Study of biofilm formation among uropathogens isolated from catheter-associated UTI patient from a tertiary care hospital

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ABSTRACT

Background: Catheter-associated urinary tract infection (CAUTI) is a serious health threat and challenging infection. CAUTI accounts for up to 40% of all nosocomial infections. Biofilm provides a survival strategy to microorganisms and ultimately leads to re-infections and recurrence of urinary tract infections (UTI) despite a full course of antibiotics. **Aims and Objectives:** The present study was conducted to determine the prevalence of CAUTI in suspected UTI patients and prevalence of biofilm-forming uropathogens among CAUTI patients. **Materials and Methods:** The cross-sectional study was done over a period of 6 months among 95 catheterized CAUTI patients. Biofilm production among isolated uropathogens was tested by tissue culture plate, tube test, and congo red agar method. Isolates were identified as biofilm producer if they were tested positive by any one of the all four methods, and isolates were considered biofilm non-producer in consensus with all four methods. **Results:** In this study, the prevalence of CAUTI was 68.42%. Among 65 isolates most common uropathogen was 28 (43.07%) *Escherichia coli*. In this present study, the prevalence of biofilm-forming uropathogens was 58.46% (38). Tissue culture plate was the most sensitive (97.36%) method in detecting biofilm formation followed by modified congo red agar (82.21%), congo red agar (71.05%), and tube test (65.78%). Biofilm productions were significantly associated with female gender, diabetes, and prolonged catheterization. **Conclusion:** Indwelling urinary catheter acts as a nidus for biofilm formation among microorganisms. Duration of catheterization is inversely associated with UTI. Hence, the need for catheter removal should be assessed daily to prevent infection. Periodic surveillance should be done to detect biofilm formation where prolonged catheterization is inevitable.

Key words: Biofilm; CAUTI; Uropathogens

INTRODUCTION

Urinary tract infections (UTI) are a serious health threat and challenging infection affecting all age group patients which is frequently encountered in clinical practice.[1] Catheter associated urinary tract infection (CAUTI) account for up to 40% of all nosocomial infections.[2] Urinary catheters are made up with tubular latex or silicone, when inserted, they rapidly acquire biofilms on both outer and inner surfaces.

There are some organisms that commonly colonize over the device and develop biofilms. The common biofilm-forming organisms are *Staphylococcus epidermidis*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and other gram-negative bacteria.[3] Prolonged catheterization increase the risk of biofilm formation which may lead to UTI. Up to 60% human infections caused due to biofilm formation which is difficult to eradicate with routine antimicrobial therapy.[4]

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Biofilms are architectural colony of microorganisms within a matrix of extracellular polymeric substances.[5] Biofilm provides a survival strategy to the bacteria by preserving nutrients and also show significant resistance towards various antimicrobial agents by restricting the diffusion of substances and binding of antimicrobial.[6] Proximity of cells within a biofilm can facilitate plasmid exchange and transfer of antimicrobial resistant gene from one organism to another and ultimately making the biofilms resistant to most of the antimicrobial agents.[7]

In this scenario, commonly using antibiotics might not have a similar effect toward biofilm-forming bacteria, which ultimately leads to re-infections and recurrence of UTI with further complications despite the full course of antibiotics. To overcome the therapeutic problem, we require some diagnostic methods for the detection of biofilm-forming uropathogens. Despite having many patients with indwelling catheter admitted to hospital, limited study conducted for the detection of biofilm formation among uropathogens in CAUTI patients in this region of West Bengal.

Aims and objectives
The present study was conducted to determine the prevalence of CAUTI among suspected UTI patients and to determine biofilm production among uropathogens in CAUTI patients.

MATERIALS AND METHODS

A cross-sectional observational study was conducted from July to December 2022 among the UTI-suspected indoor catheterized patients admitted in various wards of our institution after taking clearance from Institutional Ethics Committee (REF. NO. F-24/PR/COMJNMH/ICE/22/603). All patients at or above 18 years of age who had indwelling catheter for more than 48 h and had symptomatic UTI (fever >38°C and or urgency, frequency, dysuria) were included in the present study. All non-catheterized patients with symptoms of UTI were excluded from the study. After taking informed consent, a brief clinical history was taken – regarding demography, present complaints, and associated risk factors.

