Indian Journal of Microbiology Research 2021;8(2):163–167

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: https://www.ijmronline.org/

Original Research Article

A study of virulence factors of E. coli strains isolated from cases of urinary tract infection

Krati Varshney[1], **Sanjeev Dimri**[1,*]

[1] *Dept. of Microbiology, Saraswathi Institute of Medical Sciences, Hapur, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 12-05-2021
Accepted 17-05-2021
Available online 30-07-2021

Keywords:
UTI
E coli
UPEC
MRHA
MSHA
Hemolysin
CSH

ABSTRACT

Aim: The aim of this study was to demonstrate virulence factors namely adhesins, hemolysin and cell surface hydrophobicity of E. coli strains isolated from cases of urinary tract infection.
Materials and Methods: A total of 210 E. coli strains were isolated from 602 culture positive cases of urinary tract infections. UPEC strains were screened for virulence factors namely hemolysin, hemagglutination and cell surface hydrophobicity by recommended methods.
Result: Out of 210 E. coli strains tested, 70 (33.34%) were hemolytic, 94 (44.76%) showed hemagglutination and 46 (21.90%) were cell surface hydrophobicity positive.
Conclusion: Our study shows that a large number of uropathogenic E. coli strains had one or more virulence factors. The methods of detection of above-mentioned virulence factors are reasonably easy and screening them in a clinical microbiology laboratory is a worthwhile exercise.

1. Introduction

Urinary tract infection (UTI) is the most common form of extra-intestinal Escherichia coli (E. coli) infection and E. coli is the most common cause of UTI.[1] At some point during their lives, at least 12% of men and 10-20% of women experience an acute symptomatic UTI and even greater number develop asymptomatic bacteriuria (ABU).[2,3] In India, UTI accounts for 9.3 million doctor visits and nosocomial UTI accounts for more than 1 million cases.[4] E. coli is the most common pathogen isolated from urinary tract infections and frequently originates from patients' own intestinal flora.[5] This common inhabitant of intestinal tract usually remains in symbiotic relationship with the host and plays a role in homeostasis of intestinal tract.[6] Some strains of E. coli can diverge from their common cohorts and take a more pathogenic nature by acquiring specific virulence factors via DNA horizontal transfer of transposons, plasmids, bacteriophages and pathogenicity islands which confer increased ability to adapt to new niches and allow bacteria to increase the ability to cause broad spectrum of diseases.[7] The pathogenic E. coli strains are broadly classified into 3 pathotypes namely enteric/diarrheagenic pathotype, neonatal meningitis E. coli pathotype and uropathogenic E. coli pathotype.[8,9] It has been traditionally described that certain serotypes of E. coli are consistently associated with uropathogenicity and were designated as Uropathogenic E. coli (UPEC).[10] UPEC strains encode a number of virulence factors that enable bacteria to colonize the urinary tract and persist in face of highly effective host defenses.[7] These isolates exhibit a high degree of genetic diversity due to possession of specialized virulence genes located of mobile genetic elements called pathogenicity islands.[11,12] Virulence factors of E. coli that have been potentially implicated as important to establish urinary tract infection can be divided into 2 groups a) surface associated virulence factors, b) secreted virulence factors.[5] Virulence factors of UPEC include adhesins (fimbrial & non-fimbrial), flagella, lipopolysaccharide, capsule, α-hemloysin, cytolytic necrotizing factor, secreted autotransporter toxin, aerobactin, colicin and cell surface

* *Corresponding author.*
E-mail address: sdimri1966@yahoo.co.in (S. Dimri).

https://doi.org/10.18231/j.ijmr.2021.033

hydrophobicity.[7,13] These virulence markers of UPEC are expressed with different frequencies in different disease states ranging from asymptomatic bacteriuria to chronic pyelonephritis.[10] UPEC strains cause 75-90% of community acquired and about 50% of nosocomial UTI.[4] Uroepithelial adherence is critical for establishment of urinary tract infection and UPEC strains possess an impressive repertoire of fimbrial & non-fimbrial adhesins that enable the bacteria to aggregate and adhere to cellular surfaces.[14–18] These adhesins exhibit mannose-sensitive hemagglutination (MSHA) (type 1 fimbrae) and mannose-resistant hemagglutination (MRHA) (P-fimbrae).[1] The present study was conducted to demonstrate virulence factors namely adhesins, hemolysin and cell surface hydrophobicity of E. coli strains isolated from cases of urinary tract infection.

