

Original research article

## Biofilm production and antimicrobial resistance in catheter associated urinary tract infection (CAUTI) pathogens isolated from ICU patients

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### Abstract

**Background & Method:** Biofilm production is considered to be a major virulence factor associated with health care associated infections particularly with Catheter associated urinary tract infection (CAUTI) pathogens. This study is done to find out prevalence of antimicrobial resistance and biofilm production capacity in CAUTI pathogens in patients admitted to various ICUs of Shree Krishna Hospital, Karamsad, and diagnosed to be suffering with CAUTI based on NHSN definitions were included in study and subjected for antimicrobial susceptibility testing and in-vitro biofilm production test using micro-titre plate method. On the basis of same criteria 55 patients were included in this study. The clinical history of the patients and other details taken for various patient variable factors like age, gender, co-morbid conditions, indoor days, device days, final patient outcome and other lab based investigations done as indicator of active infection or sepsis from the electronic hospital database available on hospital information system, to determine the incidence of antimicrobial resistance, biofilm forming capacity, to determine risk factors associated and final patient outcome with biofilm formation in CAUTI pathogens isolated from ICU patients. Chi-square test was used to check the relation between the categorical variables while t test was applied in case of continuous variables. A p value less than 0.05 was considered as statistically significant.

**Result:** Among total 61 isolates recovered from 55 patients, 52.4% were biofilm producer. Most common isolates were *pseudomonas aeruginosa* (22.95%) followed by *Enterococcus faecium* (13.11%). *Candida tropicalis* and *Klebsiella pneumoniae* were seen among 11.47% each. Among gram negative organisms, most common resistant drug was ciprofloxacin (95.12%), followed by cefipime (87.80%), meropenam, piperacillin-tazobactem (85.37%). Deaths were seen more in biofilm negative patients (14.29%) as compared to biofilm positive (7.41%).

**Conclusion:** It is evident that the CAUTI remains major indwelling device associated infection in ICU patients. The biofilm production is associated with 32 (52.4%) out of total 61 isolates of CAUTI in present study.

**Keywords:** Biofilm production; Drugs resistance; Catheter associated UTI; ICU patients; Hospital-acquired infections, Indwelling device associated infection.

## Introduction

One of the reasons for the rise in morbidity and mortality among patients in healthcare setup is the Hospital-acquired infections (HAIs). Patients admitted under critical care units are vulnerable individuals with decreased immunity due to two reasons: one due to damage of protective anatomic shielding due to various invasive procedures such as intubation and mechanical ventilation, urinary and intravascular catheters, etc. and second due to the administration of drugs which may predispose to certain infections.<sup>1</sup> ICU is considered as an epicentre of the infections<sup>2</sup>. Among all indwelling devices used in ICU, usage of urinary catheters is six times higher compared to other devices; a reason why urinary tract infection is one of the major culprits in hospital acquired infections.<sup>3</sup> Around thirty six percent of all HAIs are urinary tract infections and 80% of these are due to indwelling catheters.<sup>3,4</sup> Catheter related urinary tract infection (CAUTI) is responsible for the increased morbidity, hospital cost and length of stay however mortality due to CAUTI is less than 5%. According to research, around 10-20% of hospitalized patients are catheterized. For bacteria, urinary catheters are comfortable route of admission following which they cause urinary tract infection and possible gram negative bacteraemia in hospitalized patients.<sup>4,5,6</sup> Biofilms are the sessile polymicrobial communities that adhere to biotic and abiotic surfaces and are encased within a self-produced extracellular polymeric matrix. Biofilms permits seepage of pathogen from the host defences.<sup>7</sup> It also boosts antimicrobial resistance due to gradual penetration, resistant phenotype, and altered microenvironment. After attaching to the uroepithelium, biofilms can penetrate the renal tissue which may lead to the complications like pyelonephritis and prostatitis.<sup>7,8</sup> Previous researches reveals positive correlation between the duration of catheterization and biofilm formation.<sup>4,9</sup> The frequent organisms that colonize indwelling urinary catheters and create biofilm are *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and other gram-negative organisms.<sup>10</sup>

The objective of our study was to determine the incidence of antimicrobial resistance, biofilm production capacity in CAUTI pathogens and to determine risk factors associated with biofilm formation in CAUTI including final patient outcome.

