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Study of Virulence Factors and Antimicrobial Susceptibility Pattern of Uropathogenic Escherichia Coli in a Tertiary Care Hospital

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ABSTRACT

Introduction: Urinary tract infections are the most commonly encountered bacterial infections both in community and health care settings. Uropathogenic Escherichia coli (UPEC) is the single most common pathogen, accounting for 70-75% of all cases of Urinary Tract Infections (UTIs). The aim of the present study is to isolate UPEC from urine samples from UTI patients, to evaluate the virulence factors and antimicrobial susceptibility pattern.

Material and methods: A total of 100 E.coli isolates out of 292 urine samples collected from clinically suspected UTI were taken for the study and detection of virulence factors such as haemolysin production, haemagglutination and cell surface hydrophobicity were done. Antibiotic susceptibility test was done by Kirby Baur disc diffusion method as per the CLSI guidelines.

Results: The incidence of UTI was more in females 52% and haemolysis was seen in 32%, haemagglutination in 40%, and cell surface hydrophobicity in 24% of strains. The UPEC strains were more resistant to Ampicillin (52%) followed by Cotrimoxazole (46%), Norfloxacin (42%) and Ceftriaxone (40%). Most of the strains were sensitive to Meropenem (80%) followed by Amikacin (78%) and Nitrofurantoin (72%).

Conclusion: As there is a significant association between the virulence and antimicrobial resistance of UPEC, a routine testing of these factors is recommended and further studies at molecular level are necessary.

Key words: Urinary tract infections, uropathogenic Escherichia coli, virulence factors, antimicrobial susceptibility testing, multidrug resistance

INTRODUCTION

UTIs are the most commonly encountered bacterial infections both in community and health care settings[1]. Common UTI causing bacterial pathogens belong to family Enterobacteriaceae, Escherichia coli being the most common member isolated. It has been known that certain serotypes of E.coli are consistently associated with uropathogenicity and are designated as uropathogenic E.coli that expresses chromosomslly encoded virulence markers[2]. The common virulence factors include surface hydrophobicity, colonization factor, capsule, serum resistance, resistance to phagocytosis, haemolysin, enterotoxin and siderophore, fimbriae and haemagglutination[3].

Uropathogenic strains account for 90% of all UTIs among ambulatory patients and upto 50% of all nosocomial UTIs[4]. The virulence of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them and also by the environmental conditions in the host[5]. The markers of UPEC are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis[6]. Considering the high degree of morbidity and mortality due to UTIs caused by UPEC, the present study was conducted in a tertiary care hospital in Visakhapatnam to study the virulence factors and the antibiotic sensitivity pattern of the UPEC strains isolated from the urine samples of patients suffering from urinary tract infections.

MATERIAL AND METHODS:

This prospective study was conducted in the Department of Microbiology at a tertiary care hospital in Visakhapatnam in 2019. Patients of age group 18 and above were included and paediatric age group was excluded from the study. A total of 100 E.coli strains isolated from 292 urine samples were taken for the study. The samples were processed immediately as per the standard guidelines in the lab. The isolates were taken for the detection of virulence factors and antibiotic susceptibility testing.

Haemolysin production:

The isolates were inoculated onto 5 %sheep blood agar and incubated overnight at 37 degree Celsius and observed for a zone of complete lysis around the colony (Fig.1). *E.coli* ATCC 25922 was used as a negative control.

Figure 1 – Haemolysis

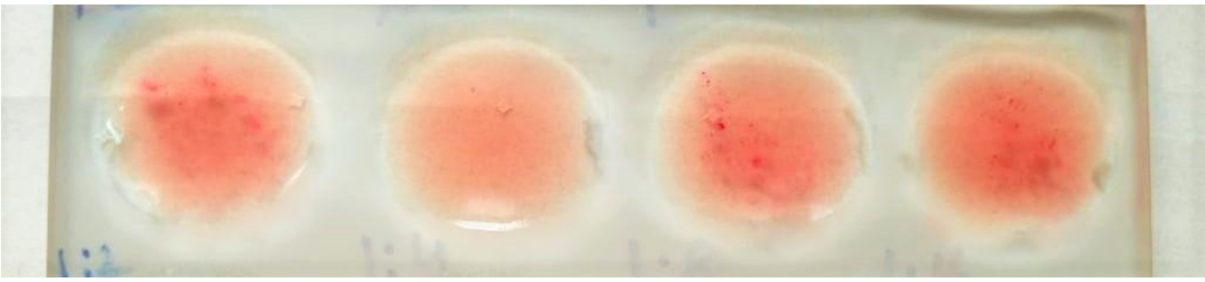
Haemagglutination:

