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Original Research Article

Study of Biofilm Formation and Antibiotic Resistance in Urinary Isolates at a Tertiary Care Hospital in South India

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ABSTRACT

Introduction: Bacteria adhere to the surfaces, initially in a reversible association and then through irreversible attachment, and eventually develop into an adherent biofilm of highly structured and cooperative consortia. Bacteria have been shown to form intracellular bacterial communities with many biofilm like properties within the bladder epithelium. Antibiotic treatment removes the planktonic bacteria only to have the symptoms return as a result of regrowth of the planktonic population from a nidus of infection consisting of biofilm bacteria displaying a higher level of resistance to the antibiotic. Biofilm infections in urogenital tissue are associated with significant morbidity and mortality.

Materials and Methods: A total of 250 midstream urine samples from symptomatic patients were collected aseptically. The samples were cultured on MacConkey agar and incubated at 37°C for 24 hrs. Urine culture yielding colony counts of>10^5organisms/ml, along with >10pus cells/HPF of a centrifuged urine sample were interpreted as diagnostic of bacteriuria. Identification of isolates was performed by colony morphology, gram staining and standard biochemical tests. Antibiotic sensitivity testing against commonly used antibiotics was done by Kirby Bauer disc diffusion method. Extended spectrum Beta lactamase (ESBL) producers were detected by disc potentiation method. Detection of biofilms was done by the Tissue Culture Plate assay described by Christensen *et al* considered as standard test for detection of biofilm formation. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 570 nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms.

Results: Out of 250 urine samples investigated for UTI, 100 samples showed significant bacteriuria. Out of 100 samples showing significant bacteriuria, the prevalence rate of uropathogenic organisms was found to be in order of E.coli 88 (88%), *Staphylococcus aureus* 4(4%), *Pseudomonas aeruginosa* 2 (2%), *Klebsiella pneumoniae* 2 (2%), *Enterococcus* sps 3 (3%) and *Citrobacter koseri* 1 (1%). Results indicated that quinolones and fluoroquinolones groups of drugs were most potent of all the antibiotics. ESBL production was detected among 17 (17%) out of 100 uropathogenic isolates. Biofilms production was detected from Uropathogenic organisms by Tissue Culture Plate in 20 (20%).

Conclusion: Significant correlation between Biofilm production and multidrug resistance was seen in the study. It is therefore recommended that routine microbiological analysis, antibiotic sensitivity test of mid-stream urine samples and biofilm detection of patients with symptoms of UTI and other

asymptomatic patients be carried out so as enhance in the administration of drugs for the treatment and management of UTIs.

Key Words: Biofilm, Antibiotic resistance, Urinary tract infection.

INTRODUCTION

Bacteria adhere to the surfaces, initially in a reversible association and then through irreversible attachment, and eventually develop into an adherent biofilm of highly structured and cooperative consortia. [1-3] Characteristics are an increased resistance to antibiotic treatment, persistence, evasion of host immune systems (thus exhibiting an altered immune response), expression of different proteins and of quorum-sensing molecules. The presence of biofilms also explains the nature of chronic infections that keep recurring after antibiotic treatment ceases. It is clear, however, that biofilms are associated with urinary tract infections (UTIs) where indwelling devices are not the cause. Bacteria have been shown to form intracellular bacterial communities with many biofilm like properties within the bladder epithelium. [4] These intracellular biofilm like pods allow bacteria to outlast a strong host immune response to establish a dormant reservoir of pathogens inside the bladder cells. One can imagine the recovery from the symptoms of cystitis following antibiotic treatment, which removes the planktonic bacteria, only to have the symptoms return as a result of regrowth of the planktonic population from a nidus of infection consisting of biofilm bacteria displaying a higher level of resistance to the antibiotic. [5] Clearly, biofilm infections in urogenital issue are associated with significant morbidity and mortality.