## Materials and Methods:

### *Study type, study setting and study period:*

It was an observational study conducted in the microbiology department of a tertiary care teaching institution. Isolates of those patients declared as CAUTI between March 2018 and December 2019 were collected. Study was duly approved by Institutional Ethics Committee of Shree Krishna Hospital (Ref no. IEC/HMPCMCE/2015/337/15).

### *Inclusion criteria:*

The isolates from catheterised urine identified as pathogens of CAUTI from cases admitted in various ICUs of Shree Krishna Hospital were included in the present study.

**Exclusion criteria:**

Patients in whom the catheterised urine grew an isolate, that had been previously isolated from the same specimen and that was suggestive of persistent infection were excluded from the present study.

**Sample size, Sampling Techniques and data collection:**

Purposive sampling of all patients were included who fulfil the inclusion criteria and fall in the study duration. All clinical isolates recovered from patients of CAUTI were obtained from diagnostic microbiology lab were preserved for further testing of in-vitro biofilm production assay. These isolates were processed for antimicrobial susceptibility testing and in-vitro biofilm production assay. Further details of the patients collected included like co-morbid conditions, inpatient-days, ventilator-days and total WBC count, were collected from the electronic hospital information system with due permission.

**Protocol for Biofilm production assay using microtiter plate method:**

All isolates were screened for their ability to form biofilm by microtiter plate (Flat bottom 96 well sterile plates, Tarson) method. Overnight cultures of study isolate in trypticase soya broth (TSB, Himedia) were taken and the turbidity was adjusted to 0.5 McFarland standards using fresh TSB. From this suspension, aliquots of 100µl were dispensed in 96 well microtiter plate containing 100µl of TSB, negative control wells were inoculated with plain TSB. Each isolate and negative control was tested in triplicate. The plates were incubated for 48 hours at 37°C. After incubation, the content of each tube was aspirated and then washed three times with phosphate buffered saline (PBS pH 6.8) to remove any non – adherent bacteria. 200µl of 99% ethanol was added to each well and kept for 15 minutes to fix biofilm. The wells were decanted, left to dry, and stained with 200µl of 0.1% (w/v) crystal violet for another 15 min. Excess stain was rinsed off gently by distilled water or tap water. The plates were air dried. The Optical Density was measured at 570 nm using spectrophotometer (Tulip Lisaquant ELISA reader). Based on the average optical density of three wells, the tested isolates were classified based on ratio of the OD. Ratio = Test isolate OD / negative control OD is calculated and they are classified as, Biofilm non producer (BFNP) having ratio <2 and Biofilm producer (BFP) having ratio  $\geq$  2. For internal quality control, Biofilm-producing reference strain of *Pseudomonas aeruginosa* (ATCC 27853) and non-biofilm forming reference strains of *Staphylococcus aureus* (ATCC 25923) were used.<sup>11,12</sup>

**Statistical analysis:**

Data were entered and analysed with Epi info 7 CDC. Categorical data were expressed in percentages while continuous data were expressed with mean and standard deviation. Chi-square test was used to check the relation between the categorical variables while t test was applied in case of continuous variables. A p value less than 0.05 was considered as statistically significant.

**Results**

In the present study, a total of 55 patients with CAUTIs were diagnosed from various intensive care units, out of them total of 61 isolates were recovered. Out of these 55 total patients, 27 (49.1%) patients were having BFP while 28 (51.9%) were having BFNP isolates. In the present study, 18 (32.73%) of the patients were between age of 18 to 45 years. Among them 37.04% were BFP while 17 (30.91%) of the study participants were more than 60 years of age and among them 40.74% were BFP. Mean age among participants were shown in table 1. Out of total 55 CAUTI patients, 30 (54.54%) were male and 25 (45.46%) were females.