5 ml of group A positive venous blood was collected and washed 3 times in physiological saline and a 3% suspension in fresh saline was made

A drop of very dense bacillary deposit was mixed with an equal drop of red cell suspension on a VDRL slide and it was rocked gently for 5 min. The haemagglutination produced was seen with naked eye as coarse clumps and microscopically. A drop of 2% solution of D-mannose (specifically inhibits type 1 fimbrial haemagglutination) was then added.

Observation: If haemagglutination occurs in the presence of D-mannose, it is considered as mannose resistant (MRHA) (fig.2). If haemagglutination is inhibited by D-mannose, it will be mannose sensitive haemagglutination (MSHA) (fig.3).

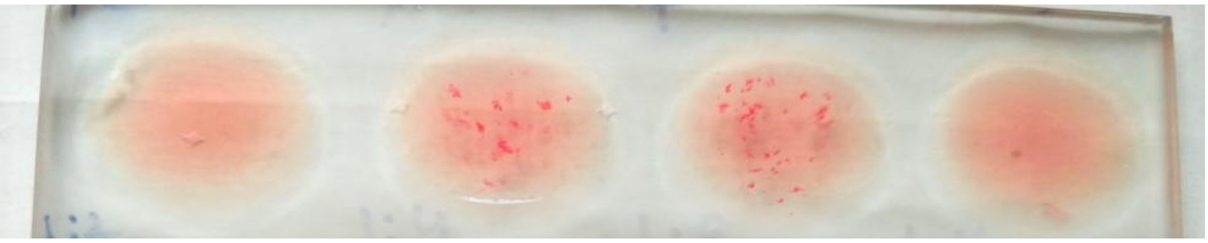
MRHA



Positive control **Negative control** **Test** **D-Mannose**

Figure 2

MSHA



Positive control **Negative control** **Test** **D-Mannose**

Figure 3

E coli ATCC 25922 were used as a positive control for mannose sensitive haemaglutination.

Cell Surface Hydrophobicity: Different molar concentrations of ammonium sulphate – 1M, 1.4M, 2M was prepared.

40μl of PBS (Phosphate Buffer solution) was taken in the first well of the VDRL slide. 40μl of 1M, 1.4M, 2M concentrations of ammonium sulphate were taken in each of the other wells of VDRL slide. 40μl of *E.coli* suspension was added to each well. The clumps found in different molar concentrations of ammonium sulphate observed under naked eye. Strains were considered hydrophobic if they aggregated with 1.4M concentration of ammonium sulphate. *E.coli* ATCC 25922 was used as a negative control for cell surface hydrophobicity (fig 4 and fig 5).

CSH Test Negative

PBS **1M** **1.4M** **2M**

Figure 4

CSH Test Positive

PBS **1M** **1.4M** **2M**

Figure 5

Antimicrobial susceptibility:

Antimicrobial susceptibility testing was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method as per CLSI guidelines.

RESULTS

A total of 292 urine samples received from symptomatic cases of urinary tract infection with significant bacteriuria were processed. Out of these, 100 E.coli isolates were obtained and studied for the virulence factors and antibiotic susceptibility pattern.

Out of the 100 E.coli isolated samples, 52% were from females and 48% were from males. The incidence of UPEC was more in the age group of 20-39 years.

Among 100 isolates, 32% showed haemolysis, 40% showed mannose resistant haemagglutination, 4% showed mannose sensitive haemagglutination and 24% showed cell surface hydrophobicity.

The UPEC strains were more resistant to Ampicillin (52%) followed by Cotrimoxazole (46%), Norfloxacin (42%) and Ceftriaxone (40%). Most of the strains were sensitive to Meropenem (80%) followed by Amikacin (78%) and Nitrofurantoin (72%) (Table 1).

