https://doi.org/10.29309/TPMJ/2022.29.09.6599

The Professional Medical Journal
www.theprofesional.com
2022, Volume, 29 Issue, 09

ORIGINAL ARTICLE

Catheter Tip or Direct Urine Culture – Choosing the Better Specimen for Biofilm Detection.

Sidra Zaman[1], Ayesha Khushi[2], Munazza Yasmeen[3], Majid Rauf[4], Saleha Maqsood[5]

Article Citation: Zaman S, Khushi A, Yasmeen M, Rauf M, Maqsood S. Catheter Tip or Direct Urine Culture – Choosing the Better Specimen for Biofilm Detection. Professional Med J 2022; 29(9):1373-1378. https://doi.org/10.29309/TPMJ/2022.29.09.6599

ABSTRACT… Objectives: To assess the better specimen for biofilm detection among catheter tip and direct urine culture and to evaluate the effect of age on biofilm forming capacity of organisms isolated. **Study Design:** Cross Sectional Descriptive study. **Setting:** Lahore General Hospital and Department of Microbiology, Post Graduate Medical Institute. **Period:** August 2018 to February 2019. **Material & Methods:** Convenient sampling technique was used to collect 75 Catheter tips and corresponding Urine samples from catheterized patients. Catheter tips were cultured by Brun-Buisson technique and Quantitative method was used for culture. The isolates were identified using standard operating procedures and the MDR isolates recovered were subjected to Microtiter plate assay to determine their biofilm forming capacity. **Results:** The mean age of catheterization was calculated to be 55.8 years. Statistically, similar number of isolates were recovered from catheter tip and urine sample. However, significantly lesser number of urine samples were found positive for growth ($p<0.05$). MTP assay of catheter tip and urine sample revealed maximum isolates exhibit strong biofilm forming capacity (56.1% vs 47.6%) while minimum number of organisms display no biofilm formation (1.8% vs 2.4%). Catheter tips and urine culture both detect biofilm forming capacity of isolates similarly ($p> 0.05$). Maximum biofilm formation (100%) is seen in extremes of age. **Conclusion:** Less number of urine samples were found positive for growth compared to catheter tips but there is no significant difference in detecting biofilm by catheter tip and urine sample.

Key words: Biofilm, CA-UTI, Catheter Tip, Catheterized, Urine.

INTRODUCTION

Over the past few decades' modern medical technologies have all dramatically modified the structure of health care systems globally, leading to the emergence of multidrug-resistant organisms and surge in the number of hospital acquired infection (HAI)[1] However, there is a scarcity of surveillance data from middle income countries, where HAIs continues to be a hidden but serious problem for patients and a huge burden on the health system.[2] The most important category of HAIs are device-associated HAI (DA-HAI) and among the DA-HAI, Catheter-associated UTI (CAUTI) are the most common cause, of infections in acute care hospitals and nursing care homes.[3,4] Center for Disease Control (CDC) has defined Hospital Acquired-CAUTI (HA-CAUTI) as a UTI where an indwelling urinary catheter was in place for >2 calendar days on the date of event, with day of device placement being day 1, and an indwelling urinary catheter was in place on the date of event or the day before. If an indwelling urinary catheter was in place for more than 2 consecutive days in an inpatient location and then removed, the date of event for the UTI must be the day of device discontinuation or the next day for the UTI to be catheter-associated.[5]

On these devices bacteria mostly exist as biofilms which are surface-adhered communities or suspended aggregates of bacteria that have increased tolerance to environmental stresses and antibiotics.[6] Biofilm formation on indwelling medical devices is influenced by factors such as device material, duration of its use, nutrient availability, number and type of organisms to which the device is exposed, flow rate and composition of the medium.[7]

1. MBBS, M.Phil (Microbiology), Assistant Professor Pathology, Avicenna Medical and Dental College, Lahore.
2. MBBS, Demonstrator Pathology, Avicenna Medical and Dental College, Lahore.
3. MBBS, M.Phil (Chemical Pathology), Assistant Professor Pathology, Avicenna Medical and Dental College, Lahore.
4. MBBS, M.Phil (Microbiology), Assistant Professor Pathology, Avicenna Medical and Dental College, Lahore.
5. MBBS, Demonstrator Pathology, Sharif Medical and Dental College, Lahore.

