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A Comparative Study of Virulence Factors of *Escherichia coli* Isolated from Urinary Tract Infections with that of Intestinal *Escherichia coli* Isolated from Apparently Healthy Adults

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A B S T R A C T

This study has been under taken to know the virulence factors associated with *E. coli* causing Urinary Tract Infection (UTI) in comparison to intestinal commensal *E. coli*. Out of 600 UTI patients 100 had *E. coli* isolates which were studied for virulence factors. A total of 100 isolates of E coli from stool samples of apparently healthy individuals were included as controls. All the *E. coli* isolates from urine and stools were tested for the following 4 virulence factors- Hemolysin production, Haemagglutination, Serum resistance, Cell surface hydrophobicity. Among case hemagglutination (61%) was the most common virulence factor followed by serum resistance (55%), Cell surface hydrophobicity (CSH) (43%), and hemolysis (27%). In hemagglutination cases 39 were MRHA and 22 were MSHA. Only (21%) of the controls showed hemagglutitation. Most of/all the virulence factors were significantly less among controls compared to cases (P value= 0.000). The expression of virulence factors were more in UTI cases than fecal controls. Hemagglutination was the most frequently observed virulence factor followed by Serum resistance, Cell surface hydrophobicity, and Hemolysin.

Introduction

Escherichia coli (*E. coli*) is one of the most important members of the family Enterobacteriaceae (Chrichton, 2006) and a commensal in the human intestinal tract. In the intestinal tract it resides harmlessly and poorly adapted to cause disease in healthy individuals. However, there exist a plethora of pathotypes that can cause specific type of illness both in normal hosts and those with compromised nonspecific defence mechanisms. Pathogenic strains differ from commensal organisms in that they possess Virulence Factor (VF) specific for each pathotype which may be coded by bacteriophages, plasmids or pathogenicity islands. The diseases caused by Escherichia coli include: extra intestinal and intestinal infections. Among extra intestinal sites urinary tract is the site most frequently infected by *E. coli*. *E. coli* is the most frequent pathogen isolated from 50-90% of all uncomplicated urinary tract infections (Steadman and Topley, 1998; Raksha *et al.*, 2003). Certain serotypes of *E. coli* are

consistently associated with uropathogenicity and are designated as uropathogenic *E. cloi* (UPEC). UPEC strains are believed to display a variety of virulence properties that help them to colonise host mucosal surface and circumvent host defence to allow invasion of the normal sterile urinary tract.

The recognized virulence factors of *E. coli* to cause urinary tract infections include increased adherence to uroepithelial cells, resistance to serum bactericidal activity, a higher quality of K antigen (K1, K5) in capsules, the presence of aerobactin, cytotoxic necrtotising factor type 1, haemagglutination, cell surface hydrophobicity, siderophore and gelatinase production (Donnenberg, 2005; Donnenberg and Welsh 1996; Bhat *et al.*, 2007).

These virulence factors are responsible initially for the colonisation of the organisms and subsequently for the tissue damage. The pathogenic strains adhere to the urinary tract and survival of the complement action appears to contribute to the urinary tract virulence, which is not as frequently observed among the normal fecal flora. The treatment of *E coli* infections is increasingly becoming difficult because of the multidrug resistance exhibited by these organisms (Bhat *et al.*, 2007).

This study was conducted in KVG Medical College & Hospital**,** Sullia, Karnataka. Sullia is a small township situated in the Western Ghats. The patients attending the hospital are majority rural population. No studies have been conducted in this region to assess the virulence factors of *E. coli* causing UTI. Hence this study has been under taken to know the virulence factors associated with *E. coli* causing UTI in comparison to intestinal commensal *E. coli* and also to find out the drug resistance patterns exhibited by both the groups.

Materials and Methods

This study was conducted in KVG Medical College & Hospital in 2011. A total of 600 urine samples from patients of UTI were processed by Microscopy and culture. Out of these 600 urine samples from UTI patients, 100 samples had *E. coli* isolates which were studied for virulence factors.

A total of 100 isolates of E coli from stool samples of apparently healthy individuals were included as controls.

Clean catch midstream urine specimens were collected in sterile wide mouthed universal container and stools samples were collected in sterile wide mouthed leak proof container. All the samples were transported to the laboratory within 1 hour and processed.

Urine samples were subjected to wet film examination (uncentrifuged), and cultured semi quantitatively by standard loop method on Sheep blood agar and Mac Conkey agar. Stools were inoculated on Mac Conkey agar.