MATERIALS AND METHODS

The study was conducted in Microbiology department of J.N Medical College and Dr Prabhakar Kore Hospital, Belgaum. A total of 250 midstream urine samples from symptomatic patients from OPD and IPD were collected aseptically. Subjects comprised of both the sexes and varying age group. Urine samples were observed macroscopically with naked eye for colour change, presence of turbidity and haemorrhagia. Findings were recorded. Urine samples collected were centrifuged in a sterile conical centrifuge tube at 2000 rpm for 15 minutes. The supernatant was discarded and the wet preparation of sediment was examined under low and high power to observe inflammatory cells, epithelial cells, RBC, casts and crystals. More than 10 inflammatory cells per high power field were considered significant. [6] All the samples were cultured by semiquantitative method. A calibrated loop with an internal diameter of 4mm was used for plating. The samples were cultured on Mac Conkey agar, Blood agar and incubated at 37°C for 24 hrs. Urine culture yielding colony counts of $>10^5$organisms/ml, along with >10pus cells/HPF of a centrifuged urine sample were interpreted as diagnostic of bacteriuria. Bacterial counts of less than this were considered insignificant and growth of more than 3 types of organisms was considered as contamination. [6] Identification of isolates was performed by colony morphology, gram staining and standard biochemical tests. Antibiotic sensitivity testing against commonly used antibiotics was done by Kirby Bauer disc diffusion method using Clinical Laboratory Standards Institute (CLSI) guidelines. Individual colonies were suspended in peptone water. Turbidity was matched to 0.5 McFarland and using sterile swabs the suspensions were inoculated on Mueller Hinton agar and incubated for 18-24 hrs at 37°C. Following discs were tested: Amoxy/clav (25µg), Cefotaxime (30µg), Gentamicin (30µg), Co-trimoxazole (23.75µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Nalidixic acid (30µg) and Norfloxacin (5µg). Extended spectrum Beta lactamase (ESBL) producers were detected by disc potentiation method in which the discs of Amoxy/clav (25µg) and

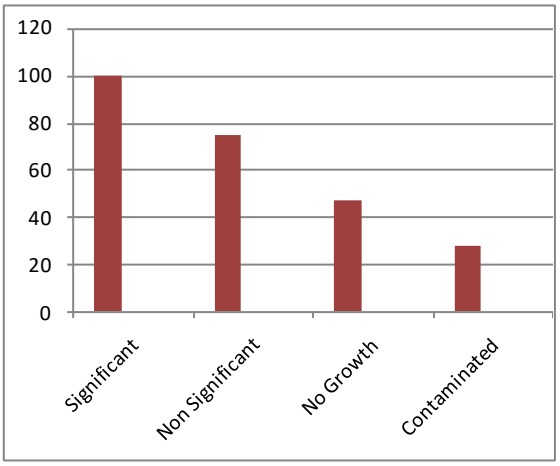
Table 1: Classification of Bacterial adherence by TCP method.		
Mean OD Values	Adherence	Biofilm Formation
<0.183	Non	Non/weak
0.183-0.238	Moderately	Moderate
>0.238	Strong	High

Cefotaxime (30µg) were placed 15mm apart from each other and looking for zone of accentuation towards Amoxy/clavulanic acid. After overnight incubation at 37°C, a positive result was interpreted as more than 5 mm increase in a zonedia meter for amoxicillin/clavulanic acid combination versus its zone when tested with Cefotaxime alone. These isolates were considered multidrug resistant (MDR). [7]

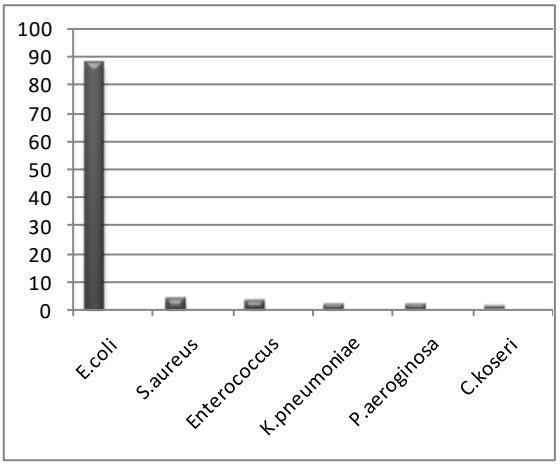
Detection of biofilms was done by the Tissue Culture Plate assay described by Christensen *et al* considered as standard test for detection of biofilm formation. Isolates from fresh agar plates were inoculated in Trypti case soy Broth with glucose and incubated for 18-24 hour at 37°C and diluted 1in100 with fresh medium. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates wells were filled with 0.2 ml aliquots of the diluted cultures and only broth served as control to check sterility and non-specific binding of media. The tissue culture plates were incubated for 18 to 24 hours at 37°C. After incubation, content of each well was gently removed by tapping the plates. The wells were washed four times with 0.2 ml of phosphate buffer saline (PBS pH 7.3) to remove free-floating 'planktonic' bacteria. Biofilms formed by adherent 'sessile' organisms in plate were stained with crystal violet (0.1%). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent bacterial cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 570 nm (OD 570 nm). These OD values were considered as an index of bacteria adhering to surface and forming biofilms. Experiment was performed in duplicates and repeated two times, the data was then averaged and standard deviation was calculated. The mean OD value obtained from media control well was deducted from all the test OD values. [8-10]

