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Study on Biofilm Producing Bacterial Isolates in Catheter Associated Urinary Tract Infection

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Abstract : *Catheter Associated Urinary Tract Infections (CAUTI) are one of the most common cause of Healthcare associated infections. Cause of CAUTI is the formation of pathogenic microbial biofilm in the indwelling urinary catheters which provides survival benefit to the colonized organism by providing protection from environmental stresses and also causing decreased susceptibility to antibiotics leading to persistent and recurrent infections. The present study was conducted to detect biofilm production by isolates from patients with CAUTI, and their association with antibiotic resistance. This prospective study was done on 484 hospitalized patients in different wards with indwelling urinary catheter of more than 2days. CAUTI was diagnosed based on the CDC guidelines 2017. Uropathogens were identified and their antibiotic susceptibility was performed following standard microbiological methods. Biofilm detection was done by Tissue Culture Plate method. Among 484 patients catheterized, 134 developed CAUTI. The most common organism isolated was E.coli (39%), followed by Klebsiella pneumoniae (28%). Among the isolates 63 (47%) were biofilm producers. Biofilm producing strains showed relatively higher antibiotic resistance than non-producers. Among the 65% of multi drug resistant (MDR) isolates, 62% were biofilm producers, showing significant association between biofilm production and multidrug resistance. Emerging multidrug resistance (MDR) of the uropathogens due to this biofilm formation is a big challenge in the management of catheter associated urinary tract infection (CAUTI). Hence early detection and newer management protocols may be deployed.*

Keywords - *Biofilm, CAUTI, E.coli, Healthcare associated infections ,MDR*

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I. Introduction

Catheter Associated Urinary Tract Infections (CAUTI) are one of the most common causes of Health Care Associated infections (HAI's). Urinary tract infections constitute about 30% of HAI's and about 75% of nosocomial urinary tract infections are associated with urinary catheters [1].Most cases of CAUTI are associated with biofilm formation which is a representative type of biofilm associated infection usually composed of multi drug resistant microorganisms [2].

.Biofilms are composed of clusters of diverse microorganisms and extracellular matrix (primarily polysaccharide materials) secreted by the colonized bacteria which promote attachment on contact with the surface**.** These bacteria then multiply and spread over the surface, both the extra & intraluminal surfaces of urinary catheters, forming colonies embedded in a gel-like polysaccharide matrix.These bacterial microcolonies in biofilms are protected from antimicrobial chemotherapy as well as host defence mechanisms, establishing chronic persistent infections, urosepsis and death if not treated [3] .

Many bacterial species especially *Acinetobacter, Pseudomonas, Klebsiella, Staphylococcus, Enterobacter* and *E. coli* shows biofilm mode of growth for their survival benefit in a wide range of clinical settings and the same may be the common cause of colonization and biofilm production in indwelling urinary catheters [4] . The incidence of bacteriuria in catheterized patients increases with increase in duration of catheterization [2] .

Although catheter-associated urinary tract infections (CAUTI) may not result in excess mortality but they significantly increases burden in the health care system by increasing both morbidity and treatment costs. However, no study has been conducted in our hospital with regards to the role of bacteria with biofilm producing properties in patients with indwelling urinary catheter associated infections. Hence, the present study was conducted to detect biofilm producing bacterial isolates from CAUTI in our hospital and their antibiotic resistance pattern.

II. Materials & Methods

This prospective analytical study was conducted in the Department of Microbiology, Coimbatore Medical college & Hospital over the period of 6 months after obtaining the approval from Institutional human ethics committee (IHEC) A total of 484 patients were on silicone coated latex catheter from different wards were included in this study.

The diagnosis of CAUTI was made based CDC- 2017 guidelines [5] (. *i.e.* those on indwelling urinary catheters for at least 2 days plus any one of the following symptoms of UTIs (fever > 38ºC, urgency, frequency, dysuria or suprapubic tenderness) and presence significant bacteruria and a positive urine culture by standard methods - semi quantitative culture using calibrated inoculating loop followed by antibiotic sensitivity testing using disk diffusion Method as per CLSI-M100/2017 standards[6] .

