International Journal of Enhanced Research in Medicines & Dental Care (IJERMDC),
ISSN: 2349-1590, Vol. 13 Issue 5, May 2026

Biofilm Production among Catheter-Associated Uropathogens and Its Relationship with Antimicrobial Resistance in a Tertiary Care Centre, Indore

Ms. Divya Mishra[1], Dr. Ramanath K[2]

[1]Research Scholar, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India
[2]Professor of Microbiology, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India

ABSTRACT

Background: Biofilm formation is central to the pathogenesis of catheter-associated urinary tract infection (CAUTI). Once microorganisms adhere to an indwelling urinary catheter, they produce an extracellular matrix, form protected communities and become less susceptible to host defences and antimicrobial exposure. Phenotypic detection of biofilm is therefore relevant for understanding treatment failure, recurrent infection and antimicrobial resistance in catheterized patients.

Objective: To evaluate biofilm production among bacterial CAUTI isolates by Congo red agar, tube method and tissue culture plate method, and to assess the relationship between biofilm production, antimicrobial resistance and beta-lactamase production.

Methods: This cross-sectional study was conducted among 100 adult in-patients catheterized for more than 48 hours at Index Medical College Hospital & Research Centre, Indore. Catheter urine specimens were collected aseptically after ethics approval and informed consent. Significant isolates were identified using standard microbiological methods and tested for antimicrobial susceptibility by modified Kirby–Bauer disc diffusion. ESBL and MBL production were detected phenotypically. Biofilm production was evaluated using Congo red agar (CRA), tube method (TM) and tissue culture plate (TCP) method. TCP results were interpreted quantitatively by optical density at 570 nm and classified as non, weak, moderate or strong biofilm production.

Results: Nineteen bacterial isolates were recovered from 100 catheterized patients. Escherichia coli was the predominant isolate, followed by Klebsiella pneumoniae and single isolates of Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella oxytoca and Acinetobacter baumannii. Biofilm was detected in 5 isolates (26.3%) by CRA, 12 isolates (63.2%) by TM and 16 isolates (84.2%) by TCP. By TCP, 5 isolates were weak producers, 8 were moderate producers and 3 were strong producers. P. aeruginosa and A. baumannii were strong biofilm producers by TM and TCP, while E. coli isolates were commonly moderate producers. Biofilm-positive isolates generally showed numerically higher resistance to several antibiotics, but no statistically significant association was observed between biofilm production and resistance to individual antibiotics. All MBL-positive isolates were biofilm producers by TCP.

Conclusion: TCP detected substantially more biofilm-positive CAUTI isolates than CRA or TM, supporting its use as a more sensitive phenotypic method. Although statistical association between biofilm and individual antibiotic resistance was not demonstrated in this small dataset, the high biofilm burden combined with high ESBL and MBL rates indicates that catheter-associated uropathogens can act as persistent reservoirs of multidrug resistance. Laboratory detection of biofilm, when interpreted alongside culture and susceptibility results, may strengthen CAUTI surveillance and infection-control planning.

Keywords: biofilm, catheter-associated urinary tract infection, tissue culture plate method, Congo red agar, tube method, ESBL, MBL, antimicrobial resistance

INTRODUCTION

Catheter-associated urinary tract infection is fundamentally a device-associated infection. Indwelling urinary catheters are clinically useful, but they provide an abiotic surface that permits bacterial attachment, migration and biofilm development. CAUTI risk increases with duration of catheterization, and surveillance guidance defines catheter association in relation to an indwelling urinary catheter present for more than two consecutive days in an inpatient location.[1] The CDC also identifies prolonged catheter use as the most important risk factor for CAUTI.[2] Once microorganisms colonize the catheter surface, they may persist despite urine flow, host immune response and antimicrobial therapy.

