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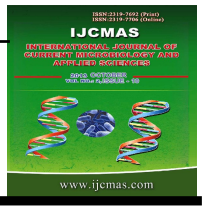

Original Research Article

Virulence-associated factors in *Escherichia coli* strains isolated from urinary tract infections

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A B S T R A C T

To study the occurrence of *Uropathogenic Escherichia coli (UPEC)* in cases with urinary tract infections. A total of 75 cases from urinary tract infections and 35 stool samples from apparently healthy individuals were included. The colonies identified as *Escherichia coli* were screened for virulence factors namely haemolysin, Mannose Resistant and Mannose Sensitive Haemagglutination (MRHA, MSHA) by recommended methods. Among 75 cases 30 (40 %) were haemolytic, 21 (28%) showed MRHA, 23 (31%) showed MSHA. In 35 controls 3(6%) were haemolytic, 2 (5.71 %) showed MRHA, 9 (18%) and 3 (8.57%) showed MSHA. The difference between cases and controls for haemolysis and MRHA were significant ($p<0.001$ and $p<0.01$ respectively). Out of 75 urinary isolates, 44 (58%) could be labelled as UPEC.

Introduction

Escherichia coli is one of the most frequent causes of extra-intestinal diseases including urinary tract infections (UTI). *E.coli* is the most frequent urinary pathogen isolated from 50% - 90% of all uncomplicated urinary tract infections. (Steadman and, Topley, 1998). It is now recognized that there are subsets of faecal *E. coli* , which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as uropathogenic *E. coli* .(Vagarali *et al*., 2008). The different virulence factors include adhesins, hemolysin production and siderophore production. Fimbriae mediate the ability of *E. coli* to adhere to the uroepithelium, thereby resisting elimination by the flow of urine. Adhesion is therefore considered to be an important step in the pathogenesis of UTI (Struve and Krogfelt, 1999).

The information on characterization of urovirulence factors in different clinical conditions is less studies. So, the present study was designed to determine the urovirulence factors of *E. coli* isolated

from the patients of UTI and to study their antimicrobial susceptibility pattern.

Materials and Methods

The study was conducted in the Department of Microbiology, ASRAM Medical college, Eluru. Seventy five Escherichia coli strains isolated from urine samples and 35 faecal isolates were studied for the detection of virulence markers of *E. coli*. *Identification of E. coli was based on biochemical properties.* The isolates were maintained by inoculating nutrient agar butts and stored at room temperature and tested for haemagglutination and Hemolysin production.

Haemagglutination assay

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 haemagglutination tests (Duguid *et al*., 1979). 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 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 at 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.

Hemolysin assay

The cytolytic protein toxin secreted by most hemolytic *E. coli* isolates is known as alpha haemolysin (Cavalieri *et al*., 1984). All Escherichia coli isolates were inoculated on to 5% sheep blood agar plates and incubated overnight. Presence of zone of emolysis around each colony is estimated to produce hemolysin.

Result and Discussion

There is a preponderance of females (57.33%) when compared to males (42.66%) in this study. 0 – 19 yrs 11 cases. 20 –39 yrs 21 cases, 40 – 59 yrs 24 cases and > 60 yrs 19 cases.

Among various clinical entities, 30 cases presented with lower UTI (40%), 20 cases with asymptomatic bacteriuria (26.6%) and 15 with Pyelonephritis (20%) and the rest 10 cases are UTI associated with renal calculi (13.33%).

Virulence markers of UPEC obtained in cases and controls are as follows, among 75 cases tested 44 (58.6 %) were positive haemagglutination virulence markers and 40% were positive for hemolysin marker. Out of 25 controls, 5 (20%) were positive for haemagglutination marker and 2 (8%) positive for hemolysin marker. (Out of 35 controls, 5 (14.28%) were positive for haemagglutination marker and 2(5.71%) were positive for hemolysin marker)

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Among the total isolates tested, the most common virulent marker is Haemolysin 30 (40 %) followed by Mannose Sensitive Haemagglutination (MSHA) 23 (31%) and Mannose Resistant Haemagglutination 21 (28%).

In control group, the occurrence of Haemolysin was 2 (8%), MSHA 3 (12%) and MRHA 2 (8%) shown in Table 1.

