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Original Research Article

Biofilm Formation as an Uropathogenic Marker of *E. Coli* Isolates Causing Urinary Tract Infections among Pregnant Women

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ABSTRACT

Background: Urinary tract infection is a major health concern in human society and also represents one of the most frequent hospital-acquired infections. Prevalence of UTI among pregnant women is more due to the hormonal and physiological changes during pregnancy. One of the bacterium mostly incriminated in UTI include *Escherichia coli* having several uropathogenic markers those allow them to colonise and persist in the urogenital tract.

Objectives: The present research work was performed to,

- Study the occurrence of *E. coli* isolates causing UTI among pregnant women.
- Detect the presence of biofilm formation in *E. coli* isolates as an uropathogenic marker.

Materials and Methods: A total of 136 no. of *E. coli* isolates were recovered out of 300 urine samples collected from pregnant women suspected to having UTI. The *E. coli* isolates were screened for biofilm formation by the method described by O'Toole and Kolter. Hemolysin production was also tested on 5% sheep blood agar.

Results: Only 19 *E coli* isolates found to have biofilm formation as a pathogenic marker and hemolysin production was seen in 57 cases.

Key Words: Urinary tract infection, Pregnancy, uropathogenic markers, Biofilm formation.

INTRODUCTION

Anatomically, urinary tract is divided into an upper portion composed of kidneys, renal pelvis, and ureters and a lower portion made up of urinary bladder and urethra. UTI is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria. Most urinary tract infections are due to bacteria that enter the opening of the urethra, where bacteria stick to the walls of the urethra, multiplying and moving up the urethra to the bladder. Pregnant women are more susceptible to UTI due to a number of factors including ureteral dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux. Development of glycosuria seen in 70% of pregnant women encourages bacterial growth in the urine. [1] UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second

most common cause of bacteraemia in hospitalized patients. [2-4]

Most uncomplicated UTIs are caused by *E. coli*, accounting for up to 90% of community-acquired and approximately 50% of nosocomial UTIs. [5] The virulence factors of *E. coli* are multiple and unusually complex affecting pathogenicity in combination with one another. [6] UPEC present several virulence factors that allow them to colonize host mucosal uro-epithelium, injure and invade host tissues, overcome host defence mechanisms, incite a host inflammatory response and eventually proceed from the lower urinary tract to the renal cavities and tissues. [7-9] They generally exhibit several characteristics which contribute to their virulence including alpha hemolysin and biofilm formation, biofilm which is a hydrated matrix of polysaccharide and protein, forms a slimy layer. In medicine, biofilm associated infections have a major impact on permanent and temporary artificial implants placed in the human body, often with devastating consequences. [10] Moreover, biofilms associated with implants often serve as a source for recurrent infections. Many persistent and chronic bacterial infections are now believed to be linked to the formation of biofilms. [11]

The enumeration of biofilm formation as one of the uropathogenic marker contribute an approach that will help in better understanding about complications of UTI in pregnancy and in the treatment procedure.

Aim and Objectives: - The aim of the present study was to

1. Determine the occurrence of *E.coli* isolates causing UTI among pregnant women.
2. Detect the presence of biofilm formation in *E. coli* isolates as an uropathogenic marker along with hemolysin production.
3. Study the antibiotic resistance pattern of biofilm forming *E coli* isolates.

MATERIALS AND METHODS

An investigational study was conducted on 300 urine samples obtained by informed consent of the pregnant women attending different antenatal clinics at Bhubaneswar and Puri. The pregnant women who are on antibiotic therapy within last two weeks were excluded from the study.

Processing for the *E. coli* Isolates:-

The samples were processed by standard microbiological techniques. [12] Then the identification and characterization of isolated bacteria was done by the morphological characterization followed by Gram stain and biochemical tests according to the recommended methods. The isolates were identified by Bergey's Manual for Determinative Bacteriology. [13]

After identification of the uropathogens, biofilm formation and hemolysin production tests were performed for the 136 number of *E. coli* isolates.

Tests for Biofilm formation-Tissue Culture Plate method (TCP) [14]

Biofilm formation was assessed by following methods as described below:

The TCP assay described by Christensen et al., (1985) is most widely used and was considered as standard test for detection of biofilm formation. Isolates from fresh agar plates were inoculated in brain heart infusion (BHI) broth (Himedia , Mumbai, India) with 2% sucrose dispensed in 2ml amount in the test tubes and incubated for 18-24 hours at 37 ºC in stationary condition. Then the broth with the growth (visible turbidity) was diluted to 1 in 100 with fresh medium. Individual wells of sterile, polystyrene, 96 well –flat bottom tissue culture plates (Himedia, Mumbai, India) wells were filled with 0.2ml aliquots of the diluted cultures and only broth served as control to check sterility and non – specific binding of medium.