Correspondence Address:
Dr. Sidra Zaman
Department of Pathology,
Avicenna Medical and Dental College, Lahore.
ccydra@hotmail.com

Article received on: 19/05/2021
Accepted for publication: 17/01/2022

Traditional microbiology falls short where biofilm detection is concerned. Therefore, new and different methods are being used to evaluate the microbial population embedded in biofilms of indwelling medical devices. Semi quantitative method such as roll plate or quantitative methods involving disruption of biofilm by sonication, vortexing, swabbing, scrubbing, rinsing, scraping or using nitric oxide are generally employed. However, there is no unanimously recognized gold standard for quantification of microbes in biofilms.[8,9] Timely recognition and management of potentially pathogenic microbes is a critical step towards prevention and management of disease.[10] Consequently, there is a dire need to evaluate a simple and economical method for the detection of biofilm producers.[11]

The objective of this study is to evaluate which specimen is better for biofilm detection among catheter tip and urine culture, so as to allow early detection of biofilm forming bacteria and improve the therapeutic outcomes for catheterized patients.

MATERIAL & METHODS

A hospital based cross sectional descriptive study was performed at Lahore General Hospital and Department of Microbiology, Post Graduate Medical Institute from August 2018 to February 2019. A sample size of 75 was estimated by keeping the confidence interval equals to 95%, margin of error equals to 10% and anticipated proportion of biofilm formation on urinary catheter tips at 26.3%.[12] Convenient sampling technique was used to collect 75 Catheter tips and corresponding Urine samples from patients catheterized for more than 3 days, with or without UTI. SPSS version 25.0 was used for data analysis. Number and Frequencies were calculated as descriptive analysis. Chi-square was computed to determine the association between the independent and dependent variables of the study. p-value$\leq$0.05 were used to signify the differences between the variables.

COLLECTION

Urine samples were collected prior to removal or change of catheter. Using aseptic technique, drainage tubing was kinked a minimum of 3 inches below the sampling port. Sampling site was then cleansed with alcohol swab and 5 ml of urine was withdrawn from the connecting tubing using a sterile syringe.[13]

After the collection of urine sample, the catheter was removed aseptically and 5 to 7 cm of the catheter tip was cut off and transferred to a dry, sterile urine container.[14]

Processing

Quantitative tip culture technique by Brun-Buisson was used. Inner surface of the catheter tip was washed with 1.0 ml distilled water in syringe. Sample was then agitated for 1 minute in vortex machine.[15]

Culture Inoculation

Quantitative method was used for inoculation of Urine culture and for Catheter tip 0.1 ml aliquot obtained after vortexing was cultured on CLED agar plates using calibrated (1 µL) wire loop. Both Plates were incubated at 37 □ C. After 24 hours of incubation the isolates were identified only if less than 3 types of growths were found. Criteria for defining significant growth in urine was presence of 10^4-10^5 cfu/ml of urine or more for each pathogenic bacteria isolated. For catheter tip cutoff point $\geq 10^3$ cfu/ml was taken as significant catheter colonization. Isolates were identified by Standard Operating Procedures and organisms which were resistant to 3 or more drugs were subject to MTP for biofilm detection.[16]

Microtiter Plate Method (MTP)

Microtiter plate assay was performed following the technique given by Tiwari et al.[17] Strains of positive biofilm producers were used as positive control while negative control wells contained sterile broth only.

RESULTS

The age distribution of the patients showed that maximum catheterizations were done in the age group 41-60 years while least number of catheterizations were performed in the age group 1-20 years. The mean age of catheterization was calculated to be 55.8 years.

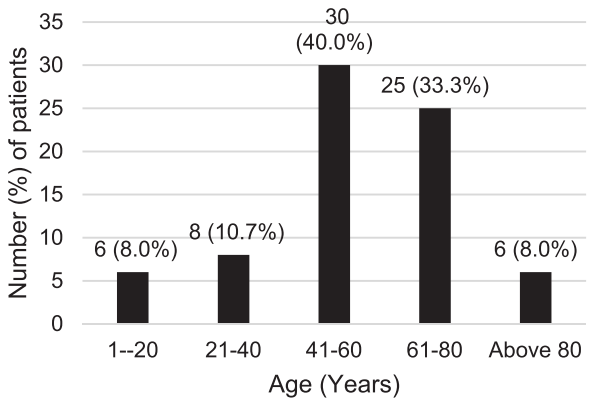

Figure-1. Age wise distribution of catheterized patients (n=75).

Catheter tips and urine cultures revealed one growth, two growths, polymicrobial growths and no growths, which are shown as in Table-I. Statistical analysis revealed there was no significant disparity in recovered isolates from catheter tip or urine sample (p- value=0.194). However, significantly lesser number of urine samples were found positive for growth.