RESULTS

Out of 250 urine samples investigated for UTI, 100 samples showed significant bacteriuria, 75 samples showed non significant bacteriuria, 47 samples were no growth and the remaining 28 samples were contaminated (Graph 1).



Graph 1:- Detection of bacteriuria in urine cultures of patients suffering from UTI. Significant bacteriuria (40%), Non significant bacteriuria (30%), No growth (18.8%) & Contaminated (11.2%).

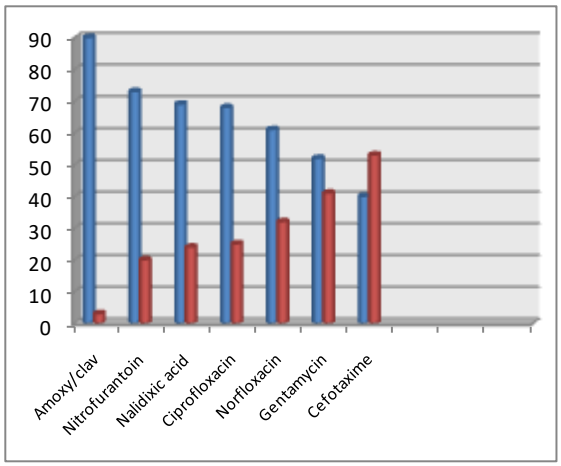


Graph 2:-Bacterial isolates from urine samples of patients suffering from UTI. *E.coli* was the predominant isolate 88(88%) followed by *Staphylococcus aureus* 4(4%), *Enterococcus* sps 3(3%), *Pseudomonas aeruginosa* 2(2%), *Klebsiella pneumoniae* 2(2%) and *Citrobacter koseri* 1(1%).

Out of 100 samples showing significant bacteriuria, the prevalence rate of uropathogenic organisms was found to be in order of E.coli 88 (88%), *Staphylococcus*

aureus 4 (4%), *Pseudomonas aeruginosa* 2 (2%), *Klebsiella pneumoniae* 2 (2%), *Enterococcus* sps 3 (3%) and *Citrobacter koseri* 1 (1%) were isolated (Graph 2).

Of the 100 samples showing significant bacteriuria, 93 were Gram negative isolates and 7 were Gram positive isolates (Graph 2). Out of 93 Gram negative isolates 73 (78.49%) were sensitive to Nitrofurantoin, 61 (65.59%) to Norfloxacin, 68 (73.11%) to Ciprofloxacin, 69 (74.19%) to Nalidixic acid, 26 (27.95%) to Cotrimoxazole, 52 (55.91%) to Gentamicin and 33 (35.48%) to Amoxyclav. The gram positive isolates *Enterococcus* sps and *Staphylococcus aureus* showed good sensitivity to all antimicrobial drugs. (Graph 3 & Table 2).



Graph 3:- Antibiogram pattern of Gram negative Uropathogens isolated from patients suffering from UTI.
Blue: Sensitive
Red: Resistant

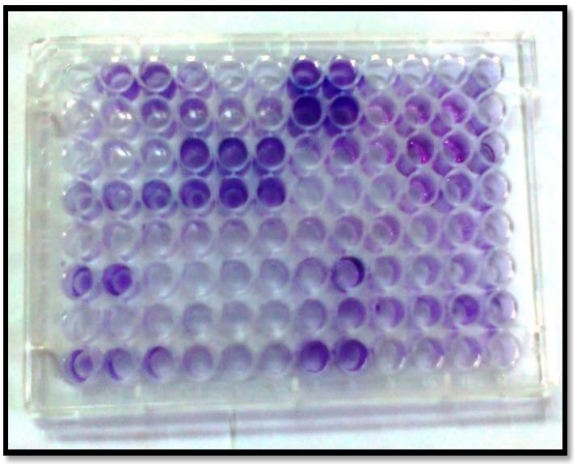


Figure 1:-Tissue Culture Plate Method.