Biofilm is an organized microbial community attached to a surface and embedded in extracellular polymeric substances. The biofilm life cycle includes initial attachment, irreversible adhesion, microcolony formation, maturation and dispersal.[3] In the urinary catheter environment, organisms are exposed to continuous urine flow, conditioning films, catheter material and host molecules, all of which can promote adhesion. Reviews of CAUTI pathogenesis emphasize that biofilm formation is a major reason why catheter-associated infections are difficult to eradicate and why bacteriuria frequently recurs if the device remains in place. [4,5]

Biofilm-associated bacteria differ from planktonic bacteria in clinically important ways. The extracellular matrix can restrict antimicrobial penetration, bind antibiotic molecules and reduce immune access. Cells inside the biofilm may grow slowly, experience nutrient gradients and express stress-response pathways that increase tolerance. Biofilms can also facilitate horizontal gene transfer and persistence of multidrug-resistant organisms. [6,7] These mechanisms help explain why in vitro susceptibility of planktonic isolates does not always predict eradication of device-associated infection. For CAUTI, the catheter itself may therefore function as both the infection focus and the reservoir from which organisms repeatedly seed the urine.

Several phenotypic methods are used to detect biofilm production in clinical microbiology research. Congo red agar is simple and inexpensive, and identifies slime-producing colonies by colour change, but it is relatively subjective and may miss weak producers.[8] The tube method is also simple and detects adherence to glass or plastic tube walls after staining, yet interpretation may vary between observers. The tissue culture plate method is quantitative, measures optical density of stained adherent biomass and is widely considered more reliable for comparative evaluation. [9,10] Comparative studies have shown that TCP often detects more biofilm producers than CRA and TM, especially when weak and moderate producers are included. [11,12]

The present study used all three methods to evaluate biofilm production among bacterial CAUTI isolates obtained from catheterized adult patients. The study also examined whether biofilm production was associated with antimicrobial resistance, ESBL production and MBL production. This focus is important because CAUTI isolates in tertiary care centres often combine biofilm-forming capacity with resistance to commonly used antibiotics, creating challenges for treatment and infection control.

MATERIALS AND METHODS

This cross-sectional study was conducted over three years in the Department of Microbiology, Index Medical College Hospital & Research Centre, Indore. The study population comprised 100 adult in-patients above 18 years of age who had urinary catheters in place for more than 48 hours. Patients with urinary tract abnormalities were excluded. Institutional ethics committee approval was obtained, and informed consent was taken from eligible participants.

Urine specimens were collected aseptically from the catheter sampling port. The catheter tubing was clamped above the port, and the port and tubing were cleaned with 70% alcohol. Urine was aspirated with a sterile syringe while preserving the closed drainage system and transferred to a sterile screw-capped container. Direct Gram staining and semi-quantitative culture were performed. Blood agar and MacConkey agar plates were incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified by Gram reaction, colony morphology and standard biochemical tests described in diagnostic microbiology references. [13]

Antimicrobial susceptibility testing was performed by modified Kirby–Bauer disc diffusion on Mueller–Hinton agar and interpreted according to CLSI guidance. [14] ESBL detection was performed using double-disc synergy testing with ceftazidime and ceftazidime-clavulanate. MBL detection was performed among imipenem-resistant isolates using imipenem screening and imipenem–EDTA combined disc testing. The antibiotic panel included beta-lactams, beta-lactam/beta-lactamase inhibitor combinations, carbapenem, fluoroquinolone, aminoglycosides, nitrofurantoin, cotrimoxazole, tetracycline and chloramphenicol as applicable.

Biofilm production was tested by three phenotypic methods. In the CRA method, isolates were inoculated on Congo red agar medium and incubated. Black, dry crystalline colonies were interpreted as biofilm positive, while pink or red colonies were considered non-producers. In the tube method, isolates were grown in broth, incubated, decanted, washed and stained; visible stained film lining the wall and bottom of the tube indicated biofilm production. In the TCP method, isolates were incubated in microtitre plate wells, washed to remove planktonic bacteria, stained and read at OD570. Isolates were categorized as non, weak, moderate or strong biofilm producers according to optical density thresholds in the source protocol.