Table-II reflects the biofilm forming capacity of MDR organisms isolated. Maximum isolates exhibited strong biofilm forming capacity (56.1% vs 47.6%) while minimum number of organisms displayed no biofilm formation (1.8% vs 2.4%). There was statistically no significant difference in detecting biofilm by catheter tip and urine sample (p-value=0.858).

Furthermore, statistical analysis revealed that there was no significant association (p-value= 0.978) between age and biofilm forming capacity of organisms although maximum number of biofilms were recovered from patients at extremes of age in the age group 1-20 and above 80 years.

DISCUSSION

Age range in present study was from 1-80 years with mean age being 55.8 years (Figure-1) This is in similarity with study conducted by Vidyasagar and Nagarathnamma[18] (mean age 58) and Ramadan et al[19] (mean age 50.8)

Our results (Table-I), showed no difference in detecting isolates by catheter tip or catheter urine culture (p-value=0.194).

Sample	One Isolate	Two Isolates	Multiple Isolates	No Growth
	No. (%)	No. (%)	No. (%)	No. (%)
Catheter Tip (n=75)	42* (56.0%)	09* (12.0%)	11* (14.7 %)	13 (17.3 %)
Urine (n=75)	34 (45.3%)	06 (8.0%)	11 (14.7 %)	24** (32.0 %)

Table-I. Results of culture of catheter tip and urine samples (n=75 each). *p-value > 0.05 (0.194), **p-value<0.05 (0.03)

Biofilm Forming Capacity	Isolates From Catheter Tip (n=57)		Isolates From Urine (n=42)		P-Value
	No.	%age	No.	%age	
Strong	32	56.1	20	47.6	*
Moderate	20	35.1	18	42.9	*
Weak	04	7.0	03	7.1	*
None	01	1.8	01	2.4	*

Table-II. Biofilm forming capacity among MDR isolates from catheter tip and urine samples. *p-value > 0.05 (0.858)

Age Group (Years)	No. of Isolates	Biofilm Formation	
		No.	%age
1-20	06	06	100.0
21-40	06	04	66.0
41-60	39	38	97.4
61-80	45	39	86.7
Above 80	10	10	100.0

Table-III. Age wise distribution of patients from whose samples biofilm forming uropathogens were isolated from catheter tip and urine sample collectively. p-value >0.05 (0.978)

Comparable results were seen in studies conducted on catheter tip culture in Czechia[20] and catheter urine sample culture[21] in Taiwan.

According to our study, however, greater number of urine samples detect no growth as compared to catheter tip (32.0% vs 17.3%). A similar finding (56.3% vs 18.8%) was reported in a study from India as well.[22]

Likewise, studies by several other researchers also documented that catheter tips are inappropriate for culture use because they overestimate microbial presence and diversity.[23,24]

The isolates recovered from the urine and catheter specimens were further evaluated for biofilm forming capacity by MTP. Our study demonstrated that there was statistically no significant difference (p-value=0.858) in detecting biofilm by catheter tip and urine sample (Table-II). Comparable statistical parameters were seen in a research from India where Catheter tip samples revealed 58% of the isolates were strong biofilm producers, 26% were considered as moderate, while 16% were weak biofilm producers.[25] Similar results were seen in another Indian research as well.[26]

In contrast, a study from Nigeria, demonstrated that a higher proportion of weak biofilm producers was recovered from catheter tip and catheter stream (35.9% vs 25.0%) compared to moderate biofilm producers (6.3 vs 9.6%).[27]

This difference is results could most probably be attributed to the discrepancy among researchers about ideal conditions required for biofilm formation in laboratory. Except the culture temperature of 37°, other conditions such as presence of nutrition and time of cultivation vary significantly among authors. Furthermore, when an indwelling medical device is contaminated with microorganisms, several variables determine at what rate a biofilm develops such as flow rate, nutrient composition of the medium, antimicrobial drug concentration, and ambient temperature. Collectively, these factors contribute to variability in results.[28]

Statistical analysis of our study revealed that age had no effect on biofilm forming capacity of organisms (p-value=0.978) although maximum number of biofilms were recovered from patients in the age group 1-20 years and above 80 years (Table 3). Similar studies conducted in Pakistan, and Portugal also concluded that no significant correlation existed between biofilm formation and age.[29,30]

However, other studies conducted in India and Egypt revealed that there was a significant relation of age with acquisition of CAUTI which was more common in young and elderly > 80 years of age.[31,32] In our study, due to financial constraints, we were not able to assess the efficacy of different catheter material in preventing biofilm formation as well as role of different diagnostic modalities for biofilm detection. We suggest that focus upon diagnostic methods that incorporate routine microbiological procedures with more sophisticated low-cost, and reliable methods needs to be considered, to allow early detection of biofilms, before institution of empirical antibiotic therapy in catheterized patients.