2. Materials and Methods

This study was conducted in the Department of Microbiology of Era's Lucknow Medical College and Hospital, Lucknow, UP from November 2011 to October 2102. A total of 210 E. coli strains were isolated from 602 cases of urinary tract infection and were characterized on the basis of various virulence factors like hemolysin, hemagglutination and cell surface hydrophobicity. Urinary tract infections were classified into disease categories according to the site of infection.[7] Upper urinary tract infection (UUTI) (most severe) involving kidney & ureters was associated with fever, chills & flank pain, lower urinary tract infection (LUTI) (moderately severe) involving urinary bladder & urethra was associated with burning pain on voiding, suprapubic pain & tenderness and asymptomatic bacteriuria (ABU) (least severe) was characterized by total absence of symptoms.[1,2,19] Hemolysin was detected by determining a zone of hemolysis around each colony on 5% sheep blood agar plates after overnight incubation.[10] Hemagglutination was detected by clumping of erythrocytes by fimbrae of bacteria in presence of D-mannose following the method of Siegfried et al.[10] E. coli grown on MacConkey's agar plates were inoculated into 5 ml of phosphate buffer saline (PBS) (pH 7.4) and incubated for 5 days at 37^0C to get fimbrae enriched E. coli. The pellicle formed on the surface was noted and subcultured into Colonization factor antigen agar and incubated overnight at 37^0C. Five millilitres (5 ml) of goup A positive blood was collected from voluntary donor and added to an equal volume of Alsever's solution. This was washed three times and 3% erythrocyte suspension was made with PBS. ATCC E. coli 25922 was used as positive control for MSHA and UPEC serotypes O6 & O11 as MRHA positive ($MRHA^+$) controls. Equal volumes of erythrocyte suspension & bacterial suspension were added to VDRL slide and rotated for 3-5 minutes. These slides were then observed for hemagglutination within 10 minutes. Hemagglutination (HA) was considered mannose-resistant when it occurred with & without D-mannose to the same degree and mannose-sensitive when it was inhibited in presence of D-mannose.[20] Cell surface hydrophobicity (CSH) was determined by Salt aggregation test[10,14] where bacteria were tested for their hydrophobicity by using different molar concentrations of ammonium sulfate (1M, 1.4M, 2M). Suspension of E. coli was prepared in 0.2 M phosphate buffer (pH 6.8) to provide a colony count of $5x10^9$ cfu/ml. Forty microliters (40 ul) of bacterial suspension was mixed with equal amounts of each salt solution in VDRL slides. Strains were considered to be hydrophobic when they aggregated in ammonium sulfate at concentrations $\leq$ 1.4M. A strain of E. coli which was hemolytic, $MRHA^+$ & consistently positive for hydrophobicity was used as positive control and a strain that was non-hemolytic, MRHA negative ($MRHA^-$)& consistently negative for hydrophobicity was used as negative control.

3. Results

Hemolysin was present in significantly higher proportion in UUTI (48.5%) & LUTI (30.3%) strains than in ABU (8%) strains. Hemagglutination property was more common in UUTI (81.8%) strains than observed in LUTI (31.1%) & ABU (12%) strains. MRHA positivity was observed more commonly in UUTI (59.1%) cases than in LUTI (21%) & ABU (12%) cases. Also, the proportion of $MRHA^+$ (n=67) (31.9%) strains were found to be significantly higher than $MSHA^+$ (n=27) (12.9%) strains. Although CSH positivity was higher in UUTI (28.8%) than LUTI (20.2%) & ABU (12%) strains, no significant difference was observed with respect to CSH positivity (Table 1).

