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Original Article

Multidrug resistance among biofilm-forming uropathogens isolated from catheter-associated urinary tract infection from a rural tertiary care center in Eastern India.

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Abstract

Background: Urinary tract infections (UTIs) are prevalent nosocomial infections, accounting for 30-40% of hospital-acquired infections. Indwelling urinary catheters create conducive environments for biofilm formation, limiting antimicrobial diffusion and facilitating the transfer of antimicrobial resistance genes. Multidrug resistance (MDR) among biofilm-forming uropathogens poses significant therapeutic challenges. This study aimed to assess the prevalence of MDR among biofilm-forming uropathogens in catheter-associated UTIs (CAUTIs) and characterize their antimicrobial resistance patterns.

Methodology: The study included 95 catheterized patients over six months. Uropathogens were isolated and identified using standard methods. Biofilm production was assessed using tissue culture plates, tube tests, congo red agar, and modified congo red agar methods. The antibiotic resistance profiles of biofilm producers and non-producers were determined.

Results: The prevalence of CAUTIs was 68.42%, with Escherichia coli being the most common uropathogenic (43.07%). Among the 65 isolates, 58.46% were identified as biofilm producers using four different methods. Biofilm producers exhibited higher resistance compared to non-producers, particularly against ampicillin (100% vs. 82.35%), Amoxiclav (90.62% vs. 58.82%), and cotrimoxazole (90.62% vs. 70.58%). Multidrug resistance was significantly higher among biofilm producers (80.64%) than non-producers (38.88%).

Conclusion: The study highlights a high prevalence of biofilm formation among CAUTIs patients, contributing to antibiotic resistance. Multidrug resistance among biofilm producers exacerbates therapeutic challenges for clinicians. Strict aseptic techniques during catheterization are crucial to prevent infections by multidrug-resistant biofilm-forming uropathogens. Regular updates to institutional empirical therapy protocols are necessary to mitigate recurrence and treatment failure in UTI cases.

Keywords

Biofilm, Catheter-Associated Urinary Tract Infection, Uropathogens, Multidrug Resistance

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Introduction

Urinary tract infections (UTIs) represent a substantial portion, approximately 30–40%, of all healthcare-associated infections (HCAIs), constituting a significant burden on individual patients and public health systems[1]. Among these, catheter-associated urinary tract infections (CAUTIs) emerge as the most prevalent nosocomial infections, primarily occurring subsequent to urinary catheter insertion[2]. The impact of CAUTIs on patient morbidity and mortality is substantial, underscoring the urgent need for effective management strategies[3].

Under normal circumstances, the bladder mucosa deploys innate defense mechanisms, such as inflammation-mediated neutrophil recruitment and shedding of epithelial cells carrying attached bacteria, to resist bacterial colonization. However, urinary catheters, being inert materials, lack such inherent defense mechanisms, thereby providing an ideal environment for bacterial attachment and subsequent biofilm formation on both inner and outer catheter surfaces. The primary biofilm-forming organisms implicated in CAUTIs include Staphylococcus epidermidis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and other gram-negative bacteria[4].

Biofilms represent complex communities of microorganisms encased within an extracellular polymeric matrix, adhering to either biotic or abiotic surfaces[5]. This matrix not only shields microorganisms from antimicrobial agents but also facilitates the transfer of antimicrobial resistance genes, thereby conferring heightened resistance to commonly used antibiotics among biofilm-producing bacteria. The restricted diffusion of antimicrobials within biofilm matrices and the close proximity of microorganisms promote the exchange of plasmids and other genetic elements, contributing to the emergence of multidrug-resistant (MDR) strains[6].

The escalating threat of antimicrobial resistance poses a grave challenge to global health and poses a significant risk to humanity[7]. Microorganisms are classified as MDR when they exhibit resistance to at least one agent from three or more classes of antimicrobials[8]. MDR pathogens significantly impact healthcare delivery by limiting therapeutic options, ultimately resulting in poor patient outcomes, including high case fatality rates[9].

