Original Article

Phenotyphic Determination of Urinary Virulence Factors in *Escherichia coli*

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Abstract

Aim: To determine the virulence factors haemolysin production, hemagglutination and serum resistance in *Escherichia coli (E.coli)* of urinary isolates and *Escherichia coli* isolated from the other sites like blood, exudate and stool. **Materials and Methods:** A cross sectional study was conducted in the Department of Microbiology, Sri Ramachandra Medical College and Research Institute. A total of 120 strains of *E. Coli* isolated fromthe following specimens urine (50), Exudate (25), Blood (25) and Stool (20) were included in the study and assessed for the presence of Uropathogenic virulence factors such as Haemolysin production, Hemagglutination property both the Mannose Resistant Hemagglutination (MRHA) and Mannose Sensitive Hemagglutination (MSHA) and Serum Resistance factor. **Results**: Among the 50 urinary isolates haemolysis was observed in 10%, hemagglutinating property was seen in 20 %(MRHA 7 and MSHA 3 n=10)) and 88 % of them had serum resistance factor. Out of the 25 *E.coli* strains isolated from the exudate samples none of them was found to be haemolytic, 12% of them were hemagglutinating (MRHA 67% and MSHA 33%) and serum resistance factor was seen with 24% of isolates. Among the 25 *E. coli* isolates from the blood 8% were haemolytic, 28% of them were hemagglutinating (MRHA 100%) and serum resistance factor was observed in 72. Out of 20 *E.coli* strains isolated from stool, haemolysis was not seen in any of the them, 20% of them were hemagglutinating property (MRHA 100%) and serum resistance factor was positive in 20% of them. **Summary and Conclusion:** A total of 50 *E.coli* reported as significant bacteriuria ($>10^5$) was included in this study and at the same time 70 isolates of *E.coli* reported from other specimens (Blood, Exudates and Stool) were taken as control group. Uropathogenic virulence factor was exhibited by 45 out of 50 urinary isolates (90%). Serum Resistance is the commonest virulence factor observed in our study (88%) followed by Hemagglutination property (20%) and Haemolytic property (10%). The entire 3 virulence factor is exhibited by 3 strains of urinary isolates (6%) only. In the control group Haemolysis was observed in 2 isolates, Hemagglutination with 24 isolate, Serum Resistance in 28 E.coli isolates.

Key Words: Uropathogenic E.coli, Haemolysin, Hemagglutination, Serum resistance.

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INTRODUCTION

Urinary tract infection (UTI) is the frequent extra intestinal *E. coli* infection. Virulence factors associated with Uropathogenic *E.coli* (UPEC) include the a) Toxins like alpha- haemolysin and cytotoxic necrotizing factor 1 b) Siderophores like aerobactin and enterobactin c) Lipopolysaccharide d) Capsule and e) Number of adhesive organelles. The presentation of adhesive molecules (adhesins) by UPEC is arguably the most important determinant of pathogenicity. Adhesins enable UPEC to bind to host cells within the urinary tract and avoid rapid clearance with the bulk flow of urine. UPEC the primary causative agent of urinary tract infections can invade and replicate within uroepithelial cells, this can provide *E. coli* with a survival advantage, allowing the microbes to better resist detection and clearance by both innate and adaptive immune defence mechanisms. Adhesive organelles, including type 1, P, and S pili along

