Original Article

Biofilm Production by Uropathogens Causing Catheter Associated Urinary Tract Infection among ICU Patients

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Abstract

Background: Biofilm is slimy layer of an extracellular matrix made of polymeric substances, colony providing resistance not only against antibiotics but also against the human immune system. **AIM:** Biofilm production by uropathogens causing catheter associated urinary tract infection among ICU patients. **Objectives:** To identify & isolates the pathogen from urinary samples, determine Antibiotic Susceptibility Test of the isolated pathogens & detect the biofilm production of isolated pathogens.

Material and Methods: Uropathogens isolates from clinical samples received in the department of microbiology over a period of 1 year were included in the study. Isolates were identified and species determined by standard methods. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion test and ***Biofilm*** detected by Congo red agar method.

Result: A total of 233 isolates were used to check biofilm formation out of which 104(46.63%) showed strong biofilm formation and 98 isolates were negative biofilm producers by Congo red test. The majority of strains that formed strong biofilms were *Escherichia coli*(49) and *Enterococcus spp.* (19). On the other hand, 12 *Escherichia coli* strains showed weak slime formation with 2 *Staphylococci* strains. Furthermore, out of all samples, 15(6.43%) were indeterminate for any biofilm formation. Out of those 233 isolated strains, the pattern of antibiotic resistance indicated that the greatest proportion of isolates were resistant to NX (norfloxacin) (97%), AMP Ampicillin (90%), and followed by GEN Gentamycin (69%).

Conclusion: In conclusion, the prevalence of biofilm-dependent CAUTI was high, with *E. coli* represented the highest biofilm producer. Therefore, minimizing the duration of catheterization as possible and the usage of silicone catheter instead of latex are recommended. Using carbapenems in treatment of biofilm-dependent CAUTI should be considered.

Keywords: Biofilm; Intensive Care Unit; Congo Red Agar; Urinary Tract Infection.

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Introduction

Biofilm were structured as slimy layers of an extracellular matrix composed of polymeric substances that form colonies, offering resistance not only to antibiotics but also to the human immune system. [1] This structural complexity enables biofilms to protect bacteria from antimicrobial agents, leading to persistent and challenging-to-treat chronic infections, as well as facilitating the spread of antibiotic resistance. The establishment and spread of antibiotic resistance are greatly aided by biofilms, which are essential for the transmission of resistance genes between bacterial species. To break up biofilms and improve the efficacy of antibiotic treatments, it was crucial to comprehend the mechanisms underlying biofilm resistance. [2] [3]

Catheter-associated urinary tract infections (CAUTI) account for 80% of nosocomial urinary tract infections (UTIs) and up to 40% of all nosocomial infections. The organisms commonly contaminating these devices were *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus epidermidis, and Enterococcus faecalis*. These organisms have a higher propensity to cause urinary tract infections the longer the urine catheter is left in place. [4]

Biofilm was present in implanted foreign bodies, prostate stones, and the urothelium. Invasion of the renal tissue by bacteria adhering to the uroepithelium and creating biofilm can result in pyelonephritis and potentially chronic bacterial prostatitis. [5]

The prevalence of hospital-associated UTIs, which make up more than 40% of nosocomial infections, is particularly high among ICU patients. With 25% of hospitalized patients needing catheterization during treatment, urinary catheters are a popular equipment used in healthcare settings, making UTIs especially problematic in these patients. [6]

One important aspect of the pathophysiology of catheter-associated UTIs was the development of biofilm by uropathogens, which can result in antibiotic resistance and difficulties with treatment. Research has shown that uropathogens like Escherichia coli (E. coli) developing biofilms were the reason for the high rates of recurrence and antibiotic resistance in UTIs.[7]

Material and Methods

A cross-sectional study was conducted in the Microbiology Department at Teerthanker Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh. The study was conducted for 1year after the approval from college CRC and IEC.

Inclusion Criteria: Only adults above 18 age group, indwelling urinary catheters for at least 2 days, and had UTI symptoms among ICU patients.