5–10 mL urine samples were collected maintaining aseptic techniques with sterile syringes from distal end of urinary catheter and transferred immediately to a properly labeled sterile container. Then samples were transported immediately to the bacteriology laboratory of the microbiology department and processed within 2 h of collection. All samples were inoculated into Mac Conkey and Hichrome UTI agar media with standard

calibrated loop to determine colony forming unit (CFU). CFU $\geq 10^5$ CFU/mL were considered as significant and further processed.[8] Identification of isolates was done by colony morphology, Gram stain finding, and standard biochemical tests.[9] Hi-chrome candida agar and germ tube test were used for the speciation of all isolated *Candida* spp.[9,10] (Figure 1).

Biofilm production of isolates was tested by tissue culture, Congo red agar, modified Congo red agar, and tube test method.

Tissue culture plate method
In the tissue culture plate method brain heart infusion broth (BHI) with 2% sucrose was placed in tissue culture plates after mixing with isolates. After incubation at 37°C for overnight when turbidity appeared it was diluted with 1 in 100 fresh medium (BHI). Further 0.2 mL diluted medium placed in individual well and incubated again at 37°C for 24 h and then washed 4 times with phosphate buffer saline (PBS) to remove free-floating bacteria. Biofilm formed by adherent organisms fixed with 2% sodium acetate and stained with 0.1% crystal violet for half an hour. The excess stain was rinsed by deionized water and kept for drying. Microassay auto reader with a wavelength of 570 nm was used to determine optical density of adherent stained bacteria[11] (Figure 2).

Tube method
A loopful microorganisms were inoculated in BHI with 2% sucrose and incubated overnight at 37°C. Then tubes were decanted, washed with PBS, dried, and stained with crystal violet 0.1% for 30 min. Excess stains were removed and tube dried. Then reading for biofilm formation was taken by the visible lines at the wall and bottom of the tube[12] (Figure 3).

Congo red agar method
Congo red agar media was prepared by BHI agar along with 5% sucrose and congo red powder. Isolates were inoculated and
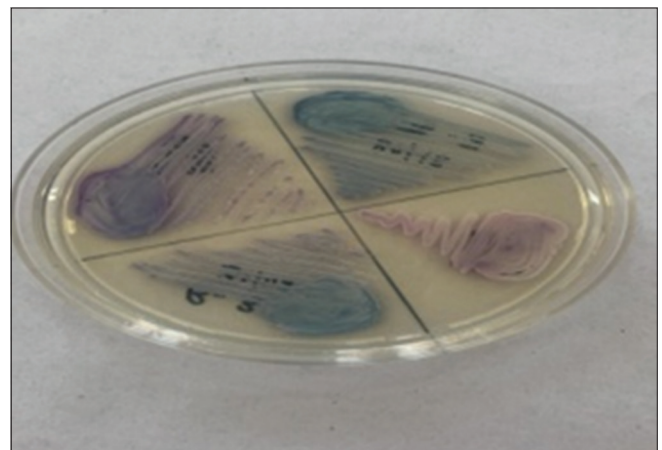

Figure 1: High chrome agar for candida speciation

incubated at 37°C for 24–48 h. Positive results were considered by black colonies with dry crystal consistency (Figure 4).[13]

Modified Congo Red Agar (MCRA) method

MCRA were basically modification of CRA by changing the concentration of Congo red dye and replacement of sucrose by glucose and BHI replaced by nutrient agar.
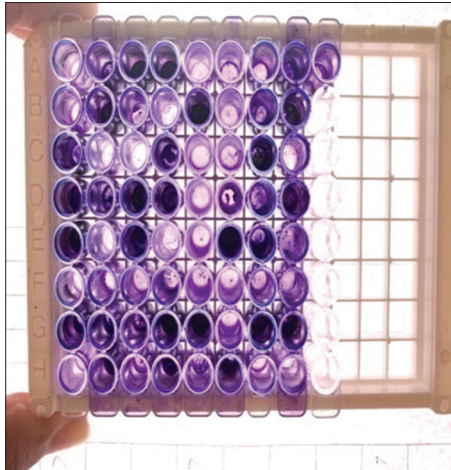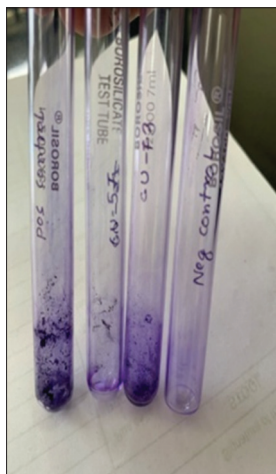