Identification of E coli was done by standard methods and susceptibility by Kirby Bauer method as per CLSI guidelines (Bauer *et al.*, 1966).

All the *E. coli* isolates from urine and stools were tested for the following 4 virulence factors.

Hemolysin production (Raksha *et al.*, 2003; Bhat *et al.*, 2007)

The *E. coli* from urine and stool were tested by plate hemolysis test. The bacteria were inoculated onto 5% sheep blood agar and incubated overnight at 35^0C. Hemolysin production was indicated by the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.

Haemagglutination (Cheryl *et al.*, 1981; Vagarali *et al.*, 2008)

Haemagglutination test was done by slide method. Group A and group O Rh positive human erythrocytes were used for the test. The blood was collected periodically from two voluntary individuals. The erythrocytes were washed thrice in normal saline and made up to a 3% suspension in fresh saline. The suspension was used immediately or within a week when stored at 3-5^0 C.

The test bacteria were freshly grown on 5% sheep blood agar. A drop of phosphate buffer saline was placed separately on 2 spots on a clean grease free dry microscopic glass slide and the test bacterium is emulsified in it so that a heavy milky white suspension is formed. A 2% weight/volume (W/V) D-mannose was added to one side and mixed. A drop of 3% red blood cell (RBC) suspension was added to both and the slide was rotated for one min and looked for agglutination of RBCs within one min. The presence of clumping of the RBCs on the side without mannose confirmed haemagglutination. The presence of clumping of the RBCs on the side with mannose confirmed mannose-resistant haemagglutinins (MRHA). The absence of clumping of the RBCs on the side with mannose confirmed mannose-sensitive haemagglutinins (MSHA). The controls included bacteria suspended in phosphate buffer saline, erythrocytes suspended in normal saline, E coli ATCC 25922 strain for MSHA and a known strain of E coli repeatedly giving positive for MRHA.

Serum resistance (SR) (Bhat *et al.*, 2007; Siegfried *et al.*, 1994; Jadhav *et al.*, 2011)

The *E coli* were freshly grown on 5% sheep blood agar for overnight. Cultures were harvested and the bacteria were suspended in Hank's balanced salt solution (HBSS). Bacterial suspension (150ul) was incubated with pooled healthy adult serum (150 ul) at 37^{0}C for 180 min. Ten microletres of samples were withdrawn and spread on blood agar plates which were then incubated at 37^{0}C for 18 hrs and the viable count was determined. Resistance of bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 180 min of incubation with serum in relation to the original count. Strains were termed serum sensitive if the viable count dropped to 1% of the initial value and resistant if more than 90% of the organisms survived after 180 min incubation.

Cell surface hydrophobicity (CSH) (Raksha *et al.*, 2003; Bhat *et al.*, 2007; Brauner *et al.*,1990; Jadhav *et al.*, 2011)

The cell surface hydrophobicity was performed by salt aggregation test using ammonium sulfate solution. Initially a 5M solution of ammonium sulfate was prepared from which different molar concentrations were prepared (i.e., 0.3, 0.6, 0.9, 1.2, 1.4, 1.8, 2.0, 2.4, 3.0, 3.6, 4.2, 5.0 M). Strains of *E. coli* were freshly grown on blood agar plates. One loopful of different molar concentrations of ammonium sulfate was placed on clean glass slides. One loopful of bacterial suspension made in Phosphate buffer (pH 6.8) was added to the ammonium sulfate and mixed and rotated for one min. The highest dilution of ammonium sulfate solution that showed visible clumping was recorded as salt aggregation test (SAT) value. The *E. coli* that aggregated in phosphate buffer alone were considered as auto aggregative and not interpreted. E coli strains that showed SAT value of 1.4 and less were considered hydrophobic.

Analysis of data

The data was analyzed on IBM SPSS version 19. *Chi-square test* was applied to test the difference between proportions, at 5% level of significance.

Results and Discussion

A total of 100 patients with UTI were included in the study of which 53 were females and 47 were males. A majority of the patients (51%) were above 50 years of age. Majority of patients presented with symptoms of fever (62%), frequency of urination/urgency/dysuria (84%). VF among cases and controls are given in table 1. Occurrence of multiple virulence factors among cases and controls are given in table 2.

Among case hemagglutination (61%) was the most common virulence factor followed by SR (55%), CSH (43%), and hemolysis (27%). In hemagglutination cases 39 were MRHA and 22 were MSHA. Only (21%) of the controls showed hemagglutitation. Most of/all the virulence factors were significantly less among controls compared to cases (P value= 0.000).

