Original Research Article

Biofilm production by uropathogens causing catheter associated urinary tract infection

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Abstract

Background: The microbial populations within urinary catheter frequently develop as biofilms, directly attaching to the surface of catheters. Bacteria in biofilm are protected from antimicrobial chemotherapy as well as host defence mechanisms, establishing chronic persistent infections, septicemia and death if not treated**. Material and Method:** The present study, includes 200 patients, catheterized for >48 hours at CIMS, Bilaspur. Urine samples were collected and inoculated in nutrient agar, blood agar and MacConkey agar plates and identification done as per standard procedure. This study was conducted to detect biofilm formation ability of uropathogens by two different methods (Tube and Congo red agar method) and compare their antibiotic sensitivity by using Kirby-Bauer disc diffusion method. **Results:** Out of 200 urine samples significant bacteriuria were detected in 148 (74%) of samples and no growth found in 52 (26%) samples and 14 samples showed growth of 2 microorganisms. A total no. of 162 microorganisms were isolated from 200 urine samples. Among these 162 isolates *E. Coli* was 29.62%, *Pseudomonas aeruginosa* 11.72%, *Klebsiella sp* 18.51%, *Citrobactor sp* 7.40%, *Staphylococcus aureus* 3.08%, and Coagulase negative *Staphylococci* 11%. Among these 162 isolates, a total of 91(56.17%) isolates showed biofilm production. Percentage of biofilm formation was highest in *P. aeruginosa* (63.15%). **Conclusion:** Tube test method was found to be more reliable method. The in vitro antibiotic susceptibility pattern of biofilm producing organisms showed less sensitivity as compared to non-biofilm producing organisms.

Keywords: Biofilm, Catheter associated urinary tract infection, Antibiotic resistance

Introduction

"A biofilm is an aggregate of micro-organisms in which cells adhere to each other on a surface embedded within a self-produced matrix of extracellular polymeric substance"[1]. Urinary tract infection, with its diverse clinical syndromes and affected host groups, remains one of the most common but widely misunderstood and challenging infectious diseases encountered in clinical practice.

Manuscript received: 26th June 2017
Reviewed: 5th July 2017
Author Corrected: 14th July 2017
Accepted for Publication: 21st July 2017

The risk of developing urinary tract infection increases significantly with the use of indwelling devices such as catheters and urethral stents or sphincters. Urinary tract infections account for an estimated 25 to 40% of nosocomial infections and represent the most common type of these infections [2]. Catheter associated urinary tract infections (CAUTI) account for up to 40% of all nosocomial infections and 80% of all nosocomial Urinary Tract Infections (UTIs) [3].

The organisms commonly contaminating these devices are *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus epidermidis* and *Enterococcus faecalis*. The longer the urinary catheter remains in place, the greater the tendency of these organisms to develop urinary tract infections [2].

Organisms causing CAUTIs require fewer recognized virulence factors to colonize and establish infection, including bacterial adhesions, degradative enzymes, toxins and capsules, lipopolysaccharides. These virulence factors help bacteria to attach on catheter surface and to induce an inflammatory response. Biofilm is one of the most important virulence factor of bacteria which causes CAUTIs [4].

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].

Materials and Methods

Study design- Cross Sectional study

Study setting- Department of Microbiology, Chhattisgarh institute of Medical Science, Bilaspur, over a period of one year

Inclusion criteria- All ages groups, both Sexes, who indwelling urinary catheter for at least 2 days, and who were suffering from symptoms of UTIs

Exclusion criteria- patients with symptoms of UTI prior to catheterization

Participants- After approval from ethical committee, samples collected from admitted patients from Chhattisgarh institute of medical science hospital

Study size- 200 Samples were inoculated in nutrient agar, blood agar and MacConkey agar plates, as per standard procedure [5].

After 24 hours of incubation at 37˚C in aerobic atmosphere, inoculated plates were observed for presence of growth and a colony count was done. The results were recorded as positive where urine culture showed colony count of 100 or more which was equivalent to 10^5 CFU/ml [5].

After identification on the basis of colony morphology, gram stain and motility test, the isolates were subjected to biochemical tests for species identification [6].

Biofilm formation was detected by Congo red agar method and Tube test method.

Congo Red Agar Method- CRA medium proposed by Freeman *et al.* was prepared with brain heart infusion broth, sucrose, agar and Congo red indicator. Congo red stain was prepared separately in sterile distilled water and was added to sterile molten agar base and then the medium was poured in the plates. CRA plates were inoculated with the test organisms and incubated at 37 ºC for 24 hours to 48 hours aerobically. Black colonies with dry crystalline consistency indicate strong biofilm formation. Brownish or reddish growth was considered as negative biofilm formation (Fig. 1) [7].

Tube Method- Biofilm production was investigated by the tube adherence test proposed by Christensen *et al.* Ten ml Trypticase soya broth with 1% glucose was inoculated with the test organism on nutrient agar individually. Broths were incubated at 37 ºC for 24 hours. The cultures were aspirated and the tubes were washed with phosphate buffer saline pH 7.3. The tubes were dried and stained with 0.1% crystal violet. Excess stain was removed. Tubes were dried in inverted position. In positive biofilm formation, a visible stained film was seen along the walls and bottom of the tube (Fig. 2) [7].