Figure 2: Tissue culture plate method

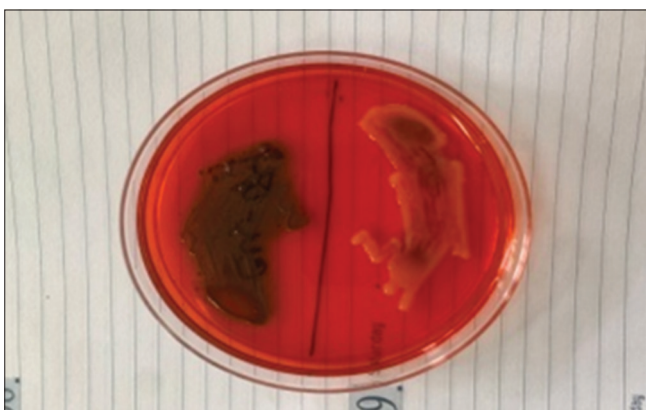**Figure 3:** Tube test method

Figure 4: Congo red agar

MCRA plates were inoculated and incubated at 37° for incubate for 48 h followed by another 2–4 days incubation at room temperature[14] (Figure 5).

Isolates were identified as biofilm producer if they were tested positive by any one of the all four methods and isolates were considered biofilm non producer in consensus with all four methods.

Statistical analysis

Analysis of all the data was performed by SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Qualitative variables were expressed as mean±standard deviation. Chi-square T-test or Fischer's exact test was used to compare two independent groups as qualitative variables in qualitative data. $P<0.05$ is considered statistically significant. Here, we considered the Pearson Chi-square value as equivalent to P-value, because it a test for categorical data.

Specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) data were calculated and analyzed by MedCalc free trial version 18.9.[15] The PPV and NPV were measured by variables data using true positive, true negative, false positive, and false-negative data analyzed by MedCalc.

Ethics

The present study was conducted after taking approval from the Institutional Ethics Committee (REF. NO. F-24/PR/COMJNMH/ICE/22/603). All patients were adequately explained about our research work and then informed consent was taken before including in our study.

RESULTS

In this study, CAUTI were detected among 65 (68.42%) patients out of 95 suspected patients (Table 1). Overall CAUTI cases were higher among female patients (71.69%) compared to male patients (64.28%).
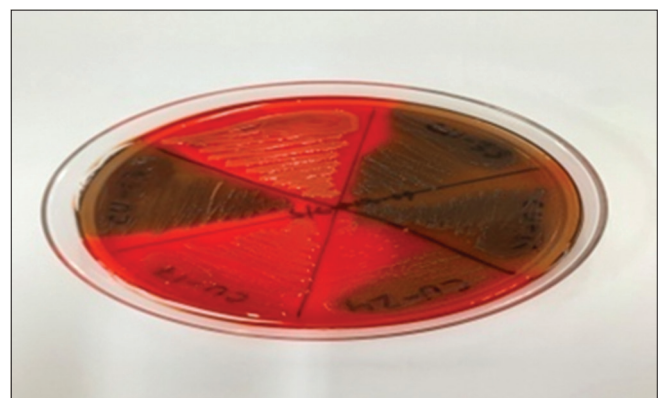

Figure 5: Modified congo red agar method

Table 1: Age- and sex-wise distribution of CAUTI patients

Age groups (years)	Total sample received	Male			Female			Total CAUTI detected (%)
		Sample received	CAUTI detected	CAUTI detected (%)	Sample received	CAUTI detected	CAUTI detected (%)	
18–30	7	3	1	33.33	4	2	50	3 (42.85)
31–45	22	8	4	50	14	9	64.28	13 (59.09)
46–60	36	14	11	78.57	22	18	81.81	29 (80.55)
61–75	18	10	6	60	8	6	75	12 (66.66)
>75	12	7	5	71.42	5	3	60	8 (66.66)
Total	95	42	27	64.28	53	38	71.69	65 (68.42)

CAUTI: Catheter-associated urinary tract infections

In the current study, most common isolates were *E. coli* (43.07%) followed by *Candida spp.* (24.61%), *K. pneumoniae* (13.84%), *Enterococcus spp* (12.30%) (Figure 6).

Out of 16 isolated *Candida* spp. most common species were *Candida albicans* (6), followed by *Candida dubliniensis* (4), *Candia krusei* (3), *Candida parasilopsis* (2), and *Candida tropicalis* (1) according to Hi-chrome Candida agar (Figure 1).