RESULTS

Of the 100 catheterized patients, 19 yielded significant bacterial growth. The isolate distribution was dominated by Gram-negative bacilli. E. coli accounted for 12 isolates (63.2%), K. pneumoniae for 3 isolates (15.8%), and Proteus mirabilis, P. aeruginosa, K. oxytoca and A. baumannii for one isolate each. This organism profile is consistent with CAUTI literature, in which E. coli and Klebsiella species are common Enterobacterales pathogens while non-fermenters contribute a smaller but clinically important proportion of infections. [4,5]

Biofilm detection varied markedly by method. CRA detected biofilm in 5 of 19 isolates (26.3%). TM detected biofilm in 12 isolates (63.2%). TCP detected biofilm in 16 isolates (84.2%), making it the most sensitive of the three methods in this dataset. TCP categorized 5 isolates (26.3%) as weak producers, 8 isolates (42.1%) as moderate producers and 3 isolates (15.8%) as strong producers. Only 3 isolates were non-producers by TCP.

Biofilm method	Positive isolates	Detection rate	Key interpretation
Congo red agar	5/19	26.3%	Lowest detection; likely under-detected weak and moderate producers
Tube method	12/19	63.2%	Intermediate detection; simple but observer dependent
Tissue culture plate	16/19	84.2%	Highest detection; quantitative categorization possible

Organism-wise results showed that CRA detected biofilm mainly among E. coli and K. pneumoniae. Four of 12 E. coli isolates and 1 of 3 K. pneumoniae isolates were positive by CRA. By TM, 8 of 12 E. coli isolates and 2 of 3 K. pneumoniae isolates were positive. P. aeruginosa and A. baumannii were strong producers by TM. TCP detected biofilm among most isolates. E. coli was most frequently classified as moderate producer, K. pneumoniae isolates were weak producers, P. mirabilis and K. oxytoca were moderate producers, and P. aeruginosa and A. baumannii were strong producers.

Organism	Number of isolates	Dominant TCP biofilm category
Escherichia coli	12	Mostly moderate producers
Klebsiella pneumoniae	3	Weak producers
Proteus mirabilis	1	Moderate producer
Pseudomonas aeruginosa	1	Strong producer
Klebsiella oxytoca	1	Moderate producer
Acinetobacter baumannii	1	Strong producer

Antimicrobial resistance was high among the CAUTI isolates. All isolates were resistant to ampicillin and amoxicillin-clavulanic acid. Resistance was also high to ceftriaxone, ceftazidime, ciprofloxacin, tetracycline, cotrimoxazole and cefepime. Amikacin showed the lowest resistance among tested antibiotics, while imipenem resistance remained substantial. When biofilm status was compared with antibiotic resistance, CRA did not show a meaningful pattern and biofilm-negative isolates appeared numerically more resistant for many antibiotics, most likely because CRA failed to detect many true biofilm producers. TM and TCP showed higher numerical resistance among biofilm-producing isolates for several drugs, but the association between biofilm production and resistance to individual antibiotics was not statistically significant.

ESBL production was detected in 12 isolates (63.2%) and MBL production in 6 isolates (31.6%). Biofilm production by CRA, TM or TCP was not significantly associated with ESBL or MBL production. However, all MBL-positive isolates were biofilm producers by TCP. This observation is clinically important because MBL positivity and biofilm production together imply reduced carbapenem susceptibility and potential persistence on catheter surfaces.

DISCUSSION

The present study demonstrates that biofilm production is common among bacterial CAUTI isolates in this tertiary care setting. The most important methodological finding is the large difference in detection rates between CRA, TM and TCP. CRA identified only 26.3% of isolates as biofilm producers, whereas TCP identified 84.2%. This pattern agrees with comparative biofilm-method literature, which has repeatedly shown that CRA is simple and inexpensive but less sensitive than quantitative adherence-based methods. [8,11] CRA interpretation depends on colony colour and morphology, which can vary with media composition, incubation conditions and strain physiology. Therefore, a negative CRA result should not be interpreted as absence of biofilm-forming capacity, especially in catheter-associated isolates.