Among males BFP were 59.26% while BFP proportion among female was 40.74% (Table 1). Dysuria was most common symptoms followed by fever among study participants. As shown in table 1, in present study 48.15% participants who were hypertensive were also BFP as compared to 35.71% among BFNP. Diabetics were higher among BFP patients (29.63% as compared to 14.29%). Deaths were seen more in BFNP (14.29%) as compared to BFP (7.41%) patients. Among 55 patients 61 organisms were isolated as shown in table 2. Most common isolates were *Pseudomonas aeruginosa* (22.95%) followed by *Enterococcus faecium* (13.11%). *Candida tropicalis* and *Klebsiella pneumoniae* were seen among 11.47% each. Table 3 shows, antimicrobial drug resistant pattern to different organisms. Among gram negative organisms, most common resistant drug was Ciprofloxacin (95.12%), followed by Cefipime (87.80%), Meropenam, Piperacillin-tazobactem (85.37%). Among gram positive organism, drug resistant was 100% against Ciprofloxacin and 90% against Ampicillin, Penicillin G, Tetracyclin, Gentamycin and Streptomycin while in case of yeast 100% resistant was observed against Amphotericin B and Voriconazole. (Table 4)

**Table 1: Characteristics of study participants (n=55)**

Characteristics	Biofilm Non-producer (BFNP) (%) (n=28)	Biofilm Producer (BFP) (%) (n=27)	p-value
Mean urinary catheter days	25.21 ± 19.05	31.03 ± 39.65	0.49
Mean Total WBC count (cumm) on day of reporting CAUTI	16357.14 ± 8384.13	12344.44 ± 5646.60	0.04
Mean Length of hospital stay (days)	35.57 ± 22.84	36.44 ± 36.66	0.91
Mean days to CAUTI after urinary catheter insertion	15.14 ± 10.21	19.81 ± 28.62	0.42
<b>Age groups in years</b>			
1-10	0 (0.0%)	1 (3.7%)	0.36
11-20	1 (3.6%)	1 (3.7%)	
21-30	1 (3.6%)	2 (7.4%)	
31-40	7 (25%)	6 (22.2%)	
41-50	5 (17.9%)	2 (7.4%)	
51-60	8 (28.6%)	3 (11.1%)	
61-70	2 (7.1%)	7 (25.9%)	
>70	4 (14.3%)	4 (14.8%)	
<b>Sex</b>			
Female	14 (50%)	11 (40.74%)	0.49
Male	14 (50%)	16 (59.26%)	
<b>Location of admission</b>			
MICU	15 (53.57%)	15 (55.55%)	0.77
PICU	1 (3.57%)	2 (7.4%)	
SICU	12 (42.85%)	10 (37.03%)	
NICU	0 (0.00%)	0 (0.00%)	
<b>Comorbidities</b>			
Hypertension	10 (35.71%)	13 (48.15%)	0.25
Diabetes mellitus	04 (14.29%)	08 (29.63%)	0.16
CKD	01 (3.57%)	01 (3.7%)	0.97
BPH	01 (3.57%)	02 (7.41%)	0.57

Patient's outcome			
DAMA	9 (32.14%)	9 (33.33%)	0.61
Death	4 (14.29%)	2 (7.41%)	
Discharge	14 (50%)	16 (59.26%)	
Transfer	1 (3.57%)	0 (0%)	

Table 2: Isolates among study participants (n=61)

Isolates	Biofilm Non-producer (BFNP) (n=29)	%	Biofilm Producer (BFP) (n=32)	%
Acinetobacter baumannii	0	0.00	1	100.00
Pseudomonas aeruginosa	6	42.86	8	57.14
Pseudomonas putida	0	0.00	1	100.00
Burkholderia Cepacia	1	100.00	0	0.00
Myroides sp.	0	0.00	1	100.00
Klebsiella pneumoniae	4	57.14	3	42.86
Escherichia coli	3	50.00	3	50.00
Providencia rettgeri	1	25.00	3	75.00
Enterobacter cloacae	0	0.00	3	100.00
Enterobacter aerogenes	0	0.00	1	100.00
Proteus mirabilis	0	0.00	2	100.00
Enterococcus faecalis	2	100.00	0	0.00
Enterococcus faecium	6	75.00	2	25.00
Candida lusitanae	0	0.00	1	100.00
Candida albicans	1	50.00	1	50.00
Candida tropicalis	5	71.43	2	28.57