All isolates were subjected to antibiotics susceptibility testing on Mueller Hinton agar by disc diffusion method of Kirby-Bauer using commercially available discs from Himedia [8].

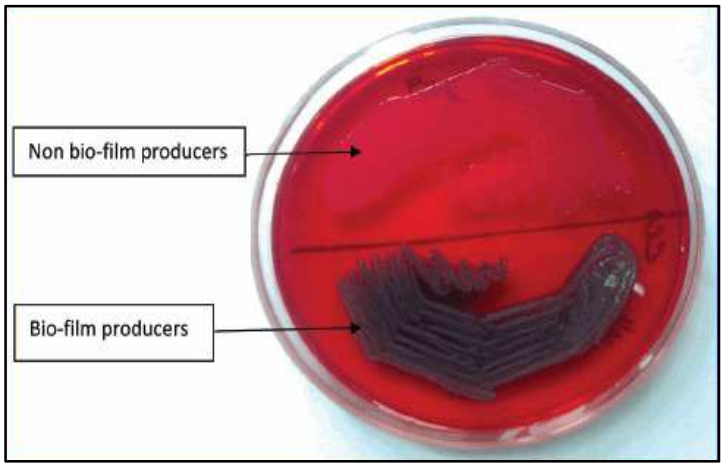

Fig-1: Congo red method for biofilm detection

Fig-2: Tube test method for biofilm detection

Results

Out of 200 urine samples significant bacteriuria was detected in 148 (74%) of samples and no growth was found in 52 (26%) samples.

As shown in the table 1, rate of developing UTI was more with increase in duration of catheterization and it was 87.5% with (7-14 days) of catheterization.

Table-1: Length of catheterization in relation to development of significant bacteriuria.

Duration	No. Of Samples	Significant bacteriuria
(2-7 days)	20	3(15%)
(7-14 days)	72	63(87.5%)
(>14 days)	8	6(75%)
Total	**100**	**72**

Among these 148 positive samples, 14 samples showed growth of 2 organisms. A total no. of 162 microorganisms were isolated from 200 urine samples.

As shown in the table 2, *E. coli* was found to be most frequently isolated pathogen (29.62%) followed by *Klebsiella sp.* (18.51%) and *enterobactor* (13.58%).

Table-2: Distribution of uropathogens from urine samples

Name of Bacteria	No.	Percentage (%)
Escherichia coli	48	29.62
Klebsiella species	30	18.51
Enterobactor	22	13.58
Pseudomonas aeruginosa	19	11.72
Enterococci	15	9.25
Citrobacter freundii	12	7.40
Coagulase –ve Staphylococci	11	6.79
Staphylococcus aureus	5	3.08
Total	**162**	**100**

Table-3: Biofilm formation among these urinary isolates

Organisms	Biofilm positive	Biofilm negative
P. aeruginosa (19)	12	7
Klebsiella sp (30)	18(60%)	12(40%)
Enterococci(15)	9	6
E. coli(48)	28(58.33%)	20(41.66%)
Enterobactor(22)	12	10
Citrobactor sp(12)	6	6
S. aureus(5)	2	3
CONS(11)	4	7
Total(162)	**91(56.17%)**	**71(43.82%)**

A total of 91(56.17%) isolates showed biofilm formation by Congo red or Tube test. Percentage of biofilm formation was highest in *P. aeruginosa* (63.15%) followed by *Klebsiella sp* (60%) and *E. coli* (58.33%). [Table 3]

Tube test methods detected 77.8% biofilm positive isolates whereas Congo red test detected 71.1% isolates producing biofilm.

The in vitro antibiotic susceptibility pattern of gram negative organisms showed very less sensitivity to gentamycin (29.1%), tobramycin (45.8%), amoxyclav (37.5%), ciprofloxacin (20.8%), doxycycline (33.3%), ceftriaxone (37.5%), ceftazidime (41.7%) and levofloxacin (37.5%).

Sensitivity to amikacin (66.6%), nitrofurantoin (70.8%) piperacillin - tazobactum (83%), imipenam and meropenam (87.5%) were good.

The in vitro antibiotic susceptibility pattern of biofilm producing Gram negative organisms showed less sensitivity as compared to non-biofilm producing organism such as gentamycin (23.68%), tobramycin (31.5%), amoxyclav (31.5%), ciprofloxacin (10.5%), doxycycline (23.68%), ceftriaxone (28.9%), and ceftazidime (36.8%). Among biofilm producing isolates, four isolates of *Pseudomonas,* two isolates of *Klebsiella* and two isolates of *E.coli* showed resistant to all drugs whereas in biofilm non-producing isolates resistant to all drugs were found only in one each isolates of *Klebsiella species* and *E. coli.*

Staphylococcal species were less sensitive to penicillin (0%), ampicillin (0%), levofloxacin (27.7%), ciprofloxacin (27.7%), ofloxacin (33.33%), norfloxacin (33.33%), doxycycline (38.9%), and cotrimoxazole (38.9%). *Staphylococcal species* showed good sensitivity to linezolid (88.8%) and nitrofurantoin (66.6%).