The detection of biofilm was done by the Tissue Culture Method.10 ml of Trypticase soy broth with 1% glucose was inoculated with a loopful of test organism from overnight culture on nutrient agar. The broth was incubated at 37deg C for 24 hours. The culture was further diluted 1:100 with fresh medium. 96 wells flat bottom tissue culture plates were filled with 0.2 ml of diluted cultures individually. Only sterile broth was served as blank. Similarly control organisms were also diluted and incubated. All three controls and blanks were put in the tissue culture plates. The culture plates were incubated at 37deg C for 24 hours. After incubation, gentle tapping of the plates was done. The wells were washed with 0.2 ml of phosphate buffer saline (pH 7.2) four times to remove free floating bacteria. Biofilms which remained adherent to the walls and the bottoms of the wells were fixed with 2% sodium acetate and stained with 0.1% crystal violet. Excess stain was washed with deionized water and plates were dried properly. The Optical densities (OD) of stained adherent bacteria in the tissue culture plates were read with ELISA reader at wavelength of 570 nm and they were graded as **(OD < 0.120 : None/weak, OD 0.120 - 0.240 : Moderate and OD > 0.240 : Strong)** as per Christensen *et al* [7] . These OD values were considered as an index of bacteria adhering to the surface and forming biofilms. It was performed in triplicate and repeated thrice. Average of OD values of sterile medium were calculated and subtracted from all test OD values.

For few cases, in addition to the catheterized urine sample, scrapings from external and internal luminal surfaces of Foley's catheter tip also collected under sterile precautions and sub cultured after enrichment in BHI broth for confirmation of the isolates and its biofilm producing property.

III. Results

Out of 484 patients on catheter during the study period, 134 (28 %) developed CAUTI. **(Table.1)** The most common organism isolated was *E. coli* (39%) followed by *Klebsiella spp.* (28%), *Pseudomonas aeruginosa* (16%), *Proteus spp.* (7.5%), *Enterobacter spp.(4.5%) Enterococcus faecalis* (3%) and *Acinetobacter baumannii* (2 %). We also observed few samples showed growth of multiple organisms. Among the isolates, sixty three (47%) were found to be positive for biofilm production by Tissue Culture Plate method. **(Table.2)**

Table.1 Bacterial colonization & Biofilm formation

Total No. of patients under study	484	(%)
No. of Patients without bacterial colonization after 5 days of indwelling Catheter	350	72 %
No. of Patients with bacterial colonization after 5 days of indwelling Catheter	134	28%
No of Bacterial isolates producing Biofilm	63	47%

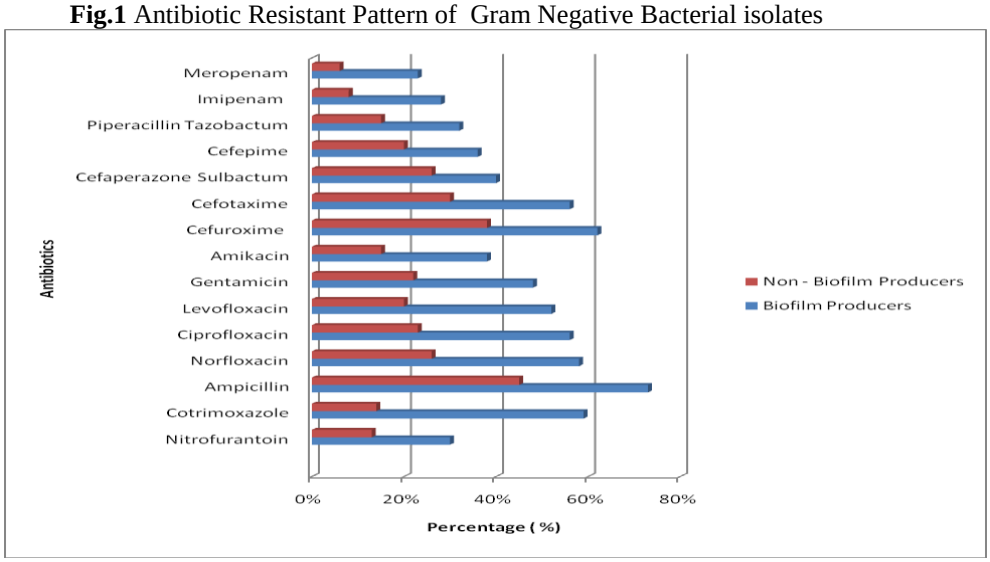
Table.2 Distribution of Biofilm producers among the Bacterial isolates