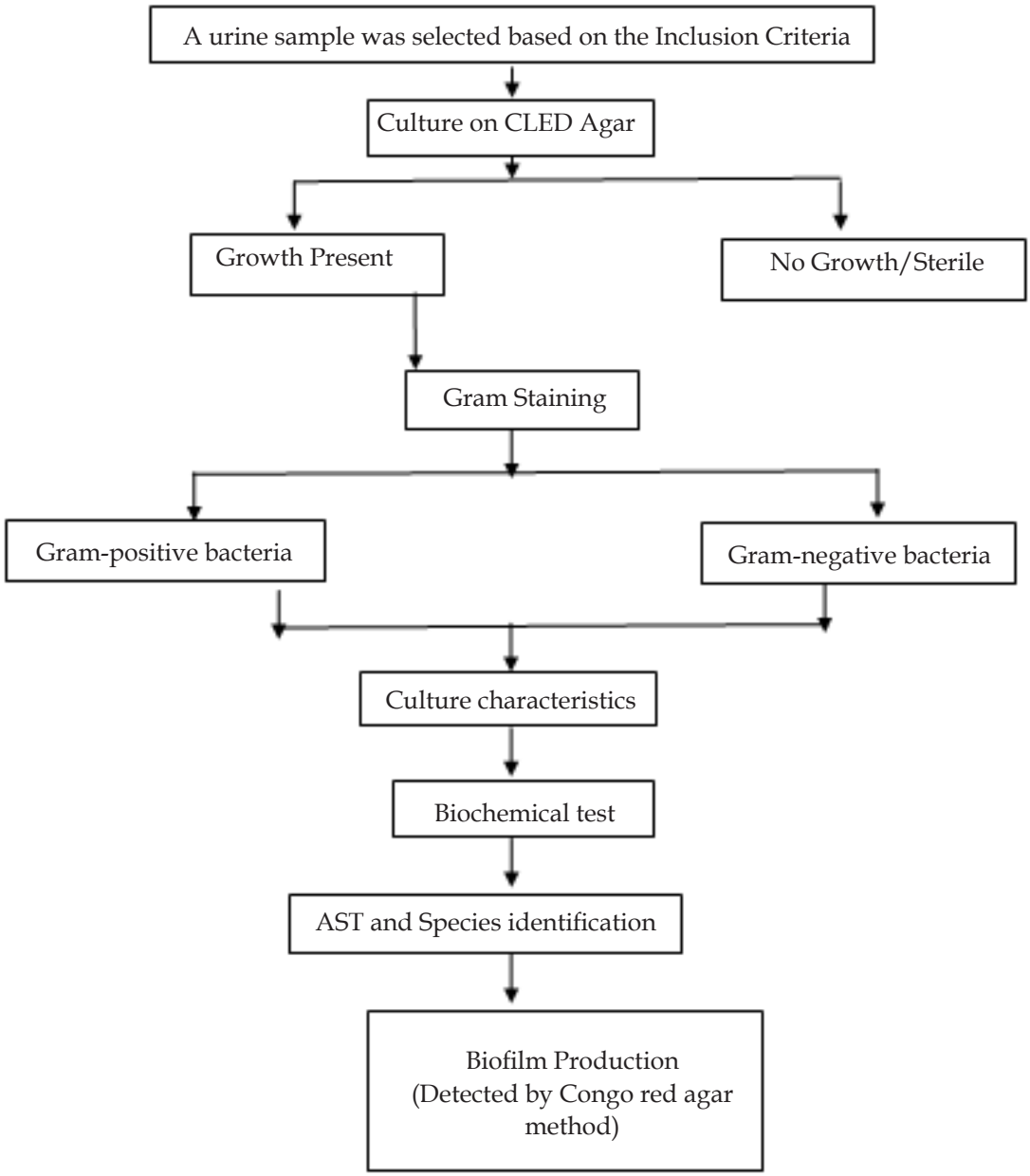
Exclusion Criteria: Patients denying consent, Individuals who had UTIs before getting catheters.

Sample Size Calculation

The required sample size (N) was determined using the formula $N=Z_{\alpha/2}P(100-P)\ /E^2$, Where$Z_{\alpha/2}$: Standard normal rate (1.96 for 95%), P: Prevalence rate (78%), E: Absolute error (5%), N: Minimum sample size. The calculated minimum sample size was 263.58, rounded up to 264. However, the authors included 264 isolates as we received more isolates during the study period.