Occurrence of uropathogenic virulence markers in UPEC was shown in table 2. The association of multiple virulence factors was 53% (8/15 cases) in pyelonephritis cases, 40% (4/ 10 cases) in UTI with renal calculi and 27% (8/30 cases) in lower UTI cases and the rest 6 % (2 / 20 cases) in asymptomatic UTI cases.

Of the total 75 Uropathogenic *Escherichia coli* tested for antibiotic susceptibility, the maximum susceptibility was shown to Imipenem 70(93.30%) followed by Amikacin 68 (90.66%), Nitrofurantoin 60(80%), Cotrimoxazole 45 (60%), ceftriaxone 25(33.33%), Ciprofloxacin 18(23.33%), Ceftazidime 8(10%) and Amoxyclav 5(6.66%) (Table 3).

Considering high degree of morbidity and mortality due to UTIs the subject of uropathogenic *E. coli* is receiving increasing attention (Raksha and srinivasa, 2003). *E. coli* express several surface structures and secrete protein molecules some of them Cytotoxic peculiar to the strains causing UTIs. Hence it is important to identify UPEC from non UPEC isolates in urinary samples (Steadman and Topley, 1998).

In our study it's observed that presence of virulence factors ranged from 40% (hemolysin) to 58% (haemagglutination) in cases when compared to controls showing a positive association with UPEC than in non UPEC.

Occurrence of multiple virulence factors observed. Its been observed that Pyelonephritis cases and complicated UTI with renal calculi were more associated with multiple virulence factors when compared to non complicated lower UTIs. Studies conducted by MA vagarali and R Raksha also showed a positive association of virulence factors with uropathogenic Escherichia coli than in non UPEC strains.

The cytolytic protein toxin secreted by most hemolytic *E. coli* as hemolysin. E. coli also produces cell associated lysin on blood agar plates and hemolysin causes clear zone of hemolysis (Smith, 1963). In this present study there was high production of hemolysin in cases when compared to controls and the difference is highly significant ($p < 0.0001$). This was similar to studies conducted by Prabath *et al*., (2010) and R Rashmi *et al*., (2003).

Haemagglutination is mediated by P fimbriae (Duguid *et al*., 1979) and also X, FIC , Dr fimbriae. Thus MRHA positive strains can be considered UPEC most likely having P fimbriae (Jonson, 1991). In this study there was higher association of MRHA in cases when compared to controls and statistically significant with p value < 0.0001.

Mannose-sensitive adherence mediated by E. coli strains is due to type 1 fimbriae (Johnson *et al*., 1987). Studies on MSHA in association with clinical disease are inadequate. In our study MSHA were

Table.1 virulence markers in uropathogenic *Escherichia coli*

S. No	Virulence markers	Cases (n=75)	Controls (n=35)	P value
1	Haemolysin production	30 (40%)	2 (5.7%)	Highly significant p<0.000299(<0.001)
2	Haemagglutination activity a) MRHA b) MSHA c) NO HA	 21 (28%) 23 (31%) 31 (41%)	 2 (5.7%) 3 (8.5%) 30 (85.7%)	Highly significant p<0.000195(<0.001)

Table.2 Correlation of virulence markers

Virulence markers	Cases	Controls
One marker Lytic strains MRHA MSHA	 22 15 21	 1 1 3
Two markers Lytic + MRHA Lytic + MSHA	 6 2	 1 0
Total	33	3

Table.3 Antibiotic susceptibility testing of uropathogenic *Escherichia coli*

Antibiotic	No sensitive	Percentage
Imepenam (10mcg)	70	93.3%
Amikacin (10mcg)	68	90.66%
Nitrofurantoin (100mcg)	60	80%
Cotrimaxozole (25 mcg)	45	60%
Ceftriaxone (30 mcg)	25	33.33%
Ciprofloxacin (5 mcg)	18	23.30%
Ceftazidime (30 mcg)	8	10%
Amoxyclav (30 mcg)	5	6.66%

more associated in cases when compared to controls i.e. fecal isolates and is statistically significant (p Value <0.001). Recently it is been shown that MSHA is associated with lower UTIs when compared to upper UTIs (Latham and Stamm, 1984) and in UTIs with indwelling bladder catheters (Mobley *et al*., 1987).

In this study the production of hemolysin, MRHA and MSHA which are considered as urovirulence markers were high among the clinical isolates of Escherichia coli when compared to the fecal isolates of Escherichia coli. Hence identification of UPEC and the further understanding of these markers in different UTIs required to understand their contribution to the antimicrobial resistance among UPEC.

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