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with Dr adhesins, promote both bacterial attachment and invasion of host tissues within the urinary tract. Interactions mediated by these adhesins can also stimulate a number of host responses that can directly influence the outcome of a urinary tract infection. Adhesins can also contribute to virulence in a number of other ways; directly triggering host and bacterial cell signalling pathways, facilitating the delivery of other bacterial products to host tissues, and promoting bacterial invasion.[1] The binding of *E. coli* to epithelial cell receptors containing globoseries glycosphingolipid accounts for the attachment of most strains causing kidney infection and is not inhibited by mannose, this binding is called mannose resistant. Fimbriae attaching to globoseries receptors are termed *P fimbriae* because the receptor is a constituent of the P blood group antigen complex present in human erythrocytes and uroepithelial cells. P fimbriae are frequently present in uropathogens and they augment the virulence of UPECat different stages of infection, including remaining longer in the intestinal tract and spreading more efficiently to the urinary tract for purposes of colonizing and producing ascending infection. P fimbriae also appear to confer enhanced ability of UPEC clones to colonize the colon and spread to the perineum. P fimbriated*E. coli* dominate as a cause of pyelonephritis and urosepsis, and especially among blood isolates. Binding of *E. coli* to mannose-containing host epithelial receptors, glycoproteins uroplakin I and II, occurs with most uropathogenic strains. In fact, strains from cystitis patients are more likely to bind than those from pyelonephritis patients. Fimbriae attaching to mannosylated proteins via FimH subunits are the common type 1 fimbriae (pili), and attachment is inhibited in the presence of mannose (MS, mannose-sensitive). Type 1 fimbriae bind mannose epitopes on secreted glycoproteins such as secretory IgA and urinary mucus, Tamm-Horsfall protein (THP), bladder uroplakin protein, and fibronectin. Expression of type 1 fimbriae is not especially prevalent among pyelonephritogenic strains. Almost all cystitis causing strains of *E. coli* express type 1 fimbria. Type 3 fimbriae in uropathogenic*E. coli* are thought to contribute to biofilm formation and to be important in urethral catheter infections.[2] Haemolytic *E. coli* produces two haemolysins, a cell-bound (beta) haemolysin and a cell-free (alpha) haemolysin (AH). Both haemolysins cause beta-haemolysis on blood agar plates. Haemolysin is an important virulence factor in extra intestinal infections and is associated with urinary tract infection (UTI), bacteraemia, peritonitis, and septicaemia. Alpha haemolysin lyses erythrocytes of all mammals In addition to lysing erythrocytes, haemolysin is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defences[3].

SERUM RESISTANCE

Bacteria are killed by normal human serum through the lytic activity of the complement system. The alternative pathway is activated by bacteria in the absence of specific antibody and plays a more important role in serum killing than does the classic pathway. Lipid A can activate the classic pathway in the absence of antibody, but its location deep within the outer membrane probably makes it inaccessible to complement components in intact bacteria (except perhaps in rough strains). Both arms of the complement cascade converge in the formation of the C5_9 membrane attack complex (MAC), Digestion of the cell wall allows the MAC to insert into the inner membrane, leading to cell lysis. Bacterial susceptibility to serum killing is measured by assessing regrowth after incubation in serum or growth rates in dilute serum[4].

MATERIALS AND METHODS

SAMPLE

The study was conducted in the Department of Microbiology, Sri Ramachandra Medical College and Research Institute. A total of 50 *E.coli* reported as significant bacteriuria (>10^5cfu/ml of urine) was included in this study and at the same time 70 isolates of *E.coli* reported from other specimens (Blood, Exudates and Stool) were taken as control group. A total of 120 strains of *E. Coli* isolated from various specimens like urine (50), Exudate (25), Blood (25) and Stool (20) were studied and compared for the presence of uropathogenic virulence factor's like Haemolysin production, Haemagglutination property (Mannose Resistant Haemagglutination and Mannose Sensitive Haemagglutination) and Serum Resistance factor.

DETECTION OF VIRULENCE FACTOR

1. Haemolysin Production:

All 120 *E.coli* isolates were inoculated into 5% sheep blood agar and incubated at 37^{o}C. Haemolysin production was detected by the presence of zone of complete lysis of the RBC'S and clearance around the colonies.

2. Haemagglutination:

The strains of *E. coli* were inoculated in 1% Nutrient broth and incubated at 37^{o}C for 48 hours for full fimbriation. Freshly prepared red blood cells obtained from human blood group 'O' were washed thrice in normal saline, and used for haemagglutination test. The slide haemagglutination tests were carried out on a multiple concavity slide. One drop of RBC suspension was added to a drop of 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 (MSH) was detected by the absence of haemagglutination in a parallel set of test in which a drop of 2% D-Mannose was added to the red cells and a drop of broth culture. Mannose Resistance Haemagglutination (MRH) was detected by the presence of haemagglutination of in the presence of 2% Mannose.