Study Procedure

The urine sample was first collected based on the inclusion criteria, The sample was processed on CLED agar, after 24-48 hours, growth was observed and then gram staining was carried out, Culture characteristics were done for gram-positive Bacteria (catalase and coagulase test), and gram-negative bacteria (biochemical test), AST was done based on CLSI guideline 2023 for species identification and the Congo Red Agar method was carried out for biofilm production. [8] [9] [10]



Catalase positive, Coagulase positive=*Staphylococcus aureus*

Catalase positive, Coagulase negative = Coagulase-negative *Staphylococcus aureus* (*CONS*)

Catalase negative = Bile esculin *Enterococcus*

Results

During the study period, outof a total of 264 different clinical samples, 111 (42.06%) samples were from males, and 153 (57.94%) samples were from females. The most commonly affected age group was 51- 60 years, with 58 cases (21.96%), followed by the 31-40 years age group with 47 cases (17.80%), as illustrated in [Table/Fig-1].

Table 1: Distribution of different genders in the study population according to different age groups and sex wise.

AGE GROUP	MALE	FEMALE	TOTAL
18-20	7	9	16
21-30	13	29	42

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31-40	19	28	47
41-50	21	22	43
51-60	26	32	58
61-70	11	20	31
>71	14	13	27
TOTAL (264)	111	153	264

Out of 264 samples taken, 233 (88.25) were growth-positive isolates whereas 31 (11.75) were sterile. 93 (39.91) isolates were obtained from males and 140 (60.09) isolates were obtained from females respectively. The data was analyzed using statistical application software IBM SPSS version 20.

Table 2: Distribution of bacterial growth according to gender of patients.

BACTERIAL GROWTH	MALE No. (%)	FEMALE No. (%)	TOTAL
GROWTH	93 (39.91)	140 (60.09)	233
STERILE	18 (58.06)	13 (41.94)	31
TOTAL	111 (42.04)	153 (57.96)	264

Out of 264 clinical samples processed, bacterial growth was detected in 233 samples only. Among all bacterial isolates, *Escherichia coli* was isolated in 99(42.48) samples which was the highest in number. Followed by *Klebsiella pneumoniae* 28 (12.01%).

Table 3: Distribution of isolates based on gender

PATHOGEN ISOLATED	MALE	FEMALE	TOTAL
Escherichia coli	37	62	99
Escherichia coli (ESBL)	7	9	16
Klebsiella pneumoniae	11	17	28
Pseudomonas spp.	11	13	24
Enterococcus spp.	6	18	24
S.aureus	3	8	11
S.aureus (MRSA)	6	5	11
Acinetobacter baumannii complex	5	2	7
CONS	3	3	6
Proteus	3	2	5
Citrobacter koseri	1	0	1
Serratia marcescens	0	1	1
Total	93	140	233

Table 4: Antibiogram of isolated Pathogen

Pathogen isolated	AMP (R)	GEN (R)	AK (R)	AZM (R)	FOS (R)	NIT (R)	NX (R)	MRP (R)	PB (R)	CL (R)	LZ (R)	VA (R)	HLG (R)	CIP (R)	CX (R)
Escherichia coli (N=99)	97	69	12	46	2	5	98	47	0	0	-	-	-	-	-
Escherichia coli (ESBL) (N=16)	16	10	4	8	2	1	16	10	0	0	-	-	-	-	-
Klebsiella spp. (N=28)	26	20	3	12	0	2	27	15	0	0	-	-	-	-	-
Pseudomonas spp. (N=24)	23	22	12	17	1	5	24	18	0	0	-	-	-	-	-
Acinetobacter spp. (N=7)	7	4	1	2	0	0	7	6	0	0	-	-	-	-	-
Proteus spp.(N=5)	5	3	3	3	0	0	5	2	2	2	-	-	-	-	-
Citrobacter koseri (N=1)	1	0	0	0	0	0	1	0	0	0	-	-	-	-	-
Serratia marcescens (N=1)	1	0	0	0	0	0	1	1	0	0	-	-	-	-	-
S. aureus (N=11)	5	5	2	3	1	0	10	-	-	-	2	0	-	6	0