These tissue culture plates were incubated for 24 hours a 37 ºC. After incubation, the content of the well was gently removed by tapping the plates. The wells were then washed four times with

0.2ml of phosphate buffer saline (PBS pH 7.2) to remove free-floating planktonic bacteria. Biofilms formed by adherent sessile organism in plate were fixed with sodium acetate for half an hour (2%) and stained with crystal violet (0.1%w/v) for half an hour. Excess stain was rinsed off by thorough washing with deionised water and plates were kept for drying. Adherent bacterial cells usually form biofilms on all sides of the wells and were uniformly stained with crystal violet. Optical densities (OD) of stained adherent bacteria were determined with a micro ELISA auto reader at wavelength of 620nm and were graded as per Christensen et al. These OD values were considered as an index of bacteria adhering to surface and forming biofilms. [11,14]

Classification of Bacterial Adherence-

Mean OD values	Adherence	Biofilm formation
<0.120	None	None/Weak
0.120-0.240	Moderate	Moderate
≥0.240	Strong	High

Plate hemolysin test was done by using 5% sheep blood agar to detect hemolysin produced by the pathogen. Each isolate was inoculated on to previously prepared sheep blood agar plates and incubated over night at 37^0C. Hemolysin production was detected by the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium. [15]

Antibiotic resistance pattern was studied for the biofilm forming *E. coli* by standard disc diffusion method, using commercially available antibiotic discs in accordance with CLSI guidelines.

RESULTS

Out of the 136 *E. coli* isolates collected from the urinary isolates of pregnant women, the frequency of hemolysin production and biofilm formation was 41.9% and 13.9% respectively (Table1).

Hemolysin production

Table-1: Incidence of biofilm formation and hemolysin production in E.coli isolates-

Pathogenic Markers	No. of positive cases(%)	No. of Nagative cases(%)
Hemolysin	57 (41.9)	79 (58.0)
Biofilm formation	19 (13.9)	117(86.0)

Table-2: Antibiotic resistance pattern in biofilm forming E.coli isolates (19)-

Antibiotics	Sensitive (%)	Resistant (%)	Intermediate Sensitive (%)
Amikacin (Ak)	14 (73.6)	4 (21.0)	1(5.2)
Ampicillin/sulbactam (A/S)	12 (63.1)	6 (31.5)	1 (5.2)
Cefuroxime (Cxm)	8 (42.1)	9 (47.3)	2 (10.5)
Ciprofloxacin (Cip)	12(63.1)	6(31.5)	1(5.2)
Gentamicin(G)	13(68.4)	5 (26.3)	1(5.2)
Nitrofurantoin(Nit)	17(89.4)	1(5.2)	1(5.2)
Norfloxacin (Nx)	10(52.6)	8(42.1)	1(5.2)
Piperacillin/Tazobactam(P/T)	16(84.2)	2 (10.5)	1(5.2)

The antibiotic susceptibility pattern was mentioned in table no 2. It shows that 89.4% of the biofilm forming E coli isolates were sensitive to nitrofurantoin followed by 84.2% were sensitive to Piperacillin/ Tazobactam (P/T). The least effective drug was Cefuroxime showing resistance in 47.3% of biofilm producing E.coli isolates.

DISCUSSION

The uropathogenic markers of E.coli are multiple and usually complex affecting pathogenicity in combination with one another. Biofilm formation is one of the bacterial pathogenic determinants which allow the bacteria to persist a long time in vivo and interfere with bacterial eradication. Biofilm producing bacteria have several advantages, such as the acquisition of antibiotic tolerance, expression of several virulence factors and an increased resistance against phagocytosis and other host defense mechanisms. There are several mechanisms responsible for the resistance activity of the

biofilm producers including failure of antibiotic penetration into biofilm as it act like a barrier. [10,14] In medicine, biofilm associated infections have a major impact on permanent and temporary artificial implants placed in the human body. Many persistent and chronic bacterial infections are now believed to be linked to the formation of biofilms. [11]

In the urinary tract, bacterial biofilms can develop on many living surfaces and virtually all artificial implants, producing chronic and often intractable infections. Biofilm production in *E. coli* may promote the colonization and lead to increase rate of UTIs and such infections may be difficult to treat as they exhibit multidrug resistance.

In the present study, only 13.9% of E.coli isolates were found to be biofilm producers. However this study is comparatively less according to the study conducted by Ponnusamy P *et al.*, done on the 100 *E. coli* strains, in which 72 strains displayed a biofilm positive phenotype the biofilm positive phenotype strains those were also classified as highly positive (6,6%), moderate positive (80, 80 %) and weakly positive (14, 14 %). [16] The rates of antibiotic resistance of biofilm producing *E. coli* were found to be 100 % for chloramphenicol and amoxyclav (amoxicillin and clavulanic acid), 86% for gentamicin and cefotaxime, 84% for ceftazidime, 83% for cotrimoxazole and piperacillin/tazobactam, 75% for tetracycline and 70% for amikacin. Same as here in this study, 73.6% were sensitive to amikacin but 89.4% were sensitive to nitrofurantoin.