CONCLUSION

Our study found that there is no significant difference in recovering isolates from catheter tip and urine sample, although significantly less number of urine samples were positive for growth. Consequently, catheter tips are considered unsuitable for culture use because they overestimate microbial growth. However, catheter tips did not detect biofilm producers more frequently than urine sample. Irrespective of age, both the samples were found equally effective for the routine detection of biofilm producers.

REFERENCES

1. Friedman ND, Levit D, Taleb E, Marcus G, Michaeli L, Broide M, Mengesha B, Zaidenstein R, Lazarovitch T, Dadon M, Kaye KS. **Towards a definition for health care–associated infection.** Open forum Infect Dis. 2018; 5:6.

2. Dhaneria M, Jain S, Singh P, Mathur A, Lundborg CS, Pathak A. **Incidence and determinants of health care-associated blood stream infection at a neonatal intensive care unit in Ujjain, India: A prospective cohort study.** Dis. 2018; 6(1): 14.

3. Rosenthal VD. **Epidemiology and prevention of HAIs worldwide in ICUs with limited resources: Experience of the International Nosocomial Infection Control Consortium Global Network.** Crit Care Update. 2019; 30: 44.

4. Pettemerides Y, Vasilios R. **Incidence rate of device-associated, hospital acquired infections in ICUs: a systematic review developed versus developing economies.** Int J Caring Sci. 2018 Sep 1; 11(3):1913-41.

5. Center for Disease Control and Prevention. **Device-associated Module UTI.** 2019. Available from https://www.cdc.gov/nhsn/pdfs/pscManual/7pscCauticurrent.pdf.

6. Lee CK, Vachier J, de Anda J, Zhao K, Baker AE, Bennett RR, Armbruster CR, Lewis KA, Tarnopol RL, Lomba CJ, Hogan DA. **Social cooperativity of bacteria during reversible surface attachment in young biofilms: A quantitative comparison of Pseudomonas aeruginosa PA14 and PAO1.** MBio. 2020 Feb 25; 11(1):1-21. e02644-19.

7. Khelissa SO, Abdallah M, Jama C, Faille C, Chihib NE. **Bacterial contamination and biofilm formation on abiotic surfaces and strategies to overcome their persistence.** J Mater Environ Sci. 2017; 8(9):3326-3346.

8. Wilson C, Lukowicz R, Merchant S, Valquier-Flynn H, Caballero J, Sandoval J, Okuom M, Huber C, Brooks TD, Wilson E, Clement B. **Quantitative and qualitative assessment methods for biofilm growth: A mini-review. Research & reviews.** Journal of engineering and technology. 2017 Dec; 6(4): 1-42.

9. Mandakhalikar KD, Rahmat JN, Chiong E, Neoh KG, Shen L, Tambyah PA. **Extraction and quantification of biofilm bacteria: Method optimized for urinary catheters.** Sci Rep. 2018; 8(1):1-9.

10. Halim RM, Kassem NN, Mahmoud BS. **Detection of biofilm producing staphylococci among different clinical isolates and its relation to methicillin susceptibility.** Open access Macedonian J. Med. Sci. 2018; 6(8):1335.

11. Haney EF, Trimble MJ, Cheng JT, Vallé Q, Hancock RE. **Critical assessment of methods to quantify biofilm growth and evaluate antibiofilm activity of host defence peptides.** Biomolecules. 2018; 8(2):29.

12. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. **Evaluation of different detection methods of biofilm formation in the clinical isolates.** Brazilian J Infect Dis. 2011; 15(4): 305-311.

13. Al-Ouqaili MT. **Biofilm antimicrobial susceptibility pattern for selected antimicrobial agents against planktonic and sessile cells of clinical isolates of staphylococci using MICs, BICs and MBECs.** Asian Journal of Pharmaceutics (AJP). 2018; 12(04): 1375-1383.

14. Kirmusaoglu S, Yurdugül S, Metin A, Vehid S. **The effect of urinary catheters on microbial biofilms and catheter associated urinary tract infections.** Urol J. 2017; 14(2):3028-3034.

15. Brun-Buisson C, Abrouk F, Legrand P, Huet Y, Larabi S, Rapin M. **Diagnosis of central venous catheter-related sepsis: Critical level of quantitative tip cultures.** Arch Intern Med. 1987; 147(5): 873-877.

16. Winn W, Allen S, Janda W, et al. **Koneman's color atlas and textbook of diagnostic microbiology.** 6th ed. New York: Lippincott Williams and Wilkins; 2006.

