ORIGINAL ARTICLE

Study of virulence factors of uropathogenic Escherichia coli and its antibiotic susceptibility pattern

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ABSTRACT

Context: Urinary tract infection (UTI) is one of the most common nosocomial infections, caused by *Escherichia coli*. This study determined the presence of virulence factors in the organism and correlates it with the multi-drug resistance (MDR). **Aims:** The aim of the following study is to assess the virulence factors of uropathogenic *E. coli* and antibiotic susceptibility pattern. **Settings and Design:** This was a prospective study conducted in the Department of Microbiology in PT. B. D. Sharma, PGIMS, Rohtak. **Subjects and Methods:** The study was conducted over a period of 1 year. Urine samples received were processed as per standard microbiological procedures. Virulence factors such as hemolysin, hemagglutination, cell surface hydrophobicity, serum resistance, gelatinase and siderophore production were studied. The antimicrobial susceptibility was done as per Clinical and Laboratory Standard Institute Guidelines. **Statistical Analysis Used:** The data was analyzed by using SPSS(Statistical Package for the social sciences) IBM Corporation version 17.0. A two sided $P \leq 0.05$ was considered to be significant. **Results:** Hemolysin production was seen in 47.4%, hemagglutination in 74.8%, cell surface hydrophobicity in 61%, serum resistance in 59%, gelatinase in 67.5% and siderophore production in 88% isolates. Nitrofurantoin was found to be most effective followed by, gatifloxacin and gentamicin. Twenty nine percent (29.62%) isolates were MDR. **Conclusions:** Therefore, the knowledge of virulence factors of *E. coli* and their antibiotic susceptibility pattern will help in better understanding of the organism and in the treatment of UTI.

KEY WORDS: Antibiotic susceptibility, multiple drug resistance, , uropathogenic *Escherichia coli*, virulence factors

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span.[1] They can be symptomatic or asymptomatic.

Escherichia coli is the most common cause of UTIs, accounting for about 85% of community acquired and 50% of hospital-acquired infections, it predominates strongly at most ages.[2]

E. coli is a commensal in the human intestinal tract, when enters into unnatural sites, it can cause a variety of infections, e.g., UTIs, sepsis, pyelonephritis etc. Serotypes which lead to UTIs are designated as uropathogenic *Escherichia coli* (UPEC).[3]

The virulence factors of *E. coli* are multiple and unusually complex affecting pathogenicity in combination with one another. The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, hemolysin, enterotoxin and siderophore, fimbriae and hemagglutination.[4] These markers of UPEC are expressed with different frequencies in different diseases states ranging from asymptomatic bacteriuria to chronic pyelonephritis.

Moreover, the drug resistance among strains has further aggravated the problem of UTI's. Therefore, the present study was carried out with aim to know the prevalence of various virulence factors in UPEC and to study their antibiotic susceptibility profile.

SUBJECTS AND METHODS

The study was carried out on a total of 135 *E. coli* isolates recovered from urine samples of patients with clinically suspected UTIs of all age groups, over a period of 1 year from January to December 2011. *E. coli* isolates were identified by the standard microbiological procedures. The antibiotic susceptibility testing was performed using the standard antimicrobial agents (Hi Media, Mumbai) amoxyclavulanic acid (30 μg), ceftizoxime (30 μg), cotrimoxazole (25 μg), gatifloxacin (5 μg), gentamicin (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), as per Clinical and Laboratory

Standard Institute Guidelines.[5] *E. coli* (ATCC 25922) was used as control strain.

Virulence factors such as hemolysin, hemagglutination, serum resistance, gelatinase test, cell surface hydrophobicity, siderophore production were detected as follows.