Against this backdrop, the current study aims to investigate the prevalence of multidrug resistance among biofilm-forming uropathogens in catheterized patients with UTIs and elucidate their antimicrobial resistance patterns. By shedding light on the interplay between biofilm formation and antimicrobial resistance, this research seeks to inform targeted therapeutic interventions and mitigate the adverse consequences associated with MDR CAUTIs.

Methodology

Study Design

This cross-sectional observational study was conducted among Indoor patients of College of medicine and JNM Hospital, Kalyani, Nadia, West Bengal for a period of 6 months from July to December 2022.

Setting

This study was conducted at College of medicine and JNM Hospital, Kalyani, Nadia, West Bengal.

Participants

Patients aged 18 years and above, of both sexes, with indwelling catheters for more than 48 hours and suspected of CAUTIs, were included.

Sample collection

Aseptic techniques were employed to aspirate 5-10 ml of urine samples from the indwelling catheter tubing. All samples were promptly transported and processed within 2 hours of collection.

Microscopic Examination

Urine samples were centrifugated at 3000 rpm for 5 minutes, and wet preparations were examined under high-power (40X) microscopy. Significant pyuria was defined as the presence of ≥5 pus cells/high-power field (HPF).

Culture and Identification

Semi-quantitative culture methods using standard loop techniques were utilized, with samples inoculated onto MacConkey and Hichrome UTI agar media. Colony-forming units $>10^5$ CFU/ml were considered significant[10]. Isolate identification was based on colony morphology, Gram stain findings, and standard biochemical tests[11]. Antimicrobial susceptibility testing of the isolates was done using the Kirby Bauer disc diffusion method using Mueller Hinton agar according to the CLSI guideline 2020[12].

Drug Susceptibility Testing

The Kirby Bauer disc diffusion method, following CLSI guideline 2020 recommendations, was employed for drug susceptibility testing. The following antibiotic discs were used: Amikacin (30mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Nitrofurantoin (300mcg), Imipenem (10mcg), Meropenem (10mcg), Cotrimoxazole (25mcg), Cefoxitin (30mcg), Cefotaxime (30mcg), Amoxicillin-clavulanic acid (10mcg), Ceftazidime (30mcg), Ceftazidime/clavulanic acid (30/10mcg), Ampicillin (10mcg), Piperacillin-tazobactam (100/10mcg), and Vancomycin (30mcg). Reference strains of E. coli (ATCC 25922), S. aureus (ATCC 25923), and E. faecalis (ATCC 29212) were employed for quality control.

Biofilm Production Assessment

Biofilm production was assessed using Tissue culture, Tube test, Congo red agar, and modified Congo red agar[13-16]. Isolates were categorized as biofilm producers if they tested positive by at least one of the four methods; those negative across all methods were considered non-producers.

Statistical Analysis

Data were analyzed using MedCalc (version 18.9)[17]. Qualitative variables were presented as mean ± standard deviation (SD). The Chi-square test was used to compare qualitative variables between two independent groups, with a p-value < 0.05 considered statistically significant. Pearson's Chi-Square value was considered equivalent to the p-value, as it is a test for categorical data.

Ethical Considerations

Before commencement, ethical clearance was obtained from the Institutional Ethics Committee (Ref. No. F-24/PR/COMJNMH/ICE/22/603).

Results

Participants

Ninety-five urine samples were collected from patients with indwelling catheters placed for more than 48 hours. The distribution of samples across different wards showed that the majority were collected from Female Medicine Ward (FMW), followed by Male Medicine Ward (MMW), Female Surgical Ward (FSW), Orthopedics, and Gynecology (Table 1).

Descriptive Data

Out of the total samples, 65 (68.42%) were positive for catheter-associated urinary tract infection (CAUTI), while 30 (31.57%) showed no growth on culture (Figure 1). Among the positive samples, 43 (66.13%) had a pus cell count of >5/HPF, while 22 (33.84%) had a count of <5/HPF. In contrast, among the negative samples, 4 (13.33%) had a pus cell count >5/HPF, and 26 (86.66%) had a count <5/HPF (Table 2).