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S. aureus (MRSA) (N=11)	9	9	1	4	1	2	11	-	-	-	1	0	-	4	11
Enterococcus spp. (N=24)	16	16	6	-	1	5	22	-	-	-	4	0	9	14	14
CoNS (N=6)	4	4	1	4	1	0	5	-	-	-	2	0	-	4	4
TOTAL (264)	210 (90%)	162 (69%)	45 (19%)	99 (47.36)	9 (3%)	20 (8%)	227 (97%)	99 (54%)	2 (1%)	2 (1%)	9 (17%)	0 (0%)	9 (17%)	28 (53%)	29 (55%)

NOTE- *AMP (Ampicillin), GEN (Gentamicin), AK (Amikacin), FOS (Fosfomycin), NIT (Nitrofurantoin), NX (Norfloxacin), MRP, (Meropenem), PB (Polymyxin B), CL (Colistin), LZ (Linezolid), VA (Vancomycin), HLG (High level Gentamicin), CIP (Ciprofloxacin) and CX (Cefoxitin).

R* (Resistance).

Table 4: summarizes the resistance of isolates to antimicrobial agents. Of those 233 isolated strains, the pattern of antibiotic resistance indicated that the greatest proportion of isolates were resistant to NX (Norfloxacin) (97%), AMP (Ampicillin) (90%), and followed by GEN (Gentamicin) (69%).

In our study, Ampicillin, Norfloxacin and Gentamicin demonstrated reduced sensitivity against gram-negative organism and Ampicillin, Norfloxacin, Gentamicin, Ciprofloxacin were showed reduced sensitivity against gram positive organism.

A total of 233 isolates were used to check biofilm formation out of which 104(46.63%) showed strong biofilm formation and 98 isolates were negative biofilm producers by Congo red test. The majority of strains that formed strong biofilms were *Escherichia coli*(49)and *Enterococcus spp.* (19). On the other hand, 12 *Escherichia coli* strains showed weak slime formation with 2 *Staphylococci* strains. Furthermore, out of all samples, 15(6.43%) were indeterminate for any biofilm formation.

Table 5: Distribution of biofilm producer pathogens

PATHOGEN ISOLATED	**BIOFILM**							
	STRONG POSITIVE		**WEAK SLIME PRODUCER**		**INDETERMI -NATE**		**NEGATIVE**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Escherichia coli **(115)**	49	42.60	12	10.43	6	5.21	48	41.73
Klebsiella spp **(28)**	13	46.42	1	3.57	2	7.14	12	42.85
Pseudomonas spp. **(24)**	8	33.33	1	4.16	0	0	15	62.5
Enterococcus spp. **(24)**	19	79.16	0	0	2	8.33	3	12.5
Staphylococci **(22)**	8	36.36	2	9.09	3	13.63	9	40.90
Acinetobacter Baumannii Complex **(7)**	2	28.57	0	0	1	14.28	4	57.14
CONS ***(6)***	2	33.33	0	0	1	16.66	3	50
Proteus Spp. ***(5)***	3	60	0	0	0	0	2	40
Citrobacter koseri ***(1)***	0	0	0	0	0	0	1	100
Serratia marcescens ***(1)***	0	0	0	0	0	0	1	100
Total (233)	104	44.63	16	6.86	15	6.43	98	42.06

Discussion

Among the most common bacterial diseases in humans was urinary tract infections or UTIs. Catheterization-associated urinary tract infections (CAUTIs) were diagnosed in patients who had fever (temperature ≥ 38°C) without any other apparent cause, urgency, or suprapubic discomfort.

The persistence of uropathogens associated with biofilms affects most facets of CAUTI diagnosis, treatment, and prevention. [(11)] Meanwhile, in patients

with underlying diseases or under intensive care, the relevant detection of biofilm producers is crucial since CAUTIs are a common nosocomial infection.