3. Serum Resistance:

Overnight cultures of *E.coli* grown at 37°C on blood agar were harvested and cells were suspended in Hank's Balanced Salt solution. The bacterial suspension 0.05ml was incubated with freshly drawn 0.05ml serum at 37°C for 3 hours using micro titre plates. 10microliters of samples were withdrawn and spread on blood agar plate and incubated over night at 37°C and the viability count of the strains were determined. Resistance of bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 3 hours of incubation with serum in relation to the original count.

RESULTS

Table 1: Virulence Factors among the *Escherichia coli* isolates

Isolates	Total	Hemolysis		Haemagglutination				Serum Resistance	
		Positive	Negative	Positive	Negative	MR	MS	Sensitive	Resistance
Urine	50	5	45	10	40	3	7	6	44
Exudate	25	0	25	3	22	1	2	19	6
Blood	25	2	23	7	18	0	7	7	18
Stool	20	0	20	4	16	0	4	16	4
Total	**120**	**7**	**113**	**24**	**96**	**4**	**20**	**48**	**72**

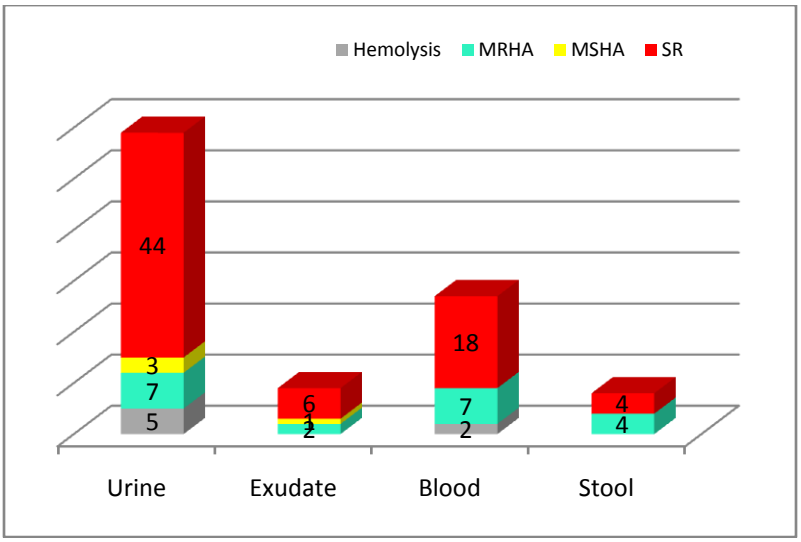

Figure 1: Virulence factors among all the isolates of *Escherichia coli*.

A total of 120 strains of *E.coli* isolated from Urine (50), Exudate (25), Blood (25) and Stool (20) were subjected to screening for virulence factors Hemolysin, Haemagglutination (MSHA and MRHA) and Serum resistance factor (Table 1) Among the 50 urinary isolates hemolysis was observed in 5 strains (10% n=50). Hemagglutinating property was seen in 10 strains (20 % n=50). Among the 10 Haemagglutination positive strains 7 of them were MRHA (70% n=10) and 3 were MSHA (30% n=10). Serum resistance factor was observed in 44 strains (88% n=50). Out of 50 urinary isolates only 3strains (6%) exhibited the all the three virulence factors. Among the 5 haemolytic urinary strains, Serum resistance was observed in all the strains and Hemagglutinating property was exhibited by 2 and both were found to be MRHA. All the Hemagglutinating strains of urinary E.coli both MRHA (7) and MSHA (3) exhibited the Serum resistance factor. Out of 44 serum resistance factors positive E.coli 10 of them was Hemagglutinating (MRHA 7 and MSHA 3) and 5 of the them were hemolytic. Out of the 25 E.coli strains isolated from the exudate samples none of the was found to be hemolytic. Serum resistance factor was seen in 6 (24% n=25) isolates. Hemagglutinating property was observed in 3(12% n=25) strains in which one isolate was MSHA and 2 were MRHA. Among the 3 Hemagglutinating strains 1

