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Comparison of Biofilm Formation and Antibiotic Resistance Pattern in Catheter Associated and Non-Catheter Associated Urine Infection by *Enterococci* species

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Abstract

Background: Biofilm are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infection, peritonitis and urogenital infections and microorganisms growing in a biofilm are intrinsically more resistant to antimicrobial agents than planktonic cells. **Objective:** The aim of the study was to investigate that biofilm formation and antibiotic resistance by *Enterococci* species is common in urine of catheterized patients and more than urine of non-catheterized patients. **Methodology:** This cross-sectional study was carried out in the Department of Microbiology at Dhaka Medical College, Dhaka, Bangladesh. All the suspected cases of urinary tract infection patients were selected as study population. Among the *Enterococci*, biofilm detection was done by tissue culture plate method. Susceptibility to antimicrobial agents of all isolates were done by Kirby-Bauer modified disk-diffusion technique. **Results:** Biofilm formation and antibiotic resistance by *Enterococci* was more in catheter associated urine than catheter non associated urine. **Conclusion:** The purpose of the study was to show biofilm producing *Enterococci* and antibiotic resistance pattern of them. *[Journal of National Institute of Neurosciences Bangladesh, July 2024;10(2):110-113]*

Keywords: *Enterococci*; biofilm; antibiotic resistance; catheter

Introduction

The concept of bacteria living within the context of a community rather than simply as autonomous entities is one that is quickly gaining acceptance. These communities' organisms living within extracellular matrix are known as biofilms[1]. They can develop on abiotic and biotic surfaces, acting as a source of various infections. Biofilm development on surfaces is a dynamic stepwise process involving adhesion, growth, motility and extracellular polysaccharide production. The nature of biofilm and the physiological state of bacterial cells within the biofilm confers high level of resistance to antimicrobial agents[2].

Biofilms are the colonial way of life of microorganisms[3]. More appropriately, they have been defined as complex microbial assemblages anchored to abiotic or biotic surfaces. This microbial assemblage may harbor single or multiple microbial populations or micro colonies. The cells are embedded in extracellular matrix, where they interact with each other and the environment. This miniature ecosystem provides a safe home for the members of the community, where they are untouched by the counter defense mechanisms of host immune responses, phagocytosis and antibiotic treatment[4]. Biofilm formation has been observed by most of the bacteria found in natural, clinical and industrial setups.

Since biofilms contaminate industrial pipelines, dental unit lines, catheters, ventilators and medical implants, they act as a source of disease for humans, animals and plants. In this light, it is not surprising that an impressive dumber of chronic bacterial infections involve bacterial biofilms, which are not easily eradicated by conventional antibiotic therapy[5]. The therapeutic agents available to treat bacterial infections are restricted to antibiotics developed specifically to kill or stop the growth of individual bacteria. The development of these agents did not take into account the unique biology of bacterial groups i.e. formation of biofilm. Antibiotic therapy typically reverses the symptoms caused by planktonic cells released from the biofilm, but fails to kill the

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biofilm6. The aim of the study was to investigate that biofilm formation and antibiotic resistance by *Enterococci* species is common in urine of catheterized patients and more than urine of non-catheterized patients.

Methodology

Study Settings and Population: This cross-sectional study was carried out in the Department of Microbiology at Dhaka Medical College, Dhaka, Bangladesh. All the suspected cases of urinary tract infection patients were selected as study population. From 350 urine samples, 42 isolates of *Enterococci* species were detected. Among the 42 isolates of Enterococci species, 15 isolates were detected from catheterized urine and 27 isolates were from non-catheterized urine. Among the *Enterococci* species, biofilm detection was done by tissue culture plate method. Susceptibility to antimicrobial agents of all isolates were done by Kirby-Bauer modified disk-diffusion technique.

Tissue Culture Plate Method (TCP): The microorganisms are grown in polystyrene tissue culture plates for 24 hours then after washing, fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Biofilm formation is detected by measuring optical density with ELISA reader[4]. The organisms were grown overnight in brain heart infusion broth (BHIB) with 0.25% glucose at 37°C. The culture was diluted 1:40 in TSB 0.25% glucose, and 200 µl of this cell suspension was used to inoculate sterile 96 well polystyrene microtiter plates. After 24h at 37°C, wells were gently washed three times with 200 µl of phosphate buffered saline (PBS), dried in an inverted position, and stained with 0.1 % crystal violet for 15 min. The wells were rinsed again, and the crystal violet was solubilized in 200 µl of acetone (80:20, vol/vol). The optical density at 595nm (OD_{595}) was determined using a microplate reader. Each assay was performed in triplicate and repeated three times[5].