Detection of virulence factors

1. Hemolysin: The *E. coli* isolates were inoculated on 5% sheep blood agar and incubated overnight at 37°C. The indicator of hemolysin production was the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.[3,6,7]
2. Hemagglutination: The test was carried out as per the direct bacterial hemagglutination test-slide method. One drop of red blood cell (RBC) suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 min. Presence of clumping was taken as positive for hemagglutination. Mannose-sensitive hemagglutination was detected by the absence of hemagglutination 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 hemagglutinating (MRHA) was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.[8]
3. Serum resistance: Overnight culture of *E. coli* on blood agar plates were suspended in Hank's balanced salt solution. Equal volume of this bacterial suspension and serum (0.05 ML) were incubated at 37°C for 3 h. Then 10 µl of this mixture was inoculated on blood agar plate and incubated at 37°C for 24 h and viable count was determined. It is termed as sensitive when colony count drop to <1% of initial value.[3,7]
4. Gelatinase test: Gelatinase production was tested using gelatin agar. The plate was inoculated with test organism and incubated at 37°C for 24 h. The plate was then flooded with 1% tannic acid solution. Development of opacity around colonies were considered as positive for gelatinase.[9]
5. Cell surface hydrophobicity: This test was carried out by salt aggregation test (SAT). One loopful of bacterial suspension in phosphate buffer was mixed with an equal volume of ammonium sulfate solution of different molarity on a glass slide and rotated for 1 min. *E. coli* strains with SAT value ≤ 1.25 M were considered cell surface hydrophobic.[6]
6. Siderophore production assay: The test was done by using chrome azurole sulfonate agar (CAS) agar diffusion assay. The CAS assay detected color change of CAS–iron complex form blue to orange after chelation of the bound iron by siderophores. A strong ligand was added to a highly colored iron dye complex, when the iron ligand complex was formed, the release of the free dye was accompanied by a color change.[,10]

RESULTS

Incidence of UTI was more common in females, i.e., 72 (53.3%) in comparison to males 63 (46.7%) and was more common in sexually active females of age group 21-30 years 37 (27%). These cases were more commonly from gynecology/obstetrics 36 (27%) followed by surgery 34 (25%) and urosurgery 26 (19%).

The most common virulence factor was siderophore production (88%) followed by hemagglutination (74.8%), serum resistance (59%) and hemolysin (47.4%) [Tables 1 and 2].

Among the 135 *E. coli* isolates, 127 (94%) were sensitive to nitrofurantoin followed by ceftizoxime 88 (65.5%) and gatifloxacin, gentamicin 73 (54%), 29.62% *E. coli* isolates were multidrug resistance (MDR) [Table 3].

Multiple virulence factors (MVF) (>4) were present in106 isolates (78.5%). Multi virulence factors producing isolates had strong positive correlation with MDR. Hemolysin producing *E. coli* isolates were more MDR ($P < .05$) which was statistically

Table 1: Detection of various virulence factors in 135 *E. coli* isolates

Virulence factors	*n (%)*
Hemolysin	64 (47.4)
Hemagglutination	101 (74.8)
Serum resistance	79 (59)
Gelatinase	91 (67.5)
Cell surface hydrophobicity	82 (61)
Siderophore production	119 (88)

E. coli: *Escherichia coli*

Table 2: Prevalence of MRHA and MSHA *E. coli* among haemaglutination positive isolates

Total hemagglutinating isolates	*MRHA (%)*	*MSHA (%)*
101	46 (45.5)	55 (54.5)

MRHA: Mannose resistant haemagglutinating; MSHA: Mannose sensitive haemagglutinating; *E. coli*: *Escherichia coli*

Table 3: Antibiotic sensitivity pattern of *E. coli* isolates (135)

Antibiotic	*Sensitivity n (%)*
Nitrofurantoin	127 (94)
Ceftizoxime	88 (65.5)
Gentamicin	73 (54)
Gatifloxacin	73 (54)
Cotrimoxazole	40 (29.5)
Norfloxacin	31 (23)
Amoxyclavulanic acid	5 (4)

E. coli: *Escherichia coli*

Table 4: Association of virulence factors and MDR (resistant to 3 or more classes of drugs) *E. coli* isolates (135)

Virulence factors	*MDR positive isolates (%)*	*MDR negative isolates (%)*	*Total*
Hemolysin	13 (20.31)	51 (79.68)	64
Haemagglutination	16 (34.80)	30 (65.20)	46
Serum resistance	32 (35.16)	59 (64.84)	91
Hydrophobicity	21 (25.30)	62 (74.70)	83
Siderophore	55 (46)	64 (54)	119
Gelatinase	48 (52.75)	43 (47.25)	91

E. coli: *Escherichia coli*; MDR: Multi drug resistant

significant. Hemagglutinating and hydrophobic isolates showed a strong positive correlation with MDR. Serum resistant *E. coli* isolates had correlation with MDR which is not statistically significant [Table 4].

DISCUSSION

UPEC are the most important group of micro-organisms responsible for UTI. 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. Besides adhesion factors, toxins, modulins, capsules ion uptake system and other bacterial products contributes to virulence of strains.[,11]

Incidence of UTI was more common in females 53.3% than in males in our study. Piatti *et al.*[][12] also reported a higher prevalence of UTI in female (77%). 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, presence of normal flora in vagina, menstrual cycle and pregnancy.

Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI.[11] It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses.[13] In the present study, 47.4% *E. coli* isolates produced hemolysin, of these isolates 13 (20.3%) were MDR. In other studies conducted by Raksha *et al*[3], Siegfried *et al.*[7] Hughes *et al* [14], Shruthi *et al* [15] hemolysin production was detected in 41.36% and 59.6%, 59.7% and 41.9% isolates respectively.

The role of bacterial adherence in the pathogenesis of UTI is that colonization of the urogenital epithelium of susceptible individuals by specific bacteria is associated with successful microbial invasion of the urinary tract.[16] and lead to UTIs. Thus, possession of MRHA by UPEC can be considered as one of the important virulence factor in the pathogenesis of UTIs. This concept has been supported in many researches, e.g., Seigfried *et al.*[7], Vagarali *et al.*[17], Raksha *et al.*[3], Kauser *et al.*[18] have reported the incidence of MRHA *E. coli* isolates as 23%, 25%, 30.9%, 30% respectively. In the present study also the rate of MRHA positive *E. coli* isolates was 45.5% and of these isolates 47.7% showed MDR.

Urinary antibodies resist UTI by preventing the adherence of bacteria to uroepithelial cells.[16] Serum resistance is the property by which the bacteria resist killing by normal human serum due to lytic action of complement system. It is likely that complete resistance to serum results from the accumulation of several distinct components at or near the cell surface.[,14] In the present study, serum resistant was found in 59% isolates and out of these isolates 57.5% showed MDR pattern. In other studies, Kauser *et al.*[18] and Sharma *et al.*[6] have demonstrated the serum resistance in 49.5% and 86.8% of the urinary *E. coli* isolates. Hughes *et al.*[,14] stated that the increased degree of serum resistance is associated with increased virulence of the organisms.

In the present study, gelatinase producing UPEC isolates (67.5%) were found to be associated with MDR (52.75%) to commonly used antibiotics.

The surface hydrophobicity is another important virulence factor, which promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells.[5] In our study 61% *E. coli* isolates were hydrophobic, out of these 49% were MDR isolates.

Siderophore production, promotes bacterial growth in the limiting iron concentrations encountered during infection and act as a virulence factor in the pathogenesis of UTI. In our study the siderophore production was seen in 119 (88%) isolates and 46% of these isolates were MDR (statistically insignificant). In other studies, the incidence of siderophore production has been reported to be 76%[19] and 98%,[17] which is in concordance with our study.

A study by Sharma *et al.*,[6] demonstrated that out of 152 *E. coli* isolates, 36 (23.7%) isolates were hydrophobic, 132 (86.8%) were serum resistant and only 4 were positive for protease. MVF were observed in 6 (44%) of isolates. There was a significant association ($P < 0.001$) between multiple factors and virulence of *E. coli*.

Raksha *et al.*[3] demonstrated among 220 urinary isolates, 91 (41.36%) were hemolytic, 68 (30.9%) showed MRHA, 58 (26.36%) were cell surface hydrophobicity positive and 72 (32.72%) were serum resistant.

In the present study, MVF (>4) were present in106 isolates (78.5%). MVF producing isolates had strong positive correlation with MDR.

Antibiotic susceptibility pattern was studied for all *E. coli* isolates. These isolates were most commonly resistant to ampicillin, amoxyclavulanic acid and cotrimoxazole. The increasing prevalence of MDR has been reported by other workers as well, which is due to dissemination of MDR strains in hospital settings. In this study, the maximum sensitivity was shown to nitrofurantoin (94%) followed by ceftizoxime (65.5%) and gatifloxacin (54%).

The present study has shown the production of various virulent factors and developing drug resistance in UPEC. Antibiotic resistance may provide a substantial advantage to the survival of the pathogen. The drug resistance among UPEC is on rise therefore the selection of appropriate antibiotics (after antibiotic susceptibility testing) is must for proper treatment of patients and to avoid emergence of drug resistance.

CONCLUSION

In the present study, MVF producing *E. coli* isolates had strong positive correlation with MDR. Hence, the study shows that virulence factors present either singly or multiple are strongly associated with pathogenesis and drug resistance. Virulence

factors of *E. coli* effect the pattern of drug resistance in these isolates. Therefore, the knowledge of virulence factors of *E. coli* and their AST will help in better understanding of organism and in treatment of UTI.

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