Out of 210 UPEC strains, 139 (66.19%) strains possessed one or more virulence markers. Majority (n=82) of cases had one virulence marker and hemolytic marker (n=26) was most common in this group followed by $MSHA^+$(n=20). There were 18 strains each of MRHA & CSH positivity. Two virulence markers were observed in 44 strains, where Hemolytic + $MRHA^+$ strains had the major proportion (n=28) followed by Hemolytic + CSH^+ strains (n=13). There was 1 strain each of $MRHA^+$+ CSH^+, $MSHA^+$+ CSH^+ and Hemolytic + $MSHA^+$. Among 3 markers positive strains (n=13), 8 strains were Hemolytic + $MRHA^+$+ CSH^+ and 5 strains were Hemolytic + $MSHA^+$+ CSH^+ (Table 2).

Hemolytic strains (60%) had higher hemagglutination positivity than non-hemolytic strains (37.14%) though CSH positivity was higher in non-hemolytic strains (22.85%) than hemolytic strains (20%), but the difference in this case was not significant (Table 3).

Significantly higher proportion of $MRHA^+$ (53.7%) strains had hemolysin as virulence factor than $MSHA^+$ (22.2%) strains & $MRHA^-$ $MSHA^-$(24.13%) strains and a higher proportion of $MRHA^+$ (31.3.%) strains were

Table 1: Distribution of virulence factors in UPEC isolates

Type of UTI n=210	Hemolytic strains n=70 (33.34%)	Hemagglutination positive strains n=94 (44.76%)	Cell surface hydrophobicity positive strains n=46 (21.90%)
UUTI (n=66) (31.43%)	32 (48.5%)	54 (81.8%) MRHA$^+$=39 (59.1%) MSHA$^+$=15 (22.72%)	19 (28.8%)
LUTI (n=119) (56.67%)	36 (30.3%)	37 (31.1%) MRHA$^+$=25 (21.0%) MSHA$^+$=12 (10.1%)	24 (20.2%)
ABU (n=25) (11.90%)	02 (8.0%)	03 (12.0%) MRHA$^+$=03 (12.0%) MSHA$^+$=00 (00.0%)	03 (12.0%)

Table 2: Occurrence of virulence markers in UPEC (n=210)

Virulence markers	Cases (n=139)
One marker (n=82)	
Hemolysis	26
MRHA$^+$	18
MSHA$^+$	20
CSH	18
Two markers (n=44)	
Hemolysis+ MRHA$^+$	28
Hemolysis+ MSHA$^+$	01
Hemolysis+ CSH	13
MRHA$^+$+ CSH	01
MSHA$^+$+ CSH	01
Three markers (n=13)	
Hemolysis+ MRHA$^+$+CSH	08
Hemolysis+ MSHA$^+$+CSH	05

Table 3: Distribution of virulence factors in relation to hemolysis

Hemolytic character	Hemagglutination	CSH
Hemolytic strains (n=70)	42 (60.0%)	14 (20.0%)
Non-hemolytic strains (n=140)	52 (37.14%)	32 (22.85%)

positive for CSH than MSHA$^+$ (22.2%) strains & MRHA$^-$ MSHA$^-$(16.4%) strains (Table 4).

Significantly higher proportion of UUTI (48.5%) cases were positive for hemolytic strains than LUTI (30.3%) and ABU (8%) cases. Although cell surface hydrophobicity was also seen in relatively higher proportion in UUTI (28.8%) cases than LUTI (20.2%) and ABU (12%) cases, the association was not significant (Table 1).

4. Discussion

Urinary tract infection (UTI) is one of the most common infection encountered in clinical practice mainly being associated with different members of family Enterobacteriaceae and E. coli is the predominant pathogen among them. Certain strains of E. coli are consistently associated with uropathogenicity and are designated as uropathogenic E. coli (UPEC).[10] UPEC strains cause 75-90% of community acquired and about 50% of nosocomial UTI.[4] UPEC strains encode a number of virulence factors that enable bacteria to colonize the urinary tract and persist in face of highly effective host defenses.[7] It was first recognized in late 1970s that E. coli strains causing UTI typically agglutinate human erythrocytes despite the presence of mannose and this was mediated mainly by fimbrae. Subsequently a number of virulence factors were proposed as virulence markers for uropathogenic isolates of E. coli.[21] An attempt was made in this study to find the association of virulence factors of E. coli strains isolated from cases of urinary tract infection namely hemolysin, hemagglutination and cell surface hydrophobicity with UTI. In this study, majority of E. coli strains were isolated from cases of LUTI (56.6%) followed by UUTI (31.43%) and ABU (11.9%). These findings were in close proximity with Lipsky[3] and Ruiz et al.[22] The possible explanation can be that the ascending route of UTI is more common than the hematogenous route. In our study, hemolysin production was seen in 33.34% strains which is similar to findings of Johnson (38%)[1] and Fatima et al. (30%).[23] However, Raksha et al. (41.36%)[10] and Brook et al. (43%)[24] found slightly higher incidence of hemolytic strains in their study. In contrast Ulleryd et al.[25] found a much higher percentage (76%) of hemolytic UPEC strains in their study. Our study also found hemolysin production in 48.5% UUTI