Outcome Data

Biofilm production was observed in 38 (58.46%) of the isolates, as detected by all four methods (Table 4). The highest biofilm production was seen in E. coli (67.85%), followed by Candida spp. (43.75%), Enterococcus spp. (62.5%), Klebsiella pneumoniae (55.55%), Acinetobacter spp. (50%), and Staphylococcus aureus (50%).

Main Results

Biofilm producers exhibited significantly higher resistance to antibiotics compared to non-biofilm producers (Table 5). Notably, biofilm-producing uropathogens showed higher resistance to Ampicillin, Amoxiclav, Meropenem, Cotrimoxazole, Levofloxacin, Nitrofurantoin, and Piperacillin-tazobactam. Additionally, biofilm producers were significantly more likely to exhibit multidrug resistance compared to non-biofilm producers (80.64% vs. 38.88%) (Table 6).

Overall, the study highlights the prevalence of multidrug resistance among biofilm-forming uropathogens in patients with CAUTIs. These findings underscore the importance of understanding biofilm formation in the management of urinary tract infections and the need for targeted antibiotic therapy to combat multidrug resistance.

Table 1: Sample distribution according to sex and ward.

Sample	Total number	MMW & FMW	MSW & FSW	Orthopedics	Gynae
Male	42	22(52.38)	09(21.42)	11(26.19)	-
Female	53	34(64.15)	12(22.64)	03(5.66)	04(7.54)
Total	95	56(58.94)	21(22.10)	14(14.73)	4(4.21)

Values are given as n(%)

MMW- Male medicine ward, FMW- Female medicine ward, MSW/FSW- Male surgical ward, FSW- Female surgical ward

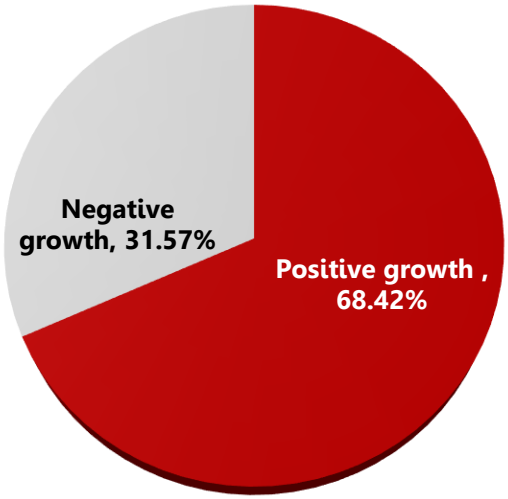

Figure 1: Distribution of study population based on the culture growth (N=95).

Table 2: Distribution of study population based on pus cell count.

Pyuria	No of cases with CAUTI	No of cases without CAUTI	Total cases	Chi-squared	df	p-value
>5 pus cells/HPF	43(66.13)	4 (13.33)	47	22.6688	1	<0.0001*
< 5 Pus cells/HPF	22(33.84)	26(86.66)	48			

*p<0.05 is considered significant.

Table 3: Distribution of CAUTI-positive isolates according to biofilm production by four different methods.

Organism	Biofilm producer		Total	Non-biofilm producer		Total
	Male	Female		Male	Female	
Bacteria	7(7.36)	24(25.26)	31(32.63)	15(15.78)	3(3.15)	18(18.94)
Fungus	1(1.04)	6(6.31)	7(7.36)	4(4.21)	5(5.26)	9(9.47)

Total	8(8.33)	30(31.57)	38(40)	19(20)	8(8.42)	27(28.42)

Table 4: Distribution of Biofilm producer organisms.

Organisms	Biofilm producer (n=38)	Non-biofilm producer (n=27)	Percentage of biofilm production respective to total isolates	Chi-squared	df	p-value
E. coli (n=28)	19(67.85)	9(32.14)	50%	2.6468	6	0.7542
Candida species (n=16)	7(43.75)	9(56.25)	18.42%			
Klebsiella species (n=9)	5(55.55)	4(44.44)	13.15%			
Enterococcus species (n=8)	5(62.5)	3(37.5)	13.15%			
Acinetobacter species (n=2)	1(50)	1(50)	2.63%			
Staphylococcus aureus (n=2)	1(50)	1(50)	2.63%			

*p<0.05 is considered significant.