was serum resistance factor positive and it was MRHA. Among the 25 *E.coli* strains isolated from the blood 2(8% n=25) were hemolytic, 7 (28% n=25) were Haemagglutinating and all were MRHA. Serum resistance factor was observed in 18(72% n=25) strains of *E.coli*. All the three virulence factors were exhibited in 2(8%) out of 25 *E.coli* Blood isolates. Out of the 7 Hemagglutinating *E.coli* strains isolated from blood all were MRHA and Serum resistance factor was seen in 5 *E.coli* strains. Among the 20 *E.coli* strains isolated from stool. Hemolysis was not seen in any of the strains. Hemagglutinating property was exhibited by 4 strains (20% n=20) and all the 4 were MRHA. Serum resistance factor was positive in 4(20%n=20) and 16(80%) of them were serum sensitive. Out of 50 urinary isolates of *E.coli* 45(90%) of them had at least one of these three virulence factors. Out of the 25 isolates of *E.coli* from exudates 20(80%) of them had at least one of the three virulence factors. Out of the 25 isolates from the Blood 18 (72%) had at least one of the three virulence factors. Out of the 20 faecal *E.coli* strain 4(20%) of them had at least one of the virulence factors(Figure 1).

DISCUSSION

There is a significant morbidity and mortality associated with urinary tract infections both in community as well as hospital associated infections. *E.coli* is one of the important causative organisms causing urinary tract infection both in the community acquired and hospital acquired urinary tract infections and is most commonly seen in all age groups especially during pregnancy. There are well defined characters of virulence factors that are associated with *E.coli* and urinary tract infection in the literature. Hence it is important to decide whether the *E.coli* isolated are Uropathogenic *E.coli* (UPEC) or Non Uropathogenic E.coli.In our study 45 (90%) strains of *E.coli* were uropathogenic exhibiting at least one of the three virulence factors Hemolysin, Haemagglutination (MRHA or MSHA) and Serum resistance factor. Serum resistance factor was one of the major Urovirulence factor observed in our study i.e., 44(88%) out of 50 urinary isolates .Urovirulence factors were absent in 5 of the urinary isolates indicative of Non-Uropathogenic *E.coli.* In a study by Kauser Y[5] with 200 *E.coli* strains from symptomatic urinary tract infection patient reported 99% of serum resistance when compared with the control strains from faecal *E.coli* strains which showed only 16% and in another study by Sharma S[6]and colleagueswith 152 isolates reported 86.8% of serum resistance. In contrast Ranjan K P[7] reported 32.72% of urinary isolates of *E.coli* exhibiting serum resistance, in his study also 24% of control group exhibited serum resistance. In our study with 70 Non urinary isolates of *E.coli* (that is from Exudates, Blood, Stool) 28(40%) strains of *E.coli* exhibited serum resistance. In the 28 isolates 24 were from blood and exudate which may be an indication of same urinary isolates causing blood stream (urosepsis) and pyogenic infection. A total of 10(20%) isolates showedthe presence of pili. Among the 10, seven were Mannose Resistance indicative of P fimbriae which are potential strain for pyelonephritis and other three were Mannose sensitive indicating presence of type 1 fimbriae. In their study Ranjan K P [7]andKauserY[5] reported 26.36%, 32.72% and 30%, 36% of Mannose Resistance Haemagglution (MRHA), Mannose Sensitive Haemagglution (MSHA) respectively. Among our control group of 70 isolates only Haemagglution property is demonstrated in 14(20%) isolates and majority of them 13 out of 14 isolates exhibited Mannose Resistance indicative of Mannose Resistance Haemagglution (MRHA) indicative of presence of P fimbriae. Haemolytic property was demonstrated by 5 (10%) of the 50 urinary isolates and 2(2.8%) isolates of 70 control group. This is in contrast with Ranjan K P [7]and Kauser Y [5] studies who had reported respectively 41.36% and 21% of Hemolysis in urinary isolates 6% and 32 % in control group.

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