Results indicated that quinolones and fluoroquinolones groups of drugs were most potent of all the antibiotics. Ampicillin, Gentamicin and Cotrimoxazole were poorly effective. ESBL production was detected among 17 (17%) out of 100 uropathogenic isolates.

Biofilms production was detected in 20 (20%) Uropathogenic organisms by Tissue Culture Plate method (Table 2).

Screening of biofilm producers by TCP method: Strong, moderate and non slime producers differentiated with crystal violet staining in 96 well tissue culture plate.

Table 2:- Correlation of Biofilm formation and ESBL production.

Organisms	No.of patients	ESBL	Biofilm Positive
E.coli	88 (88%)	17 (19.31%)	15 (17.04%)
P.aeruginosa	2 (2%)	0	0
K.pneumoniae	2 (2%)	0	0
S.aureus	4 (4%)	0	2 (50%)
Enterococcus	3 (3%)	0	3 (100%)
C.koseri	1 (1%)	0	0
Total	100	17 %	20 %

DISCUSSION

In this study significant bacteriuria was seen in 40(40%) of patients (Graph 1). A higher prevalence of Gram negative organisms as causative organisms in UTI was seen as evident in the prevalence rates of 88% for *Escherichia coli* as compared to the other studies. [1,11-13] (Graph 2). This study shows high degree of susceptibility by UPEC to various quinolones and fluoroquinolones tested as seen in Graph 3. This is similar to other studies which show quinolones are better alternatives to commonly prescribed antibiotics in Urinary tract infection. [12,14] In this study, 17 (19.31%) *E.coli* isolates were ESBL producers (Figure 3). [15] This was similar to other studies which showed high prevalence rate of ESBL (MDR) producing *E.coli*. [8,14,15] Out of the 100 isolates tested 20 were positive for biofilms production by tissue culture plate method employing criterion of blank corrected OD>0.1 (Table 1). From Table 3, it is seen that biofilm production is more predominant among the UPEC

considering TCP as the standard method (17.04%). Patients with UTI are more likely to develop acute and chronic pyelonephritis and cystitis in later stages of UTI. Hence it is essential to rule out biofilm production which indicates persistence of infection. [16-18] Table 2 shows correlation between biofilm production and multidrug resistance due to ESBL production. Among the screened isolates 17(19.31%) E.coli strains were ESBL producer. Out of these 12 (70.58%) were both ESBL and Biofilm producing. Hence the present study also showed significant correlation between biofilm production and multi-drug resistance as described by other studies. [18-20]

CONCLUSION

Significant correlation between Biofilm production and multidrug resistance was seen in the study. Biofilm production among UPEC was more predominant when compared to the other isolates. Biofilm production in *E.coli* may promote colonization and lead to increase rate of urinary tract infections. Biofilm endows bacteria with several advantages, such as acquisition of antibiotic tolerance, expression of several virulence factors and increased resistance against phagocytosis and other host defence mechanisms. The study of factors contributing to biofilms formation may be important to conceive new therapeutic solutions to treat these infections. A greater understanding of the nature of intracellular bacterial communities in chronic or recurrent UTI's will aid in the development of new and more effective treatments for this problematic diseases. It is therefore recommended that routine microbiological analysis, antibiotic sensitivity test of mid-stream urine samples and biofilm detection of patients with symptoms of UTI and other asymptomatic patients be carried out so as enhance in the administration of drugs for the treatment and management of UTIs.