In this present study, the prevalence of biofilm-forming uropathogens was 58.46% (38) (Table 2). Tissue culture (97.368%) was the most sensitive method to detect biofilm production among all tested methods.

Female CAUTI patients were significantly associated with more biofilm production than male patients (Table 3).

The presence of diabetes and prolonged catheterization (≥28 days) were significantly associated with biofilm production in CAUTI patients (Table 4).

DISCUSSION

In the present study, 95 catheterized urine samples were processed and out of which 65 (68.42%) were culture positive. Our result correlated with a study conducted by Sayal et al.,[16] reported 79% culture positivity and Vinoth et al.,[17] reported 70% culture positivity in catheterized sample. Whereas Karkee et al.,[18] and Kulkarni et al.,[19] found lower prevalence of CAUTI at 12.5% and 21.47%, respectively.

In the current study, overall CAUTI cases were higher among female patients (71.69%) compared to male patients (64.28%) (Table 1). A study conducted by Jayasukhbhai et al.,[20] and Almalki and Varghese[21] reported higher incidence (56.46% and 75% respectively) of CAUTI in female patients which are similar to our study. A study
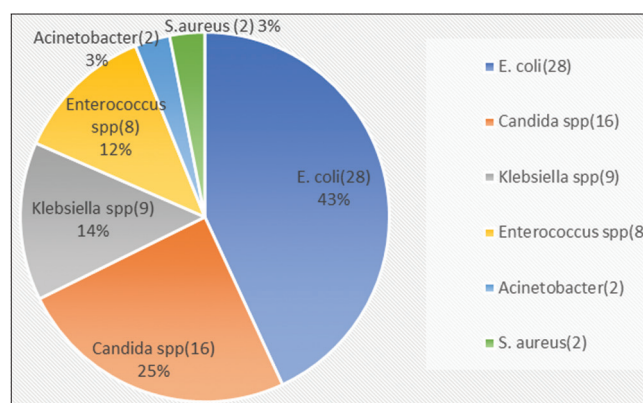
Figure 6: Frequency of distribution of causative agents of CAUTI

conducted by Vinoth et al.,[17] Kulkarni et al.,[19] and Sayal et al.,[16] found a predominance of UTI in male patients, respectively 73.07%, 68.18%, and 74.50% which differs from our study. In the present study, the prevalence of UTI is higher in female may be due to high load of periurethral flora in females which got introduced during catheterization.

In the present study. *E. coli* was the most common isolate followed by *Candida spp.*, *K. pneumoniae* and Enterococcus *spp* (Figure 6) Several other studies also revealed *E. coli* as the commonest pathogen ranging from 22 to 70%.[17,22,23] In contrast to the current study, Ghanwate et al.,[24] isolated 50% *P. aeruginosa* as the commonest agent followed by *Enterococcus spp.* (31%), *E. coli* (25%). *Candida* isolates have been also isolated by various investigators.[17,22] which was the second common isolates in the present study. Variation in the spectrum of pathogen may have happened due to differences in host immunity level, environmental conditions, geographical variance, and previous treatments with antibiotic therapy.

In the present study, 38 (58.46%) isolates were *in vitro* positive for biofilm production among them 7 were candida species (42%) (Table 2) Many studies have shown variable biofilm production ranging from 45 to

Table 2: Screening of CAUTI-positive isolates for biofilm production by four different methods

Method	Biofilm producers			Sensitivity	Specificity	Positive predictive value	Negative predictive value
	Bacteria N1=31	Fungus N2=7	Total N=38 (58.46%)				
Tissue culture method	30	7	37 (56.92)	97.368% (CI 86.190%–99.933%)	100.000% (CI 87.230%–100.000%)	100.000%	96.429% (CI 79.605%–99.467%)
Modified Congo red agar	26	6	32 (49.23)	84.211% (CI 68.747%–93.977%)	100.000%	100.000% (CI 90.5112%–100.000%)	81.818% (CI 68.348%–90.364%)
Congo red agar	22	5	27 (41.53)	71.053% (CI 54.097%–84.575%)	100.000%	100.000% (CI 89.112%–100.000%)	71.053% (CI 59.864%–80.156%)
Tube test	21	4	25 (38.46)	65.789% (CI 48.647%–80.367%)	100.000%	100.000% (CI 87.230%–100.000%)	67.500% (CI 57.199%–76.347%)

*CI=Confidence interval

71%.[16,25,26] Present study correlated well with the study conducted by Chandak et al.[27] Similar studies showed 43.3%,[28] 60%[29] biofilm-producing uropathogens causing CAUTI. In the present study, highest number of biofilm formation was detected by TCP method (n=37, 56.92%). It had missed just one biofilm producer which was detected by MCRA (49.23%) method. Tube method showed 38.46% biofilm-producing isolates in the present study which is similar to the study conducted by Mohamed and Shalakany[25] and Niveditha et al.[23] Whereas biofilm formation by CRA (41.53%) method in the present study was quite different from the study of Sayal et al.,[16] and Hassan et al.[26] (Table 5).

