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Urovirulence markers of Uropathogenic *Escherichia* coli and its antimicrobial susceptibility

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Abstract
Background*:* Escherichia coli (E. coli) is most common organism Responsible for UTI. Uropathogenic (*UPEC*) encode widespread virulence factors closely related with pathogenesis of the bacteria.
Objective: This investigation is aimed to determine the prevalence and correlation of phenotypic virulence traits and antibiotic resistance among the UPEC isolated.
Materials and Methods: A total 426 urine samples from clinically suspected UTI patients were processed by standard microbiological procedures. The virulence factors of 152 E. coli, Haemolysis, Mannose sensitive/resistant haemagglutination, serum resistance, cell surface hydrophobicity, Gelatinase were studied. The antimicrobial susceptibility was done as per Clinical and Laboratory Standard Institute Guidelines. *50* stool sample were included in study as control.
Result: Hemolysin production was seen in 30.92%, hemagglutination in 51.97%, Cell surface hydrophobicity in 38.15%, serum resistance in 52.63%, gelatinase in 40.13% and siderophore production in 63.81% isolates. Nitrofurantoin was found to be most effective followed by, imipenum and Amikacin.
Conclusion: The knowledge of virulence factors of *E. coli* and their AST will help in better understanding of organism and in treatment of UTI.

Keywords: urinary tract infection, virulence factors, antibiotic resistance, UPEC

1. Introduction
Urinary Tract Infections (UTIs) are defined as diseases which are caused by a microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus. They represent the most commonly acquired bacterial infections and they account for an estimated 25-40% of the nosocomial infections Bagshaw SM. *et al*. 2006
Uropatho genic Escherichia coli (UPEC) is the most common cause of urinary tract infections (UTIs) both in community and hospital settings with significant morbidity and mortality worldwide. Previous investigations have shown that 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, and toxins such as hemolysin. Fimbriae are categorized serologically by their hemagglutination pattern and receptor specificities as mannose sensitive (MSHA) or mannose resistance hemagglutination (MRHA). Despite the vast subclass of adhesins that have been reported in UPEC, Type I (MSHA) and P (MRHA) are the most common fimbriae found in UPEC strains. They play an important role in binding and invasion to bladder (cystitis) and kidney (pyelonephritis) epithelial cells. UPEC strains often express and secrete a labile pore-forming toxin known as a-hemolysin production that is mainly associated with more virulent UPEC strains. Tabasi M. *et al*. 2015 [15].
The surface hydrophobicity is another important virulence factor, which promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells. Sider ophore production, promotes bacterial growth in the limiting iron concentrations encountered during infection and act as a virulence factor in the pathogenesis of UTI while Serum resistance is the property by which the bacteria resist killing by normal human serum due to lytic action of complement system. Mittal S, *et al.* 2014 [9] Emergence of drug resistance to broad-spectrum

beta lactams mediated by extended spectrum beta lactamases (ESBLs) and especially multi-drug resistant (MDR) clonal groups among UPEC strains increase the serious threat to global public health Tabasi M. *et al*. 2015 [15]. There is a need of periodic survillence, antibiotic policy, careful use of empirical and target antibiotics, This will reduce incidence, chronicity and recurrence of Urinary Tract Infections.

Considering the high degree of morbidity in urinary tract infections the present study was designed to determine the urovirulence factors of *E. coli* isolated from the patients of UTI and to study their antimicrobial resistance.

2. Materials and Methods

The present study was carried out in the Department of Microbiology, Chandulal Chandrakar Memorial Medical College and Hospital, Durg, Chhattisgarh during July 2014 to August 2015. Hospitalized and OPD patients of all ages, either sexes, with a clinical diagnosis of UTI were included in the study. A total 152 E. coli, isolates recovered from 426 UTI cases. Urine samples were processed immediately and E. coli isolates were identified by the standard microbiological procedures, as per standard protocols. (Collee JG *et al*. 1996) [3].