Table 5: Antibiotic resistance pattern among biofilm producer and non-biofilm producer uropathogens.

Drugs	Resistance among all isolates (n=49)	Biofilm producer (n=31)	Biofilm non-producer (n=18)	p-value	S/NS
Ampicillin	45(91.83%)	31(100%)	14 (82.35%)	0.01	S
Amoxiclav	39(79.59%)	29(90.62%)	10(58.82%)	0.00	S
Ciprofloxacin	37(75.51%)	26 (81.25%)	11(64.70%)	0.09	NS
Imipenem	15(30.61%)	11 (34.37%)	4(23.52%)	0.52	NS
Meropenem	14(28.47%)	12(37.5%)	2(11.76%)	0.05	S
Cotrimoxazole	41(83.87%)	29(90.62%)	12(70.58%)	0.01	S
Amikacin	21(42.85%)	16 (50%)	5(29.41%)	0.13	NS
Levofloxacin	35(71.42%)	25 (78.12%)	10(58.82%)	0.01	S
Nitrofurantoin	19(38.77%)	15 (46.87%)	3(23.52%)	0.03	S
Piperacillin+Tazobactam	34(69.38%)	25 (78.12%)	9(52.94%)	0.05	S

S=significant, NS= Non-significant

Table 6: Multidrug resistance among biofilm producer uropathogens.

Variables	Multidrug Resistance		Total	Chi-squared	df	p-value
	Yes (n=32)	No (n=17)				
Biofilm producer	25(80.64)	6(19.35)	31	8.5846	1	0.003*
Non-biofilm producer	7(38.88)	11(61.11)	18			

*p<0.05 is considered significant.

Discussion

Urinary tract infections (UTIs) pose a significant challenge in clinical practice, with catheter-associated UTIs (CAUTIs) representing a common and complex manifestation. The utilization of indwelling catheters significantly elevates the risk of UTIs by disrupting normal host defense mechanisms, leading to bladder overdistension and incomplete voiding, which, in turn, create favorable conditions for bacterial growth due to residual urine. The empiric treatment of UTIs typically involves the administration of select antibiotics; however, regional variations in antimicrobial susceptibility among pathogens necessitate continuous monitoring to guide appropriate antibiotic therapy, particularly in the context of biofilm-producing uropathogens[18].

Our study observed a high prevalence of CAUTIs among suspected UTI patients, with 68.42% testing positive. Most samples were collected from medicine wards, which is consistent with previous studies. Notably, significant pyuria (>5 pus cells/HPF) was observed in 66.13% of CAUTI cases, aligning with findings from other investigations. Karkee et al.[19] and Dongal et al.[20] also reported significant pyuria in their studies which is similar to current study.

Biofilm formation emerged as a critical factor contributing to treatment failure and antibiotic resistance among uropathogens. We detected biofilm production in 58.46% of isolates, consistent with literature reports. Notably, E. coli, the most frequently isolated pathogen in our study, exhibited substantial biofilm production (67.85%), corroborating findings from various regional studies[21-24]. Different studies have demonstrated biofilm production ranging from 45-71%[21-23].

Biofilms represent dynamic communities of microorganisms encased within a protective matrix, facilitating increased resistance to antibiotics. Our study revealed significantly higher antibiotic resistance among biofilm-producing uropathogens compared to non-biofilm producers, consistent with existing literature. Notably, resistance patterns varied across different antibiotics, emphasizing the need for tailored antibiotic therapy guided by antimicrobial susceptibility testing. Almalki et al.[18] found a higher percentage of resistance among the biofilm producers of Nitrofurantoin (72%), Vancomycin (76%), and sensitivity to Imipenem (99%) and Amikacin (84%). Tiwari et al.[25] also found multidrug resistance patterns among the biofilm producers. Their study found effective therapies for biofilm producers, such as chloramphenicol, gatifloxacin, amikacin, ciprofloxacin, and nitrofurantoin. Another agreeing result reported by Savas et al.[26] and Panda et al.[27] found the most effective therapy for biofilm production was Imipenem, which is similar to the current study result. Rao et al.[28] found effective therapy for biofilm producers were Norfloxacin and Ceftazidime, and their data differs from the present data.