	No. of isolates 134 (28%)	Biofilm producers 63 (47%)
Escherichia coli	52 (39%)	28 (53%)
Klebsiella spp.	38 (28%)	15 (40%)
Pseudomonas spp.	21 (16%)	12 (57%)
Proteus spp.	10 (7.5%)	4 (40%)
Enterobacter spp.	6 (4.5%)	2 (33%)
Enterococcus faecalis	4 (3%)	1 (25%)
Acineobacter spp.	3 (2%)	1 (33%)

In this study, antibiotic sensitivity testing of biofilm producing bacterial isolates showed a relatively higher resistance against antibiotics tested **(Fig. 1)** .Out of the 134 bacterial isolates studied, 87(65%) were multidrug resistant and there was also a significant correlation between biofilm production and resistance to multiple drugs such as ampillicin, ciprofloxacin, cotrimoxazole and 2nd & 3rd generation cephalosporins were

observed. Among the 65% MDR isolates, 38% were Non - biofilm producers and the remaining 62% were biofilm producers.

Fig.1 Antibiotic Resistant Pattern of Gram Negative Bacterial isolates



IV. Discussion

The present study showed out of the 134 strains, *E. coli* was the most frequently isolated pathogen (39%) followed by *Klebsiella pneumoniae* (28%) which was similar to many other study reports done in India and in abroad [8-13] . It is explained by the known fact that presence and expression of virulence determinants like fimbrial adhesions (*Type 1 fimbriae* and *P. fimbriae*) and hemolytic toxins which are responsible for uropathogenic *E. coli* to be the most common cause of UTI as well as in CAUTI, due to the associated risk factor of indwelling catheter and lacunae of proper care bundle following catheterization in hospital settings.

Biofilm formation along the catheter surface is a contributing factor for chronic, persistent infection in CAUTI, which do not respond to the conventional antibiotic therapy and it also protects against host immune defense mechanism. Out of the 134 isolates, 63 (47%) were only found to be biofilm producers , which was relatively low when compared to observation of 60 % and 71 % biofilm producers by Sayal *et al.*, at Amritsar and Pramodhini *et al.*, at Pondicherry in their study[13,14] .

In this study, 65% of the isolates reported were MDR. Among these MDR isolates, 62 % were biofilm producers which is similar to studies by Pramodhini *et al.*, and Majumder *et al.*, at Bangladesh [14,15] . This finding indicates that larger percentage of biofilm producers may be MDR, which are always associated to persistent infections and fails to respond to treatment, but favors the spread of antibiotic resistance among nosocomial pathogens by the mutation and by the exchange of antibiotic resistance encoded genes.

Antibiotic treatment against device associated biofilm organisms often fails without the removal of the infected implant although they are not intrinsically resistant to antibiotics, an elevated expression of the efflux pump and physiological changes in their rate of growth, metabolism, interbacterial quorum signals, accumulation of toxic products and local micro environmental changes are playing a major role in the development of antibiotic resistance in biofilm producing organisms.

Our study involved patients with silicone coated latex catheter and the incidence of CAUTI was 28%. Few studies reported that not only the infection rate, also colonization by the biofilm producing organisms were significantly less with pure silicone catheter as compared to silicone coated latex catheter. However, the major disadvantage of pure silicone catheter is that it is more expensive and causes more discomfort to the patients due to its rigid nature.

V. Conclusion

E.coli was the most frequent isolate which showed the higher sensitivity to carbapenems, and lower to the quinolones & cephalosporin. Biofilm can be composed of a single or multiple organisms on various biotic and abiotic surfaces. There is association between biofilm production with persistent infection and antibiotic failure. Hence, better understanding on infections caused by biofilm producing organisms, the mechanism of biofilm production and its identification might help to modify the antibiotic therapy and to prevent infection related to biomedical devices. A suitable and reproducible method like TCP can be routinely done for screening of biofilm producers in any healthcare setting.

Since the biofilm producers are highly resistant to multiple antibiotics and difficult to treat, especially in medical device associated infections like CAUTI, knowledge regarding the safe use of indwelling urinary catheter to be updated regularly and newer management protocols need to be explored where long duration of catheter access are required, which will definitely lower the economic burden in the hospitalized patient with catheter associated urinary tract infections.

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