The antibiotic susceptibility testing was performed by using standard antimicrobial agents as per CLSI (2010) [2] guideline. E. coli (ATCC 25922) was used as control strain. 50 faecal isolates were studied as a control for the detection of virulence markers of *E. coli*. The isolates were maintained by inoculating nutrient agar butts and stored at room temperature and tested for virulence factor

Statistical Analysis

Statistical analysis was done by using SPSS version 17.0. A P-value of less than or equal to 0.05 was considered to be significant

Detection of virulence factors

I. **Hemolysin (Sharma S *et al*. 2007) [12]:** The E. coli isolates were inoculated on 5% sheep blood agar and incubated over night at 37deg 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.

II. **Hemagglutination (Ljungh A *et al*. 1979) [8]:** The test was carried out as per the direct bacterial hemagglutination test-slide method. One drop of RBC suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 minutes. Presence of clumping was taken as positive for hemagglutination. Mannose-sensitive hemagglutination was detected by the absence of hemagglutination in a parallel set of tests in which a drop of 2% W/V D-mannose was added to the red cells and a drop of broth culture. MRHA was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.

III. **Serum resistance (Siegfried L *et al*. 1995) [14]:** Overnight culture of E. coli on blood agar plates were suspended in Hank's balanced salt solution (HBBS). Equal volume of this bacterial suspension and serum (0.05 ml) were incubated at 37uC for 3 hours. Then, 10 ml of this mixture was inoculated on blood agar plate and incubated at 37uC for 24 hours and viable count was determined. It is termed as sensitive when colony count drop to 1% of initial value.

IV. **Gelatinase test (Lobefee MJ et *al*. 2010) [7]:** Gelatinase production was tested using gelatin agar. The plate was inoculated with test organism and incubated at 37uC for 24 hours. The plate was then flooded with 1% tannic acid solution. Development of opacity around colonies was considered positive for gelatinase.

V. **Cell surface hydrophobicity (Sharma S *et al*. 2007) [12]:** This was done by salt aggregation test (SAT). One loopful of bacterial suspension in phosphate buffer was mixed with equal volume of ammonium sulphate solution of different molarity on a glass slide and rotated for 1 minute. E. coli strains with SAT value # 1.25 M were considered cell surface hydrophobic.

VI. **Siderophore production assay (Vagarali MA *et al*. 2008) [16]:** The test was done by using Chrome azurole sulfonate (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.

3. Observation

Of the 152 urinary isolates from patients presenting with UTI cases, 47(30.92%) showed haemolytic activity among 50 isolates from healthy volunteers, only 7(4.6%) showed haemolysis. The difference between cases and controls for haemolysin production was highly

Significant (p<0.001). Of those 152 isolates, 79(51.97%) isolates showed Haemagglutination (HA) Out of 79 isolates tested strains positive for HA, 51(64.55%) were MRHA positive and 28(35.44%) were MSHA. Where as in the control group 4 isolates out of 50 showed HA. 3(6%) were MRHA and 1(0.65%) showed MSHA. The difference in MRHA between cases and controls was highly significant (p<0.001) (Table 1).

Table 1: Distribution of various virulence factors amongst cases and control. In *MRHA- mannose resistant Haemagglutination. MSHA- MRHA- mannose sensitive Haemagglutination.*

Virulence factors	Cases(152)	Control(50)
Hemolysin	47(30.92%)	7(4.6%)
Haemagglutination	79(51.97%)	4(8%)
MRHA	51(64.55%)	1(0.65%)
MSHA	28(35.44%)	3(6%)
Serum resistance	80(52.63%)	2(4%)
Hydrophobicity	58(38.15%)	5(10%)
Siderophore	97(63.81%)	3(6%)
Gelatinase	61(40.13%)	8(16%)

The difference between cases and controls for most of the virulence factor was found be statistically significant (p<0.05) (Table 1).

It was observed that among the different virulence markers tested, siderophore, haemagglutination and Serum resistance were found in highest number of isolates from UTI patients. In the control group, gelatinase and cell surface hydrophobicity were the two most commonly associated markers.

