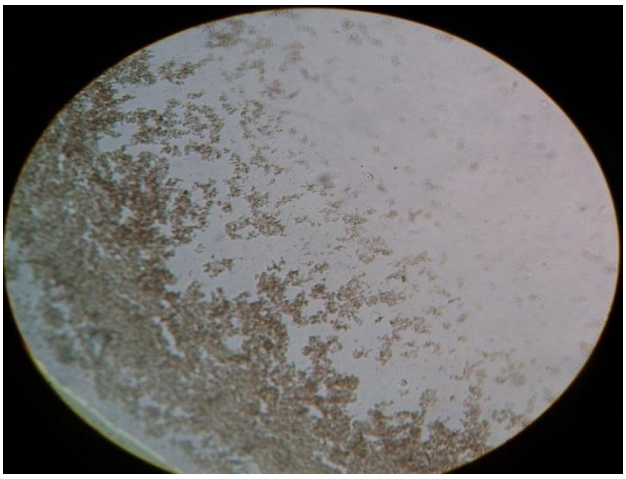

Figure 1: Salt aggregation test.
Agglutination under microscope (10X).

Cell surface hydrophobicity[6]

The cell surface hydrophobicity of *E. coli* was determined by salt aggregation test (SAT). One loopful (10µl) of bacterial suspension made in phosphate buffer was mixed with equal volume of ammonium sulphate solution of different molarities, i.e. from 0.3125 m through 5 m, on a glass slide and observed for 1 min. while rotating. The highest dilution of ammonium sulphate solution giving visible clumping of bacteria was scored as the salt aggregation test value. *E. coli* strains that had SAT value <1.25 m were considered hydrophobic (Figure 1).

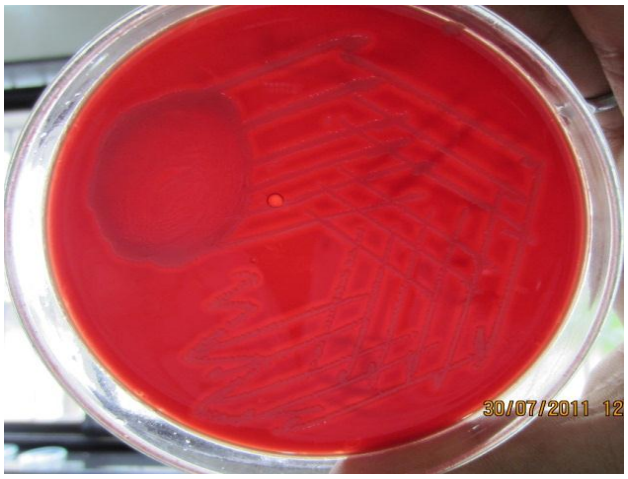

Figure 2: Hemolysis test.
E. coli shows β hemolysis on blood agar.

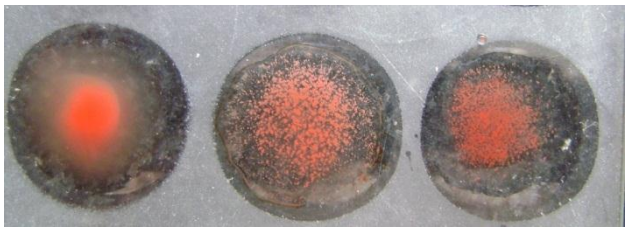

Figure 3: Haemagglutination.
Left side- Negative, Middle- Positive, Right side- MRHA.

Hemolysin production[7]

Plate hemolysis test was done for the detection of β-hemolysis produced by *E. coli*. The bacteria was inoculated onto 5% sheep blood agar and incubated overnight at 37ºC. Hemolysin productions were detected by the presence of a zone of complete lysis of the erythrocyte around the colony and clearing of the medium (Figure 2).