Discussion

Biofilm, a predominant mode of growth characteristics of bacteria, plays a central role in pathogenesis of catheter associated urinary tract infection [9].

Urinary catheterization is generally indicated to relieve urinary tract obstruction, to permit urinary drainage in patients with neurogenic bladder dysfunction and urinary retention, to aid urologic surgery and to obtain accurate measurement of urinary output in clinically ill patients. An estimated 4 million patients are subjected yearly to urinary catheterization and therefore at risk for catheter associated infection and its related sequelae [10]. In the present study, 74% of the 200 patients studied had significant bacteriuria, and 26% samples were culture negative.

As shown in table 4, our result is similar with study conducted by Asha B Patil *et al* [12].

Table-4: Incidence of CAUTI in various studies.

Study series	Year	Incidence of CAUTI
Rohan Chaudhari et al [20]	2004	44%
Taiwo SS et al10	2006	88.5%
Onipede Anthony et al [16]	2010	60.9%
S. Abaeze et al [14]	2011	41.10%
Mahabulbul Ishlam et al [11]	2014	90%
Asha B Patil et al [12]	2014	76%
Present study	2014	74%

In present study rate of developing UTI was more with increase in duration of catheterization which is similar to reports of S G Kulkarni *et al* [13], Taiwo SS *et al* [10] and Mahabulbul Ishlam *et al* [11].

The distribution of commonest bacterial organisms in the present study is close to results obtained by G F M Gad *et al* [17] and S. Abaeze *et al* [14]. *E. coli* was most frequently isolated organism 48 (29.62%) and this is similar to study conducted by S. Abaeze *et al* [14], Mahabulbul Ishlam *et al*[11] , Asha B Patil *et al* [12] and S. Niveditha 2012 *et al* [15].

Implanted prosthetic devices constitute particularly attractive surfaces for bacterial colonization, as they have none of the protective mechanisms of healthy tissue surfaces.

As shown in table 5, Our results are similar with S. Niveditha *et al* [15].

Table-5: Detection of biofilm formation in various studies.

Various studies	Biofilm formation%
G Reid *et al* [21]	73%
Narmeen Mahmoud *et al* [22]	43.3%
S. Niveditha *et al* [15]	60%
Pradeep kumar *et al* [9]	80%
Present study	56.17%

The percentage of biofilm detection of different isolates in the present study is similar to reports of S. Niveditha *et al* [15]. In the present study Tube test method was more reliable. Afreenish Hassan *et al* [7] also found that tube method is more reliable than congo red agar method.

In the present study sensitivity pattern of non-biofilm producing *E. coli* is similar to study conducted by Mahabulbul Ishlam *et al* [11].

Sensitivity pattern of *Klebsiella species* in present study is smilar to reports of Onipede Anthony *et al* [16], Taiwo SS *et al* [10] and S G Kulkarni *et al* [13]. Sensitivity pattern of *P. aeruginosa* in the present study is similar to reports of S G Kulkarni *et al* [13] and S.Abaeze *et al* [14]. In the present study sensitivity of *C. freundii* is similar to results obtained by Onipede Anthony *et al* [16].

Our study showed that imipenam, meropenam, nitrofurantoin, amikacin and piperacillin-tazobactam were most effective antibiotics against gram negative isolates. Narmeen Mahmoud *et al* [19] also found that imipenam and amikacin were most effective antibiotics against gram negative isolates.

In the present study cefotaxime, ceftriaxone, norfloxacin, ciprofloxacin, ofloxacin and the aminoglycosides showed less sensitivity against gram negative isolates. The present study showed more drug resistance in biofilm forming isolates than in non bioflim forming isolates which is similar to S. Pramodhini *et al* [18].

Our study showed that, resistance to all drugs were found more in biofilm positive isolates then in biofilm non producing isolates which is similar to reports of Mahabulbul Ishlam *et al* [11]. In the present study sensitivity pattern of *Staphylococci* is similar to reports of others.

Conclusion

Indwelling urethral catheters should be avoided whenever possible and should never be resorted to unless with absolute indications.

There is an association between biofilm production with persistent CAUTI and antibiotic therapy failure. Hence identification of infection caused by biofilm producing organisms might help to modify the antibiotic therapy and prevent infection.

In our view this is the first of its kind highlighting role and relevance of biofilm production by pathogenic bacteria isolated from catheter associated urinary tract infection in this tribal dominated region of Chhattisgarh state.

Funding: Nil, Conflict of interest: None
Permission of IRB: Yes

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How to cite this article?

Tomar R, Paviaya R, Ghosh S, Murthy R, Pradhan S, Agrawal E. Biofilm production by uropathogens causing catheter associated urinary tract infection. *Int J Med Res Rev* 2017;5(07):710-716.doi:10.17511/ijmrr. 2017.i07.09.

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