REFERENCES

1. Yasmeen Kausar, Sneha K. Chunchanur, Shobha D. Nadigir, L.H.Halesh and M.R.Chandrashekhar: Virulence factors, serotypes and antimicrobial susceptibility patterns of Escherichia coli in Urinary tract infection. Al ameen J Med Sci (2009) 2 (1) : 47-51.
2. Kelsey E. Sivick and Harry L. T. Mobley Waging War against Uropathogenic *Escherichia coli*: Winning Back the Urinary Tract. Infection and Immunity, Feb. 2010, p. 568–585.
3. Corinne K. Cusumano, Chia S. Hung, Swaine L. Chen, and Scott J. Hultgren: Virulence Plasmid Harbored by Uropathogenic *Escherichia coli* Functions in Acute Stages of Pathogenesis, Infection and Immunity, Apr. 2010, p. 1457–1467.
4. Anderson GG, Martin SM, Hultgren SJ. Host subversion by formation of intracellular bacterial communities in the urinary tract. Microbes Infect 2004;6:1094 – 101.
5. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial Biofilm-like pods in urinary tract infections. Science 2003;301:105-7
6. Koneman E W, Allen S D. Textbook of Diagnostic Microbiology New York: Wolters Kluwer Co.6th Ed. Pg 137 – 140.
7. Clinical and Laboratory Standards Institute (CLSI).*Performance standards for antimicrobial susceptibility testing*. CLSI approved standard M-100-S16 (M7). Wayne, Pa: Clinical and Laboratory Standards Institute; 2006.
8. S. Nagaveni, H. Rajeshwari, Ajay Kumar Oli : Evaluation Of Biofilm forming ability of the multidrug resistant Pseudomonas aeruginosa. The Bioscan an International Quartely Journal of Life Sciences 5(4), 2010:563-566.
9. T Mathur, S Singhal, S Khan, DJ Upadhyay, T Fatma, A Rattan, Detection of Biofilm Formation Among The Clinical Isolates Of Staphylococci: An Evalution Of Three Different Screenig Methods, Indian Journal of

Medical Microbiology, (2006) 24 (1):25-9.
10. Salwa S. Seif El-Din, Moustafa S. El-Rehewy, Mohammed M. Ghazaly: Biofilm Formation by Blood Stream Staphylococcal Isolates from Febrile Pediatric Cancer Patients at South Egypt Cancer Institute. Journal of American Science, 2011, 7(1):674-686.
11. Kolawole. A.S., Kolawole O.M., Kandaki-Olukemi Y.T.,Babatunde S.K., Durowade K.A. and Kolawale C.F. Prevalence of UTI among Patients attending Dalhatu Araf specialist hospital, Lafia, Nigeria. International Journal of Medicine and Medical Sciences Vol 1. (5) pp.163-167, May, 2009.
12. Idowu A.O and Odelola H.A.: Prevalence of Some Uropathogenic Bacterial Isolates and their Susceptibility to Some Quinolones. African Journal of Biomedical Research, Vol. 10 (2007); 269 – 273.
13. Paula L. Roesch, Peter Redford, Stephanie Batchelet, Rebecca L. Moritz, Shahaireen Pellett, Brian J. Haugen, Frederick R. Blattner and Rodney A. Welch Uropathogenic *Escherichia coli* use D-serine deaminase to modulate infection of the murine urinary tract. Molecular Microbiology (2003) 49 (1), 55–67.
14. Basharat Ali Khan, Sami Saeed, Adeel Akram, Faraz Basharat Khan, Amjad Nasim: Nosocomial Uropathogens and their Antibiotic Sensitivity patterns in a tertiary care referral teaching hospital in Rawalpindi, Pakistan. J Ayub Med Coll Abbottabad 2010;22(1).
15. Supriya S. Tankhiwale, Suresh V. Jalgaonkar, Sarfraz Ahamad & Umesh Hassani Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res 120, December 2004, pp 553-556.
16. M.Soto, A.Smithson, J.A.Martinez, J.P.Horcajada, J. Mensa and J.Vila: Biofilm formation in Uropathogenic E.coli strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. Journal of Urology; January 2007, vol 177,365-368.
17. A.I. Dadawala, Chauhan H.C., Chandel, B.S., Ranaware, P., Patel Sandip S, Khushboo Singh, Rathod P.H., Shah N.M. and Kher, H.N. Assessment of Escherichia coli isolates for In vitro biofilm production, Journal of Veterinary World Vol.3(8): 364-366.
18. E Suman, J Jose, S Varghese, MS Kotian: Study of Biofilm production in Escherichia coli causing Urinary Tract Infection. Indian Journal of Medical Microbiology 2007: 305-306.
19. J. Jayalakshmi, V.S. Jayaram: Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian Journal of Pathology and Microbiology 2008; 51(3):379-381.
20. Ponnusamy P, Natarajan V, Sevanan M: In vitro biofilm formation by uropathogenic E.coli and their antimicrobial susceptibility pattern. Asian Pac Journal of Tropical Med 2012 ; 5(3) : 210-3.

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