Haemagglutination (HA)[7]

The haemagglutination was detected by clumping of erythrocytes by fimbriae of bacteria in the presence of d-mannose. This test was carried out as per the direct bacterial haemagglutination test- slide method and mannose-sensitive and mannose-resistant haemag-glutination tests. The strains of *E. coli* were inoculated into 1% nutrient broth and incubated at 37 °C for 48 hours for full fimbriation. A panel of red blood cells was selected by obtaining blood from human (blood group 'O'). The red blood cells were then washed thrice in normal saline and made up to a 3% suspension in fresh saline. 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. One drop of the RBC suspension was added to a drop of the broth culture and slide was rocked to and fro sat room temperature for 5 minutes. Presence of clumping was taken as positive for haemagglutination. Mannose-sensitive haemagglutination was detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% w/v d-mannose was added to the red cells and a drop of broth culture. Mannose-resistant haemagglutination was detected by the presence of haemagglutination of 3% 'O' group human RBC in the presence of 2% mannose (Figure 3).

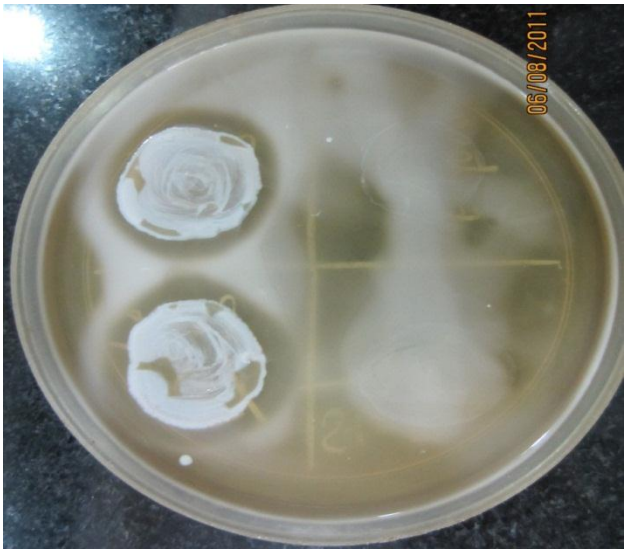

Figure 4: Gelatinase test.
Left side- Positive, Right side- Negative

Gelatinase test[6]

Gelatinase production was tested using gelatin agar. The plate was inoculated with test organism and incubated at 37ºC for 24 hrs.The plate was then flooded with mercuric chloride solution. Development of opacity in the medium and zone of clearing around colonies were considered positive for gelatinase (Figure 4).

Statistical analysis

The Statistical analysis was carried out using SPSS software version 16.0. Data were presented in proportions and percentages. Chi-square test was applied when two or more set of variables were compared. The critical value of 'p' indicating the probability of significant difference was taken as <0.05.

RESULTS

A total of 398 urine samples received over a period of one year from symptomatic cases of urinary tract infection with significant bacteriuria were processed. Out of these, 123 samples were selected for our study. Fifteen of these sample showed growth of two type of organisms including *E. coli*, while from rest 108 samples only *E. coli* were isolated. These 123 *E. coli* isolates were studied for the possession of these virulence factors.

Out of 123 patients 69 (56.09%) cases were from females and 54 (43.90%) were males. Thus female: male ratio was 1:3. The samples were received from both IPD and OPD patients (Table 1).

Table 1: Gender wise distribution of patients with uti due to *E. coli.*

Male n=53 (43.08%)		Female n=70 (56.9%)	
OPD	**IPD**	**OPD**	**IPD**
27	26	49	21

A total of 34 (27.64%) among 123 isolates showed hemolysis. Mannose resistance hemagglutination was seen in 51 (41.46%) and mannose sensitive hemagglutination in 07 (05.69%).Thirty four (27.64%) showed cell surface hydrophobicity/ salt aggregation test.

Table 2: Virulence factors of uropathogenic *E. coli.*

Tests	Positive (n)	Percentage (%)
1. Hemolysis	34	27.64
2. Cell surface hydrophobicity	34	27.64
3. Haemagglutination		
MRHA*	51	41.46
MSHA**	07	05.69
4. Gelatinase	00	00.00

*Mannose resistant haemagglutination
**Mannose sensitive haemagglutination

None of the isolates gave the gelatinase test positive (Table 2).