In the present study highest sensitivity was shown by TCP method 97.37% followed by MCRA 84.21%, CRA 71.05% tube test 65.79% (Table 2). In various studies, TCP method is considered as gold standard technique for the detection of biofilm.[30-32] In the current study although TCP is the most sensitive method, it is the most expensive, and moreover, individual isolates could not be tested by this method. In the TCP method, all isolates were tested in 96 well tissue culture plates together. Whereas MCRA are relatively easy, inexpensive method to perform a single test. Hence, in resource-poor settings MCRA can be used as an alternative method to TCP.

Female CAUTI patients were more prone to biofilm-forming uropathogens than their male counterpart in the current study (Table 3). It may be due to a higher load of periurethral flora in females which adhere and form biofilm in the presence of indwelling catheter.

In the present study statistically, significant association were found between biofilm production among uropathogens with the patient having diabetes mellitus and prolonged catheterization for ≥28 days (Table 4) Our study is comparable with the study conducted by Sayal et al.,[16] and Kulkarni et al.,[19] long term diabetes is commonly associated with altered immunological function and asymptomatic bacteriuria which may facilitate biofilm formation. Prolonged catheterization provides ideal environment for biofilm formation and thus survival benefits for pathogens which may lead to treatment failure. Detection of CAUTI may be due to inadequate precaution taken during the insertion of catheter. Preventive strategies for urinary catheter-associated infections are very limited. It can be prevented using sterile closed collection system, maintenance of aseptic technique during catheter insertion, and maintenance of hand hygiene to reduce cross-infection.[33]

Table 3: Gender-wise distribution of biofilm producer and nonproducer uropathogens

Gender	Biofilm producer	Nonbiofilm producer	Total (%)	
Male	8	19	27 (41.5)	P=0.000104 (Fisher's exact test)
Female	30	8	38 (58.5)	
Total	38 (58.5%)	27 (41.5%)	65	

Table 4: Distribution of risk factors among biofilm producer and nonbiofilm producers in CAUTI Patients

Serial no	Risk factors	Total number of patients	Biofilm producer	Non Biofilm producer	P-value
1	Diabetes	36	26	10	P=0.021895 S
2	Hypertension	32	21	11	P=0.316698 NS
3	Renal failure	14	8	6	P=1.000 NS
4	Prolonged catheterization (≥28 days)	11	10	1	P=0.021895 S

S=Significant, NS=Non-significant, CAUTI: Catheter-associated urinary tract infections

Table 5: Biofilm formation observed by TCP, TM, and CRA in other studies

Year	Study	Biofilm detection method		
		TCP (%)	TM (%)	CRA (%)
2022	Present study	56.92	38.46	41.53
2015	Mohamed and Shalakany[25]	45.6	38.11	36.9
2014	Sayal et al. [16]	71.23	56.33	9.17
2012	Niveditha et al. [23]	-	44	56
2011	Hassan et al. [26]	63.63	54	11

Limitations of the study

Molecular methods of biofilm detection couldn't be used due to unavailability of resources.

CONCLUSION

Urinary catheterization is a common procedure done for therapeutic, diagnostic AND perioperative purposes in most hospitals. Indwelling urinary catheter acts as a nidus for biofilm formation among microorganisms. Biofilm provides survival advantages to the microorganism and thus give rise to persistent and recurrent UTT in catheterized patients. The duration of catheterization is inversely associated with UTI. Hence, need for catheter removal should be assessed daily to prevent infection. Periodic surveillance should be done to detect biofilm formation where prolonged catheterizartion is inevitable. Female gender and Diabetes are important risk factors associated with biofilm formation in CAUTI patients. Every health care facilities must follow a standard care bundle approach during the insertion and maintenance of urinary catheter for the prevention of CAUTI.

ACKNOWLEDGMENT

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