17. Tiwari G, Arora DR, Mishra B, Dogra V. **Comparative evaluation of methods used for detection of biofilm production in multidrug resistant bacteria.** National J of Integrated Res in Med. 2017; 8(5): 1-8.

18. Vidyasagar K, Nagarathnamma T. **Study of catheter associated urinary tract infection and biofilm production by Escherichia coli.** Indian J of Microbiol Res. 2018; 5(3):387-392.

19. Ramadan R, Omar N, Dawaba M, Moemen D. **Bacterial biofilm dependent catheter associated urinary tract infections: Characterization, antibiotic resistance pattern and risk factors.** Egyptian J of Basic and Appl Sci. 2021; 8(1):64-74.

20. Joulaei A, Bayatmakoo Z, Mobaiyen H. **Usefulness of Urinary Catheterization in Patients Admitted to Infectious Ward of Sina Hospital, Tabriz, Iran.** Sepsis. 2015; 12(17.4): 17-4.

21. Holá V, Ruzicka F, Horka M. **Microbial diversity in biofilm infections of the urinary tract with the use of sonication techniques.** FEMS Immun & Medical Microbiol 2010; 59(3): 525-528.

22. Ko MC, Liu CK, Woung LC, Lee WK, Jeng HS, Lu SH, Chiang HS, Li CY. **Species and antimicrobial resistance of uropathogens isolated from patients with urinary catheter.** The Tohoku J Exp Med. 2018; 214(4): 311-319.

23. Chaudhari R, Deshpande A, Angadi SA, Koppikar GV. **A prielimirary study of catheter associated bacteriuria.** Hospital J. [serial online]. Available from: URL: http:// www. bhj. org/journal/2004_4603_july/july_2004/htm/ original_preliminary. htm. 2004.

24. Stark RP, Maki DG. **Bacteriuria in the catheterized patient: What quantitative level of bacteriuria is relevant?** New Eng J Med. 1984; 311(9): 560-564.

25. Xu Y, Moser C, Al-Soud WA, Sørensen S, Høiby N, Nielsen PH, Thomsen TR. **Culture-dependent and-independent investigations of microbial diversity on urinary catheters.** J Clin Microbiol. 2012; 50(12): 3901-3908.

26. Murugan K, Selvanayaki K, Al-Sohaibani S. **Urinary catheter indwelling clinical pathogen biofilm formation, exopolysaccharide characterization and their growth influencing parameters.** Saudi J Biol Sci. 2016; 23(1):150-159.

27. Chatterjee S, Maiti PK, Dey R, Kundu AK, Dey RK. **Biofilms on indwelling urologic devices: Microbes and antimicrobial management prospect.** Ann Medical and Health Sci Res. 2014; 4(1): 100-104.

28. Osungunna MO, Onawunmi GO. **Antibiotic resistance profiles of biofilm-forming bacteria associated with urine and urinary catheters in a tertiary hospital in Ile-Ife, Nigeria.** Southern Afr J Infect Dis. 2018; 33(3): 80-85.

29. Mulla SA, Revdiwala S. **Assessment of biofilm formation in device-associated clinical bacterial isolates in a tertiary level hospital.** Indian J of Pathol and Microbiol. 2011; 54(3): 561.

30. Sabir N, Ikram A, Zaman G, Satti L, Gardezi A, Ahmed A, Ahmed P. **Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance.** Amer J infect Contr. 2017; 45(10): 1101-1105.

31. Alves MJ, Barreira JC, Carvalho I, Trinta L, Perreira L, Ferreira IC, Pintado M. **Propensity for biofilm formation by clinical isolates from urinary tract infections: Developing a multifactorial predictive model to improve anti biotherapy.** J Medical Microbiol. 2014; 63(3): 471-477.

32. Bagchi I, Jaitly NK, Thombare VR. **Microbiological evaluation of catheter associated urinary tract infection in a tertiary care hospital.** Peoples J Sci Res. 2015; 8: 23-29.

33. Ramadan R, Omar N, Dawaba M, Moemen D. **Bacterial biofilm dependent catheter associated urinary tract infections: Characterization, antibiotic resistance pattern and risk factors.** Egyptian J of Basic and Appl Sci. 2021; 8(1):64-74.

AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Sidra Zaman	Final drafting of article, Methodology, Result, Discussion.	
2	Ayesha Khushi	Introduction & review of literature.	
3	Munazza Yasmeen	Review of data analysis, Proof reading.	
4	Majid Rauf	References correction, Revising manuscript, Adjustments for word count.	
5	Saleha Maqsood	Data collection and final review for corrections.	