Table 4: Resistant pattern of Gram positive organisms (BFP=Biofilm Producer, BFNP=Biofilm Non-producer, % R=% Resistance)

Organisms	Biofilm status	Tigecycline	Ampicillin	Ciprofloxacin	Levofloxacin	Nitrofurantoin	Penicillin G	Teicoplanin	Vancomycin	Linezoild	Daptomycin	Tetracycline	High Level Gentamicin	High level Streptomycin
Enterococcus faecalis (BFP)	% R (n=2)	0	50	10 0	10 0	0	50	0	0	0	0	10 0	50	50
Enterococcus faecium (BFP)	% R (n=2)	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterococcus faecium (BFNP)	% R (n=6)	16. 67	10 0	10 0	10 0	83. 3	10 0	10 0	10 0	16.6 7	83. 3	83. 3	10 0	10 0

**Table 5: Resistant pattern of Gram negative organisms (BFP=Biofilm Producer, BFNP=Biofilm Non-producer, % R=% Resistance)**

Organisms	Biofilm status	Colistin	Tigecycline	Imipenem	Meropenem	Ertapenem	Doripenem	Amikacin	Gentamicin	Amoxicillin Clavulanic acid	Ampicillin	Cefotaxime	Ceftazidime	Ceftriaxone	Cefuroxime	Cefepime	Cefoperazone-Sulbactam	Piperacillin-Tazobactam	Ciprofloxacin	Levofloxacin	Nitrofurantoin	Co-trimoxazole
Acineto. baumannii (BFP)	% R (n=1)	0	100	100	100	-	100	100	100	-	-	-	100	100	-	100	100	100	100	100	-	100
Ps. Aeruginosa (BFP)	% R (n=8)	0	-	100	100	-	87.5	100	100	-	-	-	87.5	-	-	100	100	100	100	100	-	-
Ps. Aeruginosa (BFNP)	% R (n=6)	16.7	-	66.7	83.3	-	83.3	66.7	66.7	-	-	-	83.3	-	-	66.7	66.7	83.3	83.3	66.7	-	-
Ps. Putida (BFP)	% R (n=1)	100	-	100	100	-	100	100	100	-	-	-	100	-	-	100	100	100	100	100	-	-
Burkh. Cepaciae (BFNP)	% R (n=1)	-	-	-	0	-	-	-	-	-	-	-	0	-	-	-	-	-	-	0	-	0
Myroides sp. (BFP)	% R (n=1)	100	-	100	100	-	100	100	100	-	-	-	100	-	-	100	100	100	100	100	-	100
Enterobacter aerogenes (BFP)	% R (n=1)	0	0	100	100	100	-	100	100	100	100	-	100	100	100	100	100	100	100	-	100	100
Enterobacter cloacae (BFP)	% R (n=3)	0	0	100	100	100	-	100	100	100	100	-	100	100	100	100	100	100	100	-	100	100
Esch. Coli (BFP)	% R (n=3)	0	0	0	0	0	-	0	0	33.3	100	100	-	100	100	66.7	33.3	33.3	100	-	0	33.3
Esch. Coli (BFNP)	% R (n=3)	0	0	100	100	100	-	66.7	66.7	100	100	100	-	100	100	100	100	100	100	-	33.3	100
Kleb. Pneumoniae (BFP)	% R (n=3)	0	33.3	100	100	100	-	66.7	66.7	100	100	100	-	100	100	100	100	100	100	-	100	100
Kleb. Pneumoniae (BFNP)	% R (n=4)	0	25	75	75	75	-	75	75	75	100	100	-	100	100	75	75	75	100	-	75	75
Proteus mirabilis (BFP)	% R (n=2)	50	50	100	100	100	-	50	50	50	100	100	-	100	100	100	50	50	100	-	100	100
Providencia rettgeri (BFP)	% R (n=3)	100	100	100	100	100	-	100	100	100	100	100	-	100	100	100	100	100	100	-	100	100
Providencia rettgeri (BFNP)	% R (n=1)	100	100	100	100	100	-	100	100	100	100	100	-	100	100	100	100	100	100	-	100	100