The tube method detected more producers than CRA but fewer than TCP. Its advantage is that it requires minimal equipment and can be performed in laboratories without microplate readers. However, visual grading of stained material on tube walls is subjective, and weak producers may be misclassified. TCP has the advantage of quantifying adherent biomass by optical density, allowing classification into weak, moderate and strong categories. Although TCP also has limitations, including dependence on inoculum, incubation conditions and washing technique, it provides a more reproducible framework for comparing isolates. [9,10] In the present study, TCP revealed that most isolates were not merely positive but moderate or strong producers, supporting its value in CAUTI research.

The organism-wise pattern is biologically plausible. E. coli was the most frequent isolate and showed a broad range of biofilm production, with many moderate producers. Uropathogenic E. coli can express type 1 fimbriae, P fimbriae and other adhesins that support urinary tract colonization and surface attachment.[15] Klebsiella species are known for capsule production, surface adhesins and biofilm formation, all of which contribute to device colonization and antimicrobial resistance.[16] P. aeruginosa and A. baumannii were represented by single isolates but were strong biofilm producers; this is consistent with the ability of non-fermenting Gram-negative bacilli to persist on abiotic surfaces and survive in healthcare environments.[17] Proteus mirabilis deserves attention because urease activity and crystalline biofilm formation can contribute to catheter blockage and complicated CAUTI.[18]

The relationship between biofilm and antimicrobial resistance is complex. In this study, biofilm-positive isolates showed numerically higher resistance to several antibiotics by TM and TCP, but no statistically significant association was found for individual antibiotics. This does not negate the biological role of biofilm in antimicrobial tolerance. Phenotypic disc diffusion tests measure planktonic susceptibility, whereas biofilm-associated tolerance involves matrix effects, slow growth, persister cells and local microenvironmental gradients. [6,7] Therefore, a biofilm-producing isolate may appear susceptible in standard testing but still persist clinically if the catheter remains in place. Conversely, genetic resistance mechanisms such as ESBL and MBL production can occur independently of biofilm phenotype, which may explain the absence of significant association in a small sample.

The observation that all MBL-positive isolates were TCP biofilm producers is clinically meaningful even though it was not statistically significant. MBL-producing Gram-negative bacilli threaten carbapenem efficacy, and biofilm formation can further support persistence and transmission. In practice, a catheterized patient with a biofilm-forming, carbapenem-resistant isolate requires prompt clinical review, catheter removal or replacement when feasible, targeted therapy and infection-control precautions. Biofilm status should not replace antimicrobial susceptibility testing, but it can enrich interpretation of culture results in device-associated infections.

The high biofilm detection rate also reinforces the importance of catheter stewardship. Antibiotics alone may be insufficient when a colonized catheter remains in place because the device can continue to seed organisms into the bladder. CAUTI prevention therefore depends on limiting catheter insertion, maintaining a closed drainage system, using aseptic technique and removing catheters as early as possible. [1,2] In addition, laboratories and clinicians should avoid treating asymptomatic bacteriuria indiscriminately, because unnecessary antibiotic exposure can promote resistance without addressing the device reservoir. When symptomatic CAUTI is suspected, culture-guided therapy combined with catheter management is more rational than empirical escalation alone.

This study has several limitations. The number of culture-positive isolates was small, limiting the statistical power to detect associations between biofilm and resistance. Molecular assays for biofilm genes, ESBL genes, carbapenemase genes or clonal relatedness were not performed. The TCP method measured biomass but did not directly measure viable biofilm cells or antibiotic minimum biofilm eradication concentrations. The study also did not include catheter-tip sonication or microscopy of catheter surfaces, which would have provided direct evidence of catheter biofilms. Despite these limitations, the study is useful because it compares three practical phenotypic biofilm methods in the same CAUTI isolate set and relates results to antimicrobial resistance and beta-lactamase detection.