Many studies have documented the pathogenic role of hemolysin in UPEC: Hemolysin production is associated with pathogenicity of *E.coli*, especially more severe form of infection. It has been suggested that colonization with hemolytic strains of *E.coli* is more likely to develop into UTIs. [17] In a study from Bangalore, H. Srinivasa *et al.*, showed that among 220 isolates of *E.coli* from urinary tract infections, 91 (41.36%) isolates were hemolytic. [18] In accordance to these previous studies, the present research findings also revealed 41.9% of E.coli isolates were hemolytic.

CONCLUSION

Antibiotics for empirical treatment of UTI should be applied with good to excellent activities. Consideration should be given to the sensitivity of infection, drug safety and cost effectiveness in making the appropriate choice for each patient. Controlled trials and large-scale studies are required to establish pathogenic potential of the isolates. Many studies have shown that complications during pregnancy are more common in bacteriuric women. Antibiotics like nitrofurantoin, amikacin derived as the most effective drugs for the biofilm forming uropathogenic *E. coli* isolates of pregnant women and according to the FDA guidelines these are also safe to have during pregnancy. This study has revealed the screening for biofilm producing uropathogenic *E. coli* isolates, which will reduced the complications attributed with UTI in pregnancy.

REFFERENCES

1. Poonam U. Sharma and Ulka Bidwai. Isolation and identification of bacteria causing urinary tract infections in pregnant women in Vidarbha and their drug susceptibility patterns in them. Int. J. Curr. Microbiol. App. Sci. (2013); 2(4): 97-103
2. Weinstein, M. P., Towns, M. L. and Quartey, S. M. The clinical significance of blood cultures in the 1990s: A prospective comprehensive evaluation of the microbiology, epidemiology and outcome of bacteraemia and fungemia in adults. Clinical Infectious Diseases. 1997; 24, 584-602.
3. Stamm, W. E. Scientific and clinical challenges in the management of urinary tract infections. The American Journal of Medicine 113, 2002; (supplement 1): 1-4
4. Kolawole, A. S., Kolawole, O. M., Kandaki-Olukemi, Y. T., Babatunde, S. K., Durowade, K. A. and Kolawole, C. F. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf

Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicine and Medical Sciences.2009; 1, 163-167.
5. Vila, J.; Simon, K.; Ruiz, J.; Horcajada, J.P.; Velasco, M.; Barranco, M.; et al., Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? *Journal of Infectious Diseases*, 2002; Vol. 186, No. 7, pp. 1039-1042, ISSN 0022-1899.
6. Hegde A, Bhat GK, Mallya S. Effect of exposure to hydrogen peroxide on the virulence of *Escherichia coli*. Indian J Med Microbiol. 2008; 26:25-8
7. Lai, X.H.; Arencibia, I.; Johansson, A.; Wai, S.N.; Oscarsson, J.. Cytocidal and apoptotic effects of the ClyA protein from *Escherichia coli* on primary and cultured monocytes and macrophages. Infection and Immunity. (2000); Vol. 68, No. 7, pp. 4363-4367, ISSN 0019-9567.
8. Toth, I.; Oswald, E.; Mitsumori, K.; Szabo, B.; Barcs, I.; Emody, L. Virulence markers of human uropathogenic *Escherichia coli* strains isolated in Hungary. *Advances in Experimental and Medical Biology*, 2000; Vol. 485, pp. 335-338, ISSN 0065-2598.
9. Smith, H. W. The haemolysins of *Escherichia coli*. *Journal of Pathology and Bacteriology,* (1963); Vol. 85, pp. 197-211, ISSN 0368-3494.
10. Stewart P.S, William C.J Antibiotic resistance of bacteria in biofilms. Lancet, 2001; 358:135-138.
11. Costerton J.W, Stewart P.S, Greenberg E.P, Bacterial biofilms: a common cause of persistent infection Science. (2009), 284; 1318-1322.
12. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Micobiology. 14th ed. New York: Churchill Livingstone; 1996. p. 84.
13. Buchanan, R. E. and Gribbons, N. E. Bergey's Manual of Determinative Bacteriology (8th edition). 1974; Williams & Wilkins Co. Baltimore USA.
14. O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol. (1998); 28(3):449-61.
15. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in *Escherichia coli* isolated from extraintestinal infections. Indian J Med Microbiol. 2007; 25 (4):369-73.
16. Ponnusamy P, Natarajan V, Sevanan M. I n vitro biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. Asian Pac J Trop Med 2012; 5 (3):210-3.
17. Jhonson JR. Virulence factors in *Escherichia coli* urinary tract infection. Clin Microbiol Rev 1991, Jan; 4(1):80-128.
18. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterization of uropathogenic *Escherichia coli* in urinary tract infections. Indian J Med Microbiol. 2003; 21 (2):102-7.

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