Table 4: Distribution of virulence factors in relation to hemagglutinations

Virulence factors	MRHA$^+$ (n=67)	MSHA$^+$ (n=27)	MRHA$^-$ MSHA$^-$ (n=116)
Hemolysin	36 (53.7%)	06 (22.2%)	28 (24.13%)
CSH	21 (31.3%)	06 (22.2%)	19 (16.4%)

strains, 30.3% LUTI strains and only 8% ABU strains which was similar to study of Brook et al.[25] that reported hemolytic strains in 38% cases of UUTI and 27% cases of LUTI suggesting that pyelonephritis strains are more virulent. Our study showed 31.9% uropathogenic strains to be MRHA$^+$which was similar to studies of Raksha et al. (30.9%),[10] Kausar et al. (30%)[20] and Fatima et al. (30%)[24] while Green & Thomas (56%)[26] and Johnson (58%)[1] reported higher rates of MRHA positivity. In our study, 12.9% strains were MSHA$^+$ which correlated with study by Raksha et al (18%)[10] but was in contrast to study by Kausar et al.[20] who found 72% MSHA positivity. In the present study, 31.9% uropathogenic strains were MRHA$^+$, 12.9% MSHA$^+$ and 55.2% strains non hemagglutinating (MRHA$^-$ MSHA$^-$) which was similar to study of Siegfried et al.[13] who found that among UPEC strains, 43% were MRHA$^+$, 14% MSHA$^+$and 43% MRHA$^-$ MSHA$^-$. In relation to the type of UTI, the present study found that in UUTI cases, 59.1% strains were MRHA$^+$, 22.7% MSHA$^+$ and 21.2% MRHA$^-$ MSHA$^-$. Among LUTI cases, 21% strains were MRHA$^+$, 10.1% MSHA$^+$ and 68.9% MRHA$^-$ MSHA$^-$. Among ABU cases, 12% strains were hemagglutinating and all these cases were MRHA$^+$ and rest 88% were MRHA$^-$ MSHA$^-$. It was thus concluded that E. coli associated with severe forms of UTIs exhibited mannose resistant hemagglutination (MRHA) than mannose sensitive hemagglutination (MSHA). Our study correlated with findings of Hagberg et al[27] who reported a higher proportion (77%) of pyelonephritogenic strains to be MRHA$^+$, followed by acute cystitis (36%) and ABU (18%). The present study also found 21.9% uropathogenic strains to be positive for cell surface hydrophobicity which was in close proximity with studies by Raksha et al (26.36%)[10] and Fatima et al (22%)[23] although Siegfried et al.[13] reported higher percentage (76.8%) of CSH$^+$ strains. We also studied correlation among the 3 virulence factors with each other in uropathogenic strains. A correlation between production of hemolysin and other virulence factors was also made in the present study which showed that 60% hemolytic strains were hemagglutination positive which was similar to studies by Evans et al.[28] where 63% hemolytic strains were hemagglutination positive. The study showed that significantly higher proportion of MRHA$^+$ strains (53.7%) produced hemolysin as compared to MSHA$^+$ (22.2%) and MRHA$^-$ MSHA$^-$ (24.13%) strains which correlated well with study by Siegfried et al.[13] who also found significant correlation of hemolysin production between MRHA$^+$ & MSHA$^+$strains and between MRHA$^+$ & MRHA$^-$ MSHA$^-$ strains. In our study, we found that a higher proportion of MRHA$^+$ strains showed CSH positivity than MSHA$^+$ and MRHA$^-$ MSHA$^-$ strains, though the association was not significant which correlates well study of Siegfriedet al.[13] who found no significant association between MRHA & CSH positivity and MSHA & CSH positivity. The present study found a significantly higher proportion of UUTI strains that were positive for hemolysin & hemagglutination as compared to LUTI and ABU strains which correlated well with Brook et al.[24]and Hagberg et al.[27] In our study, 66.19% UPEC strains possessed one or more virulence markers which correlated well with findings of Raksha et al.[10] who found 65% of UPEC strains with one or more virulence markers. Considering the high degree of morbidity and mortality of urinary tract infections, the subject of uropathogenic E. coli is receiving increasing attention. Various studies have revealed that uropathogenic E. coli express several surface structures and secrete protein molecules that are peculiar to the strains of E. coli causing UTI.[5,7,13] Hence, it becomes important to identify UPEC strains in urinary samples.