Antibiotic sensitivity

All the uropathogenic E. coli strains showed maximum resistance to Ampillin (100%) amoxyclav (90.78%) followed by Ciprofloxacin (98.97%) cephotaxime(86.84%), ceftazidime (78.94%) co-trimoxazole (82.23%), gentamicin (80.26%), tetracycline (80.61%) and amikacin (38.15%). (Table 2) Majority of isolates were multidrug resistant (>3 drugs) and none of the strains were found to be sensitive to all the antibiotics tested (Table 3). In our study Nitro furantion showed least resistance (9.21%)

Table 2: Distribution of Antimicrobial resistance amonst UPEC

Antibiotic	Resistant isolates
Nitrofurantoin	14 (9.21%)
Norfloxacin	84 (55.26%)
Cotrimoxazole	125 (82.23%)
Ampicillin	152(100%)
Amoxclav	138(90.78%)
Ceftazidime	120(78.94%)
Cefotaxime	132(86.84%)
Imipenem	32(21.05%)
Gentamicin	122(80.26%)
Amikacin	58(38.15%)
Tetracycline	78(80.61%)
Ciprofloxacin	97(98.97%)

Table 3: Association of virulence factors and MDR (resistant to 3 or more classes of drugs) *E. coli* isolates (152)

Virulence factors	MDR positive Isolates (%)
Hemolysin (47)	10(21.27)
Haemagglutination (79)	39(49.36)
Serum resistance (80)	39(48.75)
Hydrophobicity (58)	14(24.13)
Siderophore (97)	46(47.42)
Gelatinase (61)	33(54.09)

4. Discussion

The occurrence of virulence factors in UPEC strains strengthens the concept of association of UPEC with urinary pathogenicity. UPEC with virulence factors were significantly more in urinary isolates than in controls. (Vagarali MA *et al*. 2008) [16].

Agglutination of human erythrocytes by *E. coli* strain is an indirect evidence of the presence of fimbriae on that strain. MRHA positive strains can be considered as UPEC most likely having P fimbriae (Johnson JR (1991) [5]. *E. coli* strains associated with severe form of UTIs are reported to exhibit mannose resistant haemagglutination [14, 16]. In the present study, difference between cases and controls was highly significant (p<0.001). This was similar to studies conducted by many workers who also found significant difference in MRHA between cases and controls. Raksha R *et al*. (2003) Johnson JR (1991) [5]. In the present study MRHA positive *E. coli* isolates was 64.55% and amongst the total haemagglutinin isolates 49.36% showed MDR. 34.80% of MDR reported by Mittal S. *et al*. Finding of various virulent factor by other workers are shown in Table. 4.

Tabl 4: Finding in other studies.

Virulence factor	Present study	Other studies
Hemolysin	30.92%	Raksha *et al*, 41.36% Siegfried *et al*. 59.6%, Hughes *et al.* 59.7%, Shruthi *et al.* 41.9%
Haemagglutination	51.97%	Mittal S.*et al*. 47.7%, Seigfried *et al*, 23%, Raksha *et al*. 30.9%,
Serum resistant	52.63%	Kauser *et al*. 49.5% Sharma *et al*. 86.8%. Mittal S. *et al*. 59%
Hydrophobicity	38.15%	Sharma *et al*. 23.7%, Mittal S *et al*. 61%,
Siderophore	63.81%	Mittal S. *et al*. 88%,Santo E *et al*. 76%,Vagarali MA *et al*. 98%
Gelatinase	40.13%	Mittal S, *et al*. 67.5%

In the present day of increasing bacterial resistance there is a constant variability of antimicrobial susceptibility pattern among clinical isolates. Thus it is felt that each isolate has to be individually studied for antimicrobial susceptibility and the choice of antibiotic decided. In the present study, Imipenem, Amikacin, Nitrofurantoin are observed to be the antibiotics of choice.

The virulence factors of *E. coli* are multiple and unusually complex affecting pathogenicity in combination with one another. 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. (Mittal S. *et al*. 2014) [9]. in our study also correlation between presence of one or more virulence factor and MDR amongst uropathogenic E. coli has been noted.

5. Conclusion

Thus urovirulence amongst uropathogenic E.coli is associate with the drug resistance in these isolates and it may further provide a substantial advantage to the survival of the pathogen Therefore, the knowledge of virulence factors of *E. coli* and their AST will help in better understanding of organism and in treatment of UTI. Therefore detection of the phenotypic virulence factors could be valuable in investigations on the pathogenesis of UPEC isolates and management of UTI therapy. However, further studies of genotypic and phenotypic characteristics of UPEC isolates may help to better evaluate its pathogenesis.

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