DISCUSSION

Urinary tract infections which are not properly treated from their onset can become a renal threat in time, finally leading to renal failure. In general, the more virulence factors a strain expresses, the more severe an infection it is able to cause.[8] The occurrence of multiple virulence factors in UPEC strains further strengthens the concept of association of UPEC with urinary pathogenicity.[3] These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defence mechanisms. Virulence factors enable *E. coli* to colonize selectively the mucosal uro-epithelium, evoke an inflammatory reaction and eventually proceed from lower urinary tract to renal cavities and tissue invasion. The capacity of *E. coli* to produce many virulence factors contributes to its pathogenicity.[5]

Incidence of UTI was more common in females (56.9%) than in males (43.08%) in our study. Mittal et al also

reported a higher prevalence of UTI in females (53.3%).[9] The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having short urethra, close proximity to anal canal, presence of normal flora in vagina and pregnancy.

Hemolysin production is associated with pathogenicity of *E. coli*, especially in the more severe forms of infection. It has been suggested that colonization with hemolytic strains of *E. coli* is more likely to develop into urinary tract infections. Hemolysis, though not essential for establishment of acute pyelonephritis, may contribute to tissue injury, survival in renal parenchyma and entry into blood stream.[5] In this study 34 (27.64%) strains of *E. coli* produced hemolysin. However as per studies of Gholamhoseinian et al (reported from Kerman, Iran, 2007) hemolysin was seen in 28% which is just similar to present study.[10] Also in the other studies conducted by Kausar et al, Sharma et al, Raksha et al, Mittal et al, hemolysin was seen in 21%, 25%, 41.36% & 47.4% respectively.[3,6,10,11]

Surface hydrophobicity is another virulence factor of *E. coli* that causes intestinal infections. The high hydrophobicity of the bacterial cell surface promotes their adherence to various surfaces like mucosal epithelial cells.[5] In the current study, 34 (27.64%) of the strains were hydrophobic. According to studies of Sharma et al hydrophobicity was seen in 33.4%.[6] Also in the other studies by Raksha et al, Dorota et al, Mittal et al hydrophobicity was seen in 26.36%, 74% and 61% respectively.[3,9,12]

In our study, out of 123 isolates, 51 (41.46%) showed MRHA and only 07 (05.69%) showed MSHA. However as per the studies of Vagarali et al (reported from Karnataka, Kausar et al MRHA was seen in 25% & 30% respectively and MSHA in 34.38% and 36% respectively.[2,11] Also in the other studies by Gholamhoseinian et al (reported from Kerman, Iran, 2007), Raksha et al (2003), MRHA was seen in 48% and 30.9% respectively.[3,10] In the recent study by Mittal et al MRHA was seen in 45.5% and MSHA in 54.5%.[11]

Table 3: Different studies on virulence factors of *E. coli.*

Study	Year	Sat (%)	Hemolysin (%)	Haemagglutination	
				MRHA (%)	MSHA (%)
Raksha et al[3]	2003	26.36	41.36	30.9	-
Sharma et al[6]	2007	33.4	25	-	-
Dorota et al[12]	2007	74	-	-	-
Gholamhoseinia et al[10]	2007	-	28	48	-
Vagarali et al[2]	2007	-	-	25	34.38
Kausar et al[11]	2009	-	21	30	36
Mittal et al[9]	2014	61	47.4	45.5	54.5
Current study	2012	27.64	27.54	41.46	05.69

The present study has shown the capacity of *E. coli* to adapt and survive in different tissues by producing virulence factors and the expression of virulence factors may depend on the need and it varies in different kinds of infections.[5] A comparison of different studies of virulence factors of *E. coli* is shown in (Table 3).

CONCLUSION

UTI's are considered acute, self-limiting infections despite the prevalence of recurrent symptoms, two or more times within months of a primary infection. It is more common in middle- aged females. Uropathogenic *E. coli* the major causative agent is more common in community as compared to hospital because of poor sanitation and unhygienic practices. The knowledge of virulence factors of *E. coli* will help in better understanding of organisms and its empirical treatment.

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