5. Conclusion

Our study shows that a large number of uropathogenic E. coli strains had one or more virulence factors. The occurrence of multiple virulence markers in UPEC strains strengthen the concept of association of uropathogenic E. coli in pathogenicity of urinary tract infections. The methods of detection of above-mentioned virulence factors are reasonably easy and screening them in a clinical microbiology laboratory is a worthwhile exercise. Further studies on better understanding of interaction of different virulence factors at molecular level are necessary as most UPEC strains express multiple virulence factors simultaneously.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Johnson JR. Virulence factors in Escherichia coli urinary tract infection. *Clin Microbiol Rev*. 1991;4(1):80–128.
2. Johnson JR, Stamm WE. Urinary tract infections in women: diagnosis and therapy. *Ann Intern Med*. 1989;111:906–17.
3. Lipsky BA. Urinary tract infections in men. Epidemiology, pathophysiology, diagnosis, and treatment. *Ann Intern Med*.

1989;110(2):138–50.
4. Devi BP, Sudhakar P. A Survey on TaaS & SECaaS in Cloud Environment. *Asian J Res Soc Sci Humanit*. 2017;7:1058. doi:10.5958/2249-7315.2017.00148.4.
5. Emody L, Kerenyi M, Nagy G. Virulence factors of uropathogenic Escherichia coli. *Int J Antimicrob Agents*. 2003;22:29–33.
6. Parvez SA, Rahman D. Virulence factors of uropathogenic E. coli. In: Microbiology of Urinary Tract Infections-microbial agents and predisposing factors. IntechOpen; 2018. p. 7–21.
7. Bien J, Sokolova O, Bozko P. Role of UropathogenicEscherichia coliVirulence Factors in Development of Urinary Tract Infection and Kidney Damage. *Int J Nephrol*. 2012;2012:1–15. doi:10.1155/2012/681473.
8. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli. *Nat Rev Microbiol*. 2004;2(2):123–40. doi:10.1038/nrmicro818.
9. Russo TA, Johnson JR. Proposal for a New Inclusive Designation for Extraintestinal Pathogenic Isolates ofEscherichia coli:ExPEC. *J Infect Dis*. 2000;181(5):1753–4. doi:10.1086/315418.
10. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterisation of uropathogenic Escherichia coli in urinary tract infections. *Indian J Med Microbiol*. 2003;21(2):102–7. doi:10.1016/s0255-0857(21)03130-3.
11. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. *Exp Mol Pathol*. 2008;85(1):11–9. doi:10.1016/j.yexmp.2008.03.007.
12. Oelschlaeger TA, Dobrindt U, Hacker J. Pathogenicity islands of uropathogenic E. coli and the evolution of virulence. *Int J Antimicrob Agents*. 2002;19(6):517–21. doi:10.1016/s0924-8579(02)00092-4.
13. Siegfried L, Kmetova M, Puzova H, Molokacova M, Filka J. Virulence-associated factors in Escherichia coli strains isolated from children with urinary tract infections. *J Med Microbiol*. 1994;41(2):127–32. doi:10.1099/00222615-41-2-127.
14. Mulvey MA. Adhesion and entry of uropathogenic Escherichia coli. *Cell Microbiol*. 2002;4(5):257–71. doi:10.1046/j.1462-5822.2002.00193.x.