In most cases, urinary catheterization is recommended to treat urinary tract obstruction, allow patients with neurogenic bladder dysfunction and urine retention to drain their bladders, support urologic surgery, and collect accurate measurements of urine production in people with clinical illness.[12]

A cross-sectional observational study was conducted over 6 months among 264 catheterized CAUTI patients brought into the intensive care unit of TEERTHANKER MAHAVEER MEDICAL COLLEGE & RESEARCH CENTRE. Urine and foley tip samples were collected and cultured to identify the causative uropathogens. In the study, 233(88.25%) of the 264 patients studied exhibited notable bacteriuria, and 31(26%) samples were sterile. In the current study, overall CAUTI cases were higher among female patients 140(60.08%) compared to male patients 93(39.92%). Jayasukhbhai et al.,[13] and Almalki and Varghese[14] reported (56.46% and 75% respectively) of CAUTI in female patients which was similar to our study in this study, the prevalence of UTI was higher in females may be due to the high load of periurethral flora in females which was introduced during catheterization. We find out UTIs were more common in the age between 51-60. According to the study, *E. Coli* was the most often isolated pathogen out of these 233 strains 115(49.35%), followed by *Klebsiella spp* 28(12.01%), *Pseudomonas spp.*24(10.30%), *Enterococci* 24(10.30%) and *Staphylococci* 22(9.44%). Several other studies also revealed *E. coli*.as the commonest pathogen ranging from 22 to 70%. 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%).

In our study higher frequency of *E. Coli* was isolated from females than in males, 62 (62.62%) from females and 37(27.27%) from males. As the result shows females had a higher prevalence of *E. coli* than did males. Biofilm-producing bacteria showed comparatively better resistance against tested drugs in their antibiotic sensitivity patterns. Resistance trends among the isolates, both biofilm producers and nonproducers, are depicted in Table 4:Our study showed that imipenem, meropenem, nitrofurantoin, amikacin, and piperacillin-tazobactam were the most effective antibiotics against gram-negative isolates. Narmeen Mahmoud et al also found that imipenem and amikacin were the most effective antibiotics against gramnegative isolates.In our study, Cefotaxime, ceftriaxone, aminoglycosides, norfloxacin, ciprofloxacin, and ofloxacin demonstrated reduced sensitivity against gram-negative bacteria.Gram negative bacteria are more resistant than gram positive because of their outer membrane, which protect them from their environment and prolonged use of antibiotics. In our study Norfloxacin, Ciprofloxacin, Gentamicin show reduced sensitivity against gram positive bacteria and most effective antibacterial are Vancomycin and Linezolid for gram-positive bacteria. The present study showed more drug resistance in biofilm-forming isolates than in non-biofilm-forming isolates which is similar to S. Pramodhini et al. [15]

One element that contributed to the chronic, indolent infection in CAUTI was the development of biofilm along the catheter surface. Inthe current research, the detection of the biofilm was carried out by the Congo Red agar method. The detection of the biofilm Pfaller et al.'s research demonstrated the Congo Red method's advantages, including the colonies' ability to survive on the medium and its speed, sensitivity, and reproducibility. [16]

Microbial biofilms were associated with persistent infections that do not react to standard antibiotic treatment. [17]

The relationship between uropathogen biofilm development and antibiotic resistance has only been the subject of a small number of research. Since biofilm formation was a complicated process involving many different variables and components, more research is required to assess this correlation. However, prior research has indicated that the development of biofilms may be facilitated by early antibiotic usage. [18] To test this theory, however, more research on the consequences of exposure to sub-inhibitory antibiotic doses is required.

Conclusion

The study emphasizes how crucial early detection of biofilm-forming uropathogens in CAUTI patients to guide appropriate antimicrobial therapy and prevent the development of antibiotic resistance. Strategies to prevent CAUTI, such as daily assessment of the need for catheter removal and adherence to standard care bundle approaches during catheter insertion and maintenance, are crucial.

The study on biofilm production by uropathogens causing catheter associated urinary tract infections (CAUTI) among ICU patients has provided insightful information about the frequency and significance of biofilm formation in this particular clinical context. The results of the study highlight the role that biofilms play in the persistence and recurrence of UTIs, particularly in patients who are catheterized.

Ethical clearance: Taken from institutional ethical committee TMU Moradabad Ref no. /MC/CRC/PR/2023/488 dated: 9.06.23

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Conflict of interest: Nill

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