The association between biofilm formation and multidrug resistance was pronounced in our study, with 80.64% of biofilm producers exhibiting multidrug resistance. This finding underscores the formidable challenge posed by biofilm-producing uropathogens in clinical management and highlights the urgent need for effective therapeutic strategies. Similar study conducded by Subramanian et al.[29] and Suman et al.[30] found higher MDR pnenotype among biofilm producer. Another study conducted by Costerton et al.[31] also reported higher prevalence of MDR phenotype uropathogens among biofim producer. All three studies strongly correlated with our study.

Therapeutic approaches against CAUTIs must be informed by antimicrobial susceptibility testing, as conventional antibiotic therapy may be ineffective against biofilm producers. The development of antimicrobial resistance occurs through various mechanisms, including mutation, horizontal gene transfer, and efflux pumps, with biofilm-producing bacteria displaying heightened antibiotic resistance due to the protective nature of biofilms[32].

Limitations

Our study's inability to differentiate between catheter-associated bacteriuria and true CAUTI due

to uniform sample collection from patients with indwelling catheters for over 48 hours poses a significant limitation, potentially affecting diagnostic accuracy. Additionally, the presence of Acinetobacter spp. and Staphylococcus spp. as uropathogens raises concerns, as these organisms can survive disinfectants used during urethral catheterization, potentially leading to contamination and false-positive results. Furthermore, the underrepresentation of gram-positive organisms, likely attributed to our small sample size, limits the generalizability of our findings and warrants caution in interpreting the prevalence of antibiotic resistance. Lastly, our study's scope was constrained by our inability to comprehensively assess the antibiotic resistance patterns of all isolates, indicating the need for future research with larger sample sizes to provide more robust and informative data for guiding clinical decision-making.

Conclusion

The study highlights the prevalence of biofilm production among catheter-associated urinary tract infection (CAUTI) patients, with females being more susceptible due to anatomical factors. Biofilm formation fosters antibiotic resistance, particularly against commonly used antibiotics like fluoroquinolones and beta-lactams, posing a significant challenge in treatment. Multidrug resistance among biofilm producers is a growing concern that requires urgent attention. Various interventions, such as antibacterial or silver-coated catheters, have been proposed to mitigate biofilm formation, but their efficacy and safety need to be evalu ated in larger clinical trials. Strict adherence to aseptic techniques during catheterization is crucial to prevent biofilm-related infections. Hospital infection control committees should promote antibiotic stewardship practices to curb multidrug resistance. The study underscores the importance of regularly updating empirical therapy protocols based on local antibiogram data to minimize recurrence and treatment failure in CAUTI cases. Overall, comprehensive strategies are needed to prevent and manage biofilm-related infections effectively, thereby improving patient outcomes in CAUTI management.

Conflicts of Interest

The Author(s) declare no conflicts of interest.

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References

1. Lee JH, Kim SW, Yoon BI, Ha US, Sohn DW, Cho YH. Factors that affect nosocomial catheter-associated urinary tract infection in intensive care units: 2-Year experience at a single centre. Korean J Urol. 2013;54(1):59–65.
2. Nicolle LE. Catheter associated urinary tract infections. Antimicrob Resist Infect Control. 2014;3:23.
3. Stamm WE. Catheter-associated urinary tract infections: Epidemiology, pathogenesis, and prevention. Am J Med. 1991;91(3B):65S–71S.
4. Stickler DJ. The bacterial biofilms and the encrustation of the urethral catheters. Biofouling. 1996;9(4):293–305.
5. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics. 2020;9(2):59.
6. Schroeder M, Brooks BD, Brooks AE. The Complex Relationship between Virulence and Antibiotic Resistance. Genes (Basel). 2017 Jan 18;8(1):39.
7. Cox, G.; Wright, G.D. Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. Int. J. Med. Microbiol. 2013, 303, 287–292.
8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81.
9. Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect. Drug Resist. 2015;8:49-61.
10. Forbes BA, Sahm DF, Weissfield AS. Bailey and Scott's Diagnostic Microbiology, 10th ed, St. Louis, Missouri, USA: Mosby Inc.;1998.