15. Wullt B, Bergsten G, Connell H, Röllano P, Gebretsadik N, Hull R, et al. P fimbriae enhance the early establishment of Escherichia coli in the human urinary tract. *Mol Microbiol*. 2000;38(3):456–64. doi:10.1046/j.1365-2958.2000.02165.x.
16. Marre R, Kreft B, Hacker J. Genetically engineered S and F1C fimbriae differ in their contribution to adherence of Escherichia coli to cultured renal tubular cells. *Infect Immun*. 1990;58(10):3434–7. doi:10.1128/iai.58.10.3434-3437.1990.
17. Bouguenec CL. Adhesins and invasins of pathogenic Escherichia coli. *Int J Med Microbiol*. 2005;295(6-7):471–8.
18. Pizarro-Cerdá J, Cossart P. Bacterial Adhesion and Entry into Host Cells. *Cell*. 2006;124(4):715–27. doi:10.1016/j.cell.2006.02.012.
19. Gleckman R, Blagg N, Hibert D, Hall A, Crowley M, Pritchard A, et al. Acute Pyelonephritis in the Elderly. *South Med J*. 1982;75(5):551–4. doi:10.1097/00007611-198205000-00011.
20. Kausar Y, Chunchanur SK, Nadagir SD, Halesh LH, Chandrasekhar MR. Virulence factors, serotypes and antimicrobial susceptibility pattern of Escherichia coli in urinary tract infections. *Al Ameen J Med Sci*. 2009;2(1):47–51.
21. Ranjan KP, Ranjan N, Chakraborty A, Arora DR. An Approach to Uropathogenic Escherichia Coli in Urinary Tract Infections. *J Lab Physicians*. 2010;2(02):70–3. doi:10.4103/0974-2727.72152.
22. Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G, et al. Differences in Virulence Factors among Clinical Isolates of Escherichia coli Causing Cystitis and Pyelonephritis in Women and Prostatitis in Men. *J Clin Microbiol*. 2002;40(12):4445–9. doi:10.1128/jcm.40.12.4445-4449.2002.
23. Fatima N, Agrawal M, Shukla I, Khan PA, Fatima N, Agrawal M, et al. Characterization of uropathogenic E. coli in relation to virulence factors. *Open Access Sci Rep*. 2012;1(7):1–4.
24. Brooks HJL, O'Grady F, McSherry MA, Cattell WR. Uropathogenic properties of Escherchia coli in recurrent urinary-tract infection. *J Med Microbiol*. 1980;13(1):57–68. doi:10.1099/00222615-13-1-57.
25. Ulleryd P, Lincoln K, Scheutz F, Sandberg T. Virulence Characteristics of Escherichia coli in Relation to Host Response in Men with Symptomatic Urinary Tract Infection. *Clin Infect Dis*. 1994;18(4):579–84. doi:10.1093/clinids/18.4.579.
26. Green CP, Thomas VL. 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*. 1981;31(1):309–15. doi:10.1128/iai.31.1.309-315.1981.
27. Hagberg L, Jodal U, Korhonen TK, Lidin-Janson G, Lindberg U, Edén CS. Adhesion, hemagglutination, and virulence of Escherichia coli causing urinary tract infections. *Infect Immun*. 1981;31(2):564–70. doi:10.1128/iai.31.2.564-570.1981.
28. Evans DJ, Evans DG, Höhne C, Noble MA, Haldane EV, Lior H, et al. Hemolysin and K antigens in relation to serotype and hemagglutination type of Escherichia coli isolated from extraintestinal infections. *J Clin Microbiol*. 1981;13(1):171–8. doi:10.1128/jcm.13.1.171-178.1981.

Author biography

Krati Varshney, Associate Professor

Sanjeev Dimri, Professor and Head

Cite this article: Varshney K, Dimri S. A study of virulence factors of E. coli strains isolated from cases of urinary tract infection. *Indian J Microbiol Res* 2021;8(2):163-167.