**Table 6: Resistant pattern of yeast (BFP=Biofilm Producer, BFNP=Biofilm Non-producer, % R=% Resistance)**

Organisms	Bio film stats	Amphotericin-B	Fluconazole	Flucytosine	Voriconazole	Caspeofungin	Micafungin
Candida albicans (BFP)	% R (n=1)	0	0	0	0	0	0
Candida albicans (BFNP)	% R (n=1)	0	0	0	100	0	0
Candida lusitanae (BFP)	% R (n=1)	0	0	0	0	0	0
Candida tropicalis (BFP)	% R (n=2)	0	0	0	0	0	0
Candida tropicalis (BFNP)	% R (n=5)	0	0	0	0	0	0

**Discussion:**

UTI is one of the most critical HAIs attributed to indwelling urinary catheters. Since biofilms eventually grow on these devices, significant variables that affect biofilms' formation need to be studied<sup>4</sup>. Out of 55 catheter associated urinary tract infections detected in the present study, 27 (49.1%) were biofilm positive whereas 28 (51.9%) were biofilm negative. Biofilm

production in catheter associated UTIs have ranged from 23% to 73% in previous studies.<sup>11-14</sup>

In the present study, 30.91% of the study participants were more than 60 years of age, and among them, 40.74% were Biofilm positive. Advancing age is one of the predisposing factors for the development of CAUTI. In a study done by Sangamithra V *et al.*<sup>15</sup>, 49% of CAUTI patients were > 60 years of age which is higher compared to the present study. Although the incidence of CAUTI may have a relationship with age, the ability to produce biofilm was not influenced with age as seen in the present study as the mean age among Biofilm positive and negative participants was not statistically significant ( $p = 0.67$ ).

Out of 55 CAUTIs in the present study, 30 (54.54%) were male, and 25 (45.46%) were females. Women are at increased due to easy access of the perineal flora to the bladder along the outside of the catheter as it passes the shorter female urethra. Besides, the urethra of a woman is closer to the anus, making it possible for bacteria to spread to the urethra and cause infection.<sup>16</sup> However, gender did not influence biofilm production as biofilm production in CAUTI in males was not statistically significant ( $p = 0.5$ ) when compared to CAUTI in females in the present study.

Prolonged catheterization is one of the critical risk factors for development of CAUTI in previous studies<sup>12,13,15</sup>. In the present study, the mean duration for development of CAUTI from the day of insertion of urinary catheter, was 17 days. The mean duration for development of CAUTI was not statistically significant ( $p = 0.4$ ) between CAUTIs with biofilm producers and biofilm non producers. The mean duration of catheterization was higher in CAUTIs with biofilm producers compared to biofilm non producers; however, this difference was not statistically significant (0.4) in the present study. We follow a protocol of removal or change of urinary catheter following a CAUTI. This will ensure that catheters with biofilm producers as a source of infection, is removed, leading to early recovery of the infection as well as reduction in the duration of catheterization. CAUTI and biofilm formation can often be avoided in patients who have been catheterized for < 2 weeks using a sterile closed collection system by paying attention to aseptic procedures during insertion and application of catheters care bundles to eliminate cross-infections<sup>4</sup>.

In the present study, 41.82% patients, who developed CAUTI, were hypertensive. Bhayani P *et al.*<sup>20</sup> and Kim *et al.*<sup>21</sup> had 32% of the hypertensive patients among CAUTI in their studies. However, the relationship between hypertension and biofilms in the present study is not statistically significant ( $p$  value = 0.4).