Occurrence of two specific virulence factors and three specific virulence factors in combination are given in tables 3 and 4 respectively. Combination of two virulence factors occurrence and three virulence factors occurrence were significantly more in cases when compared to controls. The combination of MRHA and serum resistance was more commonly observed in cases.

E. coli the most prevalent facultative Gram negative bacterium in the human fecal flora, usually inhabits the colon as an innocuous commensal. It causes extraintestinal and intestinal infections. UTI is the most common form of extra intestinal infection produced by *E. coli* (Johnson, 1991).

In our study hemolysin expression was seen in 27% of cases which correlates with Bhat *et al.,* (2007) who reported 25% hemolysin. Expression of hemolysin was seen in 12% of stool *E coli* which correlates with Kauser *et al.,* (2009). We observed a significant difference (P<0.01) between cases and controls in hemolysin production. Similar significant finding was observed by previous studies (Raksha *et al.,* 2003, Johnson 1991, Jadhav *et al.,* 2011).

In this study haemagglutination was observed in 61% of cases of UTI which correlates with previous studies (Vagarali *et al.,* 2008; Kauser *et al.,* 2009). MRHA expression was seen in 39% of UTI isolates, the results correlate with Jadhav *et al.,* (2011) (40%) and Siegfred *et al.,* (1994) (43%). Expression of MSHA was seen in 22% of UTI cases which is low compared to other Indian studies.

Our study observed that 61% cases and 21% controls were positive for haemagglutination. There was statistically significant difference in occurrence of haemagglutination between cases and controls (P<0.01). This finding correlates with previous studies (Vagarali *et al.,* 2008; Kauser *et al.,* 2009). Serum resistance was seen in 55% of the UTI isolates in our study. This is similar to Jadhav *et al.,* (2011) who reported 55.3% and Kausar *et al.,* who reported 49.5%. In controls, serum resistance was seen in 32% of the isolates similar to Kausar *et al.,* (2009).

We observed a statistically significant difference (P<0.01) between the cases and controls in serum resistance. Similar findings were recorded by previous study (Kauser *et al.,* 2009). In the present study positive CSH was observed in 43% cases and 23% controls with a highly significant difference (P<0.01). The results of our study in CSH between cases and controls were significant as in Brauner *et al.,* (1990).

In the present study there was high statistical significance between cases and controls in the occurrence of following virulence factors namely hemolysin (P<0.01), haem agglutination (P<0.01), MRHA (P<0.01), CSH (P<0.01), SR (P<0.01).

Table.1 Virulence factors among cases and controls

Virulence factor	Cases (n=100)		Controls (n=100)		Chi square value	P value
	Yes	No	Yes	No		
Hemolysin	27	73	12	88	7.16	0.007
Hemagglutination	61	39	21	79	33.07	0.000
MRHA	39		09		24.67	0.000
MSHA	22		12		3.54	0.06
CSH	43	57	23	77	9.04	0.003
SR	55	45	32	68	10.76	0.001

Table.2 Occurrence of multiple virulence factors among cases and controls

Number of virulence factors(VFs)	Cases positive (n=100)	Controls positive (n=100)	Chi square value	P value
No VFs	**14**	**41**		
Any 1 VF	21	37	38.92	0.000
Any 2VFs	36	16		
Any 3VFs	22	06		
Any 4VFs	7	00		
None	14	41		

This difference of multiple virulence factors in cases and controls is statistically significant

Table.3 Occurrence of two specific virulence factors in combination

Virulence factors	Number of positive cases(n=100)	Number of positive controls(n=100)
Hemolysin + MRHA	14	02
Hemolysin + SR	12	04
Hemolysin +CSH	19	02
MRHA + CSH	22	03
MRHA + SR	27	04
CSH + SR	23	09

Table.4 Occurrence of three specific virulence factors in combination

Virulence factors	Number of positive cases(n=100)	Number of positive controls(n-100)
Hemolysin + MRHA + CSH	07	01
Hemolysin + MRHA + SR	08	01
Hemolysin + CSH + SR	07	01
MRHA + CSH + SR	13	02
Hemolysin + MSHA + SR	05	01

In our study, 14% cases and 41% controls showed no virulence factors. There was a significant difference in the occurrence of virulence factors between cases and controls and is comparable to Raksha *et al.,* (2007) who showed no virulence factor in 31 cases and 60 controls. In the present study occurrence of at least 2 virulence factors was associated with *E coli* isolated from 65% UTI cases and 22% stool controls.