11. Collee, JG Mackie and Mccartney practical medical microbiology (English), 14th edn, Churchill Livingstone, London 1996.
12. Clinical and laboratory standards institute.2020. Performance standards for antimicrobial susceptibility testing. CLSI M100- S20U. Update January 2020. Clinical and Laboratory Standards Institute, Wayne, PA, USA.
13. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, Beachey EH. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985;22(6):996-1006.
14. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun. 1982;37(1):318-326.
15. Freeman DJ, Falkiner FR, Keane CT. A new method for detecting slime production by coagulase-negative staphylococci. J Clin Pathol.1989;42(8):872-874.
16. 16.Mariana NS, Salman SA, Neela V, Zamberi S. Evaluation of modified Congo Red Agar for detection of biofilm produced by clinical isolates of methicillin-resistance Staphylococcus aureus. Afr J Microbiol Res. 2009;3(6):330-338.
17. MedCalc Software Ltd. Diagnostic test evaluation calculator. Avaiblable at: https://www.medcalc.org/calc/diagnostic_test.php (Accessed October 27, 2022)
18. Almalki MA, Varghese R. Prevalence of catheter associated biofilm producing bacteria and their antibiotic sensitivity pattern. Journal of King Saud University–Science. 2022;32(2):1427-1433.
19. Karkee P. Dhital D. Madhup SK. SherchanJBCatheter Associated Urinary Tract Infection: Prevalence, Microbiological Profile and Antibiogram at a Tertiary Care Hospital. Ann Clin Chem Lab Med. 2017;3(2):3-10.
20. Dongol A, Joshi DM , Gautam A. Detection of Pyuria versus Bacteriuria in Suspected Patients of Urinary Tract Infection. Nepal Journal of Science and Technology 2014; 15(1):129-132.
21. Sayal P, Singh K, Devi P. Detection of bacterial biofilm in patients with indwelling urinary catheters. CJM. 2014;3(3):9-16.
22. Mohamed E, Shalakany HE. Detection of biofilm formation in uropathogenic bacteria. Egypt J Med Microbiol.2015;24(1):49-57.
23. Hassan A, Usman J, Kaleem F, Omalr M, Khalid A, Iqbal M.Evaluation of different detection methods of biofilm formation in clinical isolates. Braz J Infect Dis.2015;15(4):305-311.
24. Chandak RJ, Mishra B,Thakur A, Loomba PS, Dogra V. Comparison of Methods of Biofilm Detection in Urinary Candida Isolates and Evaluating its Role in Persistent Candiduria. Natl. lab. med. 2018;7(2):6-11.
25. Tiwari AA, Ghnawate N. Detection of biofilm forming bacterial communities from urinary catheter of patients with change in its antibiotic susceptibility pattern and triclosan effect from different hospitals of Amravati City Maharashtra, India. Open J. Med. Microbiol. 2017;7(3):51-66.
26. Savas L, Guvel S, Onlen Y, Savas N, Duran N. Nosocomial urinary tract infections: micro-organisms, antibiotic sensitivities and risk factors. West Indian Med J. 2006;55(3):188-193.
27. Panda PS, Chaudhary U, Dube SK. Study of biofilm production and antimicrobial sensitivity pattern of uropathogens in a tertiary care hospital in North India. Int J Community Med Public Health.2016;3:2421-2426.
28. Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, et al. Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of Acinetobacterbaumanii. Indian J Med Microbiol. 2008;26(4):333-337.
29. Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J. 2012;5(7):344-348.
30. Suman E, Jose J, Varghese S, Kotian MS. Study of biofilm production in Escherichia coli causing urinary tract infection. Indian J. Med. Microbiol. 2007; 25:305-306.
31. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Sciences. 1999;284: 1318–1322.
32. Simon AL, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med.2008;59:415-428.