Diabetes mellitus was seen among 21.82% of the study participants. Although it was observed that patients with diabetes were higher among BFP (29.63% as compared to 14.29%), the difference was not statistically significant ( $p = 0.2$ ). Mangaiyarkarasi *et al.* (2014) observed that 66% of isolates in CAUTI from diabetic patients were biofilm producers. In a study done by Sayal P *et al.*<sup>22</sup>, biofilm formation was observed among 74.07% isolates, which is higher than the present study. As per literature, diabetes is characterized by differing degrees of insulin resistance, compromised insulin release and improved production of glucose. Patients with diabetes mellitus are at greater risk of infection, with the urinary tract being the most likely infection source<sup>23</sup>.

Effort was made to observe relationship between mortality and biofilm production in pathogen. Mortality among patients with CAUTI was 10.71% in the present study. Death was seen more in patients with BFNP compared to BFP but the difference was not statistically significant ( $p$  value = 0.6). Bhayani P *et al.*<sup>20</sup> and Danchaivijitr *et al.*<sup>24</sup> reported a mortality rate of 14.28% and 14.9%, respectively among patients with CAUTI.

In our study *Pseudomonas aeruginosa* (22.95%), *Enterococcus faecium* (13.11%), *Candida tropicalis* (11.48%), *Klebsiella pneumoniae* (11.48%) and *E.coli* (9.84%) were frequent organism to be isolated in cases of CAUTI. Similar organisms were isolated as BFP among patients with CAUTI in previous study.<sup>11</sup> Various studies have reported different organisms capable of producing biofilms in CAUTI. The study done by Alves *et al.*<sup>25</sup> reported *Acinetobacter* and *Citrobacter freundii* were the highest biofilm-forming isolates. In a study, Niveditha *et al.*<sup>13</sup>. and Pramodhini *et al.*<sup>26</sup> reported 70% of *Escherichia coli* were isolated; in contrast, Ahmed Abdallah *et al.* said 31% *Escherichia coli* in their study.

### Conclusion:

The main objective of this research work was to determine the incidence of BFP CAUTI and to determine risk factors associated with biofilm formation in CAUTI and antimicrobial resistance in CAUTI pathogens isolated from ICU patients. Out of total 55 CAUTIs 30 patients (54.54%) were male and 25 (45.46%) were females. Dysuria was most common symptoms followed by fever among study participants. Deaths were seen more in BFNP patients (14.29%) as compared to BFP (7.41%). Among 55 patients 61 organisms were isolated. Most common isolates were *Pseudomonas aeruginosa* (22.95%) followed by *Enterococcus faecium* (13.11%). *Candida tropicalis* and *Klebsiella pneumoniae* were seen among 11.47% each. Among gram negative organisms, most common resistant drug was Ciprofloxacin (95.12%), followed by Cefipime (87.80%), Meropenam, Imipenem, Piperacillin-tazobactem (85.37%). Among gram positive organism, drug resistant was 100% against Ciprofloxacin and 90% against Ampicillin, Penicillin G, Tetracyclin, high level Gentamycin and high level Streptomycin. Drug resistant pattern of yeasts were studied to both the BFP and BFNP, from that it can be concluded that only Voriconazole was shown 100% resistant against *Candida albicans* (BFNP) but others all drugs like Amphotericin-B, Fluconazole, Flucytosine, Caspofungin and Micafungin having 0% resistant. So from this study in can be concluded that to prevent the CAUTI, patients with risk factors should be monitored by urine culture to detect in advance the risk of biofilm development and the constructive conclusion of biofilm-producing pathogens in the urinary catheter could be a pointer of biofilm formation. Some researchers also reported that Biofilm formation and multi-drug resistance is significantly higher in CA-UTI than community based UTI. Schedule monitoring of antimicrobial resistance and biofilm formation is needed in all cases of UTI to improve the proper management of patients. As we know that Gram-positive bacteria are a major cause of both CA-UTI and Community acquired UTI with *Enterococcus faecalis* and *Staphylococcus aureus* as common pathogen. Since the management of CAUTI with biofilm-forming bacteria is different and difficult, hence routine surveillance of antimicrobial resistance and preferably biofilm formation capacity of isolates at some interval in one hospital is advocated to make certain the most favourable management of patients associated with CAUTI.

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