When VFs were considered in combination, our study showed combination of any two virulence factors as 36/100 and any three VF combinations as 22/100. These results are comparable to Nazish fathima *et al.,* who showed combination of 2 and 3 VFs be 67/250 and 41/250 respectively.

Findings from our study revealed that a large number of urinary isolates from UTI cases had more than one virulence factor. The occurrence of virulence factors in UPEC strains strengthens the concept of association of UPEC with urinary pathogenicity (Raksha *et al.,* 2007). Hence these isolates could be labelled as UPEC producing multiple virulence factors. In the present study 86 out of 100 UTI isolates had one or more virulence factors, our results here correlate to Kausar *et al.,* (2009) who reported that 80% of the *E coli* from UTI had one or the other virulence factors. When a comparison was done between urinary and fecal *E. coli* isolates the occurrence of hemolysin, haemagglutination, MRHA, SR and hydrophobicity was more significant in urinary isolates than fecal isolates. This combination appears to be more in our study in comparison to Raksha *et al.,* (2007). Sixty five percent of isolates from UTI cases had at least two virulence factors. In contrast to this, combination of virulence factors in controls was very low and 41% of them did not show any virulence factor. In our study the combined occurrence of two or more specific virulence factors was more in cases. Since these virulence factors were observed more in *E. coli* isolated from UTI, the isolates could be considered as uropathogenic *E. coli*.

From this study it can be concluded that, *E coli* is the most common cause of UTI and Microscopic examination of the urine is an important screening method for UTI cases. The above discussed methods for virulence factor detection are feasible and can be adapted routinely in the Clinical Microbiology laboratories. Hemagglutination was the most frequently observed virulence factor followed by SR, CSH, and Hemolysin. The expression of virulence factors were more in UTI cases than fecal controls. Combination of virulence factors were more in cases.

References

Bauer, A.W., *et al.,* 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496

Bhat, G.K., Sharrna S, Shenoy S. 2007. Virulence factors and drug resistance in *Escherichia coli* isolated from extra intestinal infections. Indian J Med Microbiol. 25:369-73.

Brauner, A., Katouli M, Tullus K, Jacobson SH. 1990. Cell surface hydrophobicity, adherence to HeLa cell cultures and haemagglutination pattern of pyelonephritogenic *Escherichia coli* strains. Epidemiol Infect. 105:255-63.

Cheryl, P., Green, Thomas V. 1981.Hemagglutination of human Type O erythrocytes, hemolysin production, and serogrouping of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis, and asymptomatic bacteriuria. Infect Immun Jan.31(1): 309-315.

Chrichton, P.B., 2006. Enterobacteriaceae: Escherichia, Klebsiella, Proteus and other genera. In: Collee JG, Fraser AG, Marmion BP, Simmon A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th edition. Churchill Livingston: New York: 361-84.

Donnenberg, M.S., 2005. Enterobacteriaceae. In: Mandell GL, Bennet JE, Dolin R, editors. Mandell, Douglas, and Bennet's Principles and Practice of Infectious Diseases. 6th edition. Churchill Livingstone. 2567-86.

Donnenberg, M.S., Welsh RA. 1996. Virulence determinants of uropathogenic Escherichia coli. In: Mobley HLT, Warren JW editors. Urinary tract infections: Molecular pathogenesis and Clinical Management. Volume 5. Washington, DC: American Society for Microbiology. 80-128.

Jadhav, S., Hussain A, Devi S, Kumar A, Parveen S, *et al.*, 2011. Virulence Characteristics and Genetic Affinities of Multiple Drug Resistant Uropathogenic Escherichia coli from a Semi Urban Locality in India. PLoS ONE. 6(3): e18063. doi:10.1371/journal.pone.0018063

Johnson, J.R., 1991.Virulence factors in Escherichia coli urinary tract infections. Clin Microboli Rev. 4:81-128.

Kausar, Y., Chunchanur SK, Nadagir SD, Halesh LH, Chandrasekhar MR. 2009. Virulence factors, Serotypes and Antimicrobial susceptibility pattern of *Escherichia coli* in urinary tract infections. Al amen J Med Sciences. 02:47-51

Siegfried, L., Kmetova M, puzova H, Molokacova M, Filka J. 1994. Virulence associated factors in *E. coli* strains isolated from children with urinary tract infections. J Med Microbiol. 41:127-32

Vagarali, M.A., Karadesai SG, Patil CS, Metgud SC, Mutnal MB. 2008. Haemgglutination and siderophone production as the uroviralence makers of uropathogenic *Escherichia coli.* Indian J Med Microbial. 26: 68-70.

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