The findings suggest several future directions. Larger multicentre studies should include catheter-day denominators, clinical outcomes, duration of catheterization, prior antibiotic exposure and device management. Molecular characterization would help determine whether ESBL and MBL producers carry biofilm-associated genes or belong to transmissible clones. Biofilm-specific susceptibility testing may also be explored, although it is not yet routine in diagnostic laboratories. For resource-limited settings, TCP can serve as a feasible research method for identifying biofilm burden and comparing interventions.

CONCLUSION

Biofilm production was highly prevalent among bacterial CAUTI isolates from catheterized adult patients at this tertiary care centre in Indore. The tissue culture plate method detected biofilm in 84.2% of isolates and outperformed the tube method and Congo red agar. E. coli was the most common organism and was often a moderate biofilm producer, while P. aeruginosa and A. baumannii were strong producers. Biofilm production was not statistically associated with resistance to individual antibiotics, ESBL production or MBL production in this small dataset; however, all MBL-positive isolates were biofilm producers by TCP. These results support the use of quantitative biofilm detection in CAUTI research and reinforce the need for catheter stewardship, culture-guided antimicrobial therapy and infection-control surveillance for multidrug-resistant biofilm-forming uropathogens.

REFERENCES

[1] Centers for Disease Control and Prevention. Urinary tract infection (catheter-associated urinary tract infection [CAUTI] and non-catheter-associated urinary tract infection [UTI]) events. NHSN Patient Safety Component Manual. Atlanta: CDC; 2026.
[2] Centers for Disease Control and Prevention. Catheter-associated urinary tract infection (CAUTI) basics. Atlanta: CDC; 2025.
[3] Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, et al. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat Rev Microbiol. 2022 Oct;20(10):608–20.
[4] Tegegne DT, Abbott IJ, Poźniak B. Catheter-associated urinary tract infections: understanding the interplay between bacterial biofilm and antimicrobial resistance. Int J Mol Sci. 2025 Sept 20;26(18):9193.
[5] Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008 Jan;21(1):26–59.
[6] Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J. Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics (Basel). 2020 Dec 23;10(1):3.
[7] Singh S, Singh SK, Chowdhury I, Singh R. Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. Open Microbiol J. 2017 Apr 28;11:53–62.
[8] Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989 Aug;42(8):872–4.
[9] Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for adherence of staphylococci to medical devices. J Clin Microbiol. 1985 Dec;22(6):996–1006.
[10] Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in clinical isolates. Braz J Infect Dis. 2011;15(4):305–11.
[11] Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in patients with catheter associated urinary tract infections. J Clin Diagn Res. 2012 Nov;6(9):1478–82.
[12] Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. J Glob Infect Dis. 2020 Aug 29;12(3):129–34.
[13] Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's diagnostic microbiology. 13th ed. St. Louis: Mosby; 2013. p. 919–930.
[14] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: CLSI supplement M100S. Wayne (PA): Clinical and Laboratory Standards Institute; 2016. 256 p.
[15] Johnson JR. Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev. 1991 Jan;4(1):80–128.
[16] Guerra MES, Destro G, Vieira B, Lima AS, Ferraz LFC, Hakansson AP, et al. Klebsiella pneumoniae biofilms and their role in disease pathogenesis. Front Cell Infect Microbiol. 2022 May 11; 12:877995.
[17] Vetrivel A, Ramasamy M, Vetrivel P, Natchimuthu S, Arunachalam S, Kim GS, et al. Pseudomonas aeruginosa biofilm formation and its control. Biologics. 2021 Oct 15;1(3):312–36.
[18] Schaffer JN, Pearson MM. Proteus mirabilis and urinary tract infections. Microbiol Spectr. 2015 Oct;3(5): 10.1128/microbiolspec.UTI-0017-2013.