Table 1

CORRELATION BETWEEN ANTIMICROBIAL RESISTANCE AND VIRULENCE FACTORS OF UPEC							
VIRULENCE FACTOR	**AK**	**AMP**	**CTR**	**COT**	**MRP**	**NIT**	**NX**
HEMOLYSIN							
Positive (n=32)	12 (37.5%)	22 (68.7%)	20 (62.5%)	24 (85.7%)	10 (31.2%)	14 (43.7%)	22 (68.7%)
Negative (n=68)	10 (14.7%)	30 (44.1%)	20 (29.4%)	22 (32.3%)	10 (14.7%)	14 (20.5%)	20 (29.4%)
HAEMAGGLUTININ							
MRHA positive (n=40)	10 (25%)	32 (80%)	24 (60%)	18 (45%)	12 (30%)	16 (40%)	22 (55%)
MRHA negative (n=60)	12 (20%)	20 (33.3%)	16 (26.6%)	28 (46.6%)	8 (13.3%)	12 (20%)	20 (3.3%)
MSHA positive (n=4)	4 (100%)	4 (100%)	2 (50%)	4 (100%)	-	2 (50%)	4 (100%)
MSHA negative (n=96)	18 (18.7%)	48 (50%)	38 (39.5%)	42 (43.7%)	20 (20.8%)	26 (27%)	38 (39.5%)
CELL SURFACE HYDROPHOBICITY							
CSH positive (n=24)	12 (50%)	16 (66.6%)	8 (33.3%)	18 (75%)	8 (33.3%)	12 (50%)	14 (58.3%)
CSH negative (n=76)	10 (13.1%)	36 (47.3%)	32 (42.1%)	28 (36.8%)	12 (15.7%)	16 (21%|)	28 (36.8%)

DISCUSSION

Considering the high degree of morbidity and mortality of UTIs, the subject of UPEC is receiving increasing attention. UTIs 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[7]. The occurrence of multiple virulence factors in UPEC strains further strengthens the concept of association of UPEC with urinary pathogenicity[8]. These virulence factors enable some members of the normal flora to elicit an infection by overcoming the host defense 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 contribute to its pathogenicity[9].

UTI is the most repeatedly diagnosed kidney and urologic disease and E.coli is by far the most common etiologic agent[10]. UTI is the second most common cause of bacterial infection in humans and thus represents a major source of human discomfort[2]. E.coli strains causing UTIs typically agglutinate human erythrocytes despite the presence of mannose and this was mediated by fimbriae[8]. The ability of E.coli to adhere to the uro-epithelium is mediated by fimbriae, thereby resisting elimination by the flow of urine. Adhesion therefore is an important step in the pathogenesis of UTI[2]. UPEC strain take advantage of a variety of virulence properties in order to colonize and establish an UTI. Bacterial adherence and colonization of UPEC are mediated by the expression of several types of fimbrial and non-fimbrial adhesions. Haemolysin is produced by many UPEC, which may be involved in kidney disease[11].

In the present study, 52% of samples were from females which correlate with Sanjay Singh Kaira et al[12] who reported 56.09%, Mittal et al[13] (53.3%) and Chhaya et al[14] (53%).

The incidence of UPEC was more in the age group of 20-39 years in the present study, which correlates with Hooton et al[15], Kabugo e tal[16] and Chhaya et al[14].

Haemolysin production was observed in 32% of isolates in our study which correlates with Chhaya etal[14] (32.3%), and Nazish Fatima et al[17] (30%) whereas Karam et al[18] reported higher incidence of 44.5% and Mittal et al[13] 47%. Kauser et al[19] reported lower incidence of 21% and Sharma etal[20] 25%.

In our study, MRHA and MSHA were observed in 40% and 4% which correlates with Kaira et al[12], who reported 41% and 5%, whereas Chhaya etal[14] reported 52.3% and 8.5%.

Cell surface hydrophobicity was observed in 24% of isolates in our study which correlates with Raksha etal[8] who reported 26.36% and Kaira et al[12] who reported 27.64% whereas Dorota et al[21] reported 74% and Mittal et al[13] reported 61%.

The antibiotic susceptibility pattern observed in our study correlates with Tabasi etal[22], Karam et al[18] and Chhaya et al[14].

CONCLUSION

Multidrug resistance was observed at a higher rate among UPEC strains. Therefore, a significant association has been found between the Virulence Factors of UPEC and antimicrobial resistance in UPEC.A routine testing of these factors and co-relation with AMR is thus recommended. Since most urovirulent strains express multiple virulent factors simultaneously, further studies at molecular level are necessary.

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