**ORIGINAL ARTICLE**

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

Sidra Zaman¹, Ayesha Khushi², Munazza Yasmeen³, Majid Rauf⁴, Saleha Maqsood⁵

**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.³⁴ 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

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**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.\(^9\)\(^,\)\(^9\) 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.
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 (mean age 58) and Ramadan et al (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).

<table>
<thead>
<tr>
<th>Sample</th>
<th>One Isolate No. (%)</th>
<th>Two Isolates No. (%)</th>
<th>Multiple Isolates No. (%)</th>
<th>No Growth No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catheter Tip</td>
<td>42* (56.0%)</td>
<td>09* (12.0%)</td>
<td>11* (14.7%)</td>
<td>13 (17.3%)</td>
</tr>
<tr>
<td>Urine</td>
<td>34 (45.3%)</td>
<td>06 (8.0%)</td>
<td>.11 (14.7 %)</td>
<td>24** (32.0 %)</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Biofilm Forming Capacity</th>
<th>Isolates From Catheter Tip (n=57)</th>
<th>Isolates From Urine (n=42)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>32 No. 56.1%</td>
<td>20 No. 47.6%</td>
<td>*</td>
</tr>
<tr>
<td>Moderate</td>
<td>20 No. 35.1%</td>
<td>18 No. 42.9%</td>
<td>*</td>
</tr>
<tr>
<td>Weak</td>
<td>04 No. 7.0%</td>
<td>03 No. 7.1%</td>
<td>*</td>
</tr>
<tr>
<td>None</td>
<td>01 No. 1.8%</td>
<td>01 No. 2.4%</td>
<td>*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>No. of Isolates</th>
<th>Biofilm Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>06</td>
<td>06</td>
</tr>
<tr>
<td>21-40</td>
<td>06</td>
<td>04</td>
</tr>
<tr>
<td>41-60</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>61-80</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>Above 80</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

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\textsuperscript{20} and catheter urine sample culture\textsuperscript{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.\textsuperscript{22}

Likewise, studies by several other researchers also documented that catheter tips are inappropriate for culture use because they overestimate microbial presence and diversity.\textsuperscript{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.\textsuperscript{25} Similar results were seen in another Indian research as well.\textsuperscript{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%).\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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


**AUTHORSHIP AND CONTRIBUTION DECLARATION**

<table>
<thead>
<tr>
<th>No.</th>
<th>Author(s) Full Name</th>
<th>Contribution to the paper</th>
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