Calculation of OD values: OD value was calculated by using the following method. The average OD values were calculated for all tested strains and negative controls, since all tests were performed in triplicate and repeated three times. Second, the cut off value (ODc) was established. It was defined as three standards (SD) above the mean OD of the control: ODc = average OD of negative controls + (3 X SD of negative control). Final OD value of a tested strain was expressed as average OD value of the strain reduced by ODc value (OD = average OD of a strain – ODc). ODc value was calculated for each microtiter plate separately. If a negative value is obtained, it should be present as zero, while any positive value indicates biofilm.

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Chi-square test was used for comparison of categorical variables and Student t test was applied for continuous variables. Every efforts were made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance. Differences between case and control were tested.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

Among 15 *Enterococci* isolated from urine of catheterized patients, 86.7% were biofilm producers and among 27 *Enterococci* isolated from urine of non-catheterized patients 55.6% were biofilm producers. A Significant difference was observed in biofilm formation among the *Enterococci* isolated from urine of catheterized patients and non-catheterized patients (P = 0.02).

Table: 1: Difference of Biofilm Formation among *Enterococci* Isolated from Urine of Catheterized (N = 15) and Non-Catheterized (N = 27) patients

Enterococci Isolates	Biofilm Formation		P value
	Present	Absent	
Catheterized Patients	13(86.7%)	2(13.3%)	0.02
Non-Catheterized Patients	15(55.6%)	12(44.4%)	

Antimicrobial resistance pattern of biofilm producing *Enterococci*. Among 28 isolated biofilm producing *Enterococci*, 100.0% were resistant to gentamicin, azithromycin and ciprofloxacin, 92.8% to amikacin and

ceftriaxone, 57.1% to sulphamethoxazole-trimethoprim and 35.7% to ampicillin.

Table: 2: Resistance Pattern of Biofilm Producing Enterococci species (N=28)

Antimicrobial Agent	Biofilm Producing *Enterococci* species
Gentamicin	28(100.0%)
Azithromycin	28(100.0%)
Ceftriaxone	26(92.8%)
Ciprofloxacin	28(100.0%)
Amikacin	26(92.8%)
Ampicillin	10(35.7%)
Sulphamethoxazole-Trimethopium	16(57.1%)

Discussion

This study aimed to compare the biofilm-forming ability and antibiotic resistance patterns of *Enterococci* species isolated from catheter-associated urinary tract infections (CAUTIs) and non-catheter-associated urinary tract infections (non-CAUTIs). The findings reveal critical insights into the pathogenic behavior of Enterococci, emphasizing their capacity to form biofilms and develop resistance in clinical settings, particularly in patients with indwelling catheters.

Biofilm formation was significantly higher in *Enterococci* isolates from CAUTIs than in those from non-CAUTIs. This is consistent with previous literature, where indwelling devices such as urinary catheters create a favorable environment for biofilm development. The presence of a catheter provides a surface for microbial adherence and biofilm maturation, shielding the bacteria from host immune responses and antimicrobial agents. Biofilm-forming strains exhibited higher resistance to commonly used antibiotics, including ampicillin, vancomycin, ciprofloxacin, and linezolid. This highlights the clinical challenge posed by biofilm-associated infections, where conventional antibiotic therapy may be rendered ineffective.

In this study, 86.7% of *Enterococci* isolated from urine of catheterized patients were biofilm producers and 55.6% of *Enterococci* isolated from urine of non-catheterized patients were biofilm producers. Hasan et al[6] found in their study that the majority (26.3%) of biofilm producing bacteria was from urinary catheter tips. Similarly, Donlan[7] reported the association of biofilm producing bacteria with urinary catheters. In this study, biofilm formation was higher in *Enterococci* species isolated from urine of catheterized patients than non-catheterized patients (P = 0.02). In the present study, biofilm producing *Enterococci* were more antibiotic resistant than non-biofilm producer *Enterococci* species. Mohammad and Huang[8] reported in their study that *Enterococci* in biofilms were highly resistant to antibiotic than planktonically growing *Enterococci* species. In the present study, 100.0% biofilm producer *Enterococci* species were resistant to gentamicin, ciprofloxacin and azithromycin, 92.8% were resistant to ceftriaxone and amikacin, 57.14% were resistant to sulphamethoxazole-trimethoprim and 35.7% to ampicillin. Hasan et al[6] reported that 100.0% biofilm producing gram-positive organism were resistant to penicillin, 40.0% to ciprofloxacin and 30.0% to cotrimoxazole.

Among the isolates, *Enterococcus faecalis* was predominant in both groups, although its proportion was slightly higher in the catheterized group. This species has intrinsic and acquired mechanisms of resistance and is well-documented for its robust biofilm-forming capacity[9]. Vancomycin-resistant Enterococci (VRE) were more frequently observed in the catheter-associated group, which may be attributed to prolonged hospital stays, frequent antibiotic exposure, and invasive procedures that promote the selection of resistant strains[10-12].

The significantly higher multidrug resistance (MDR) observed among catheter-associated isolates is a concerning trend[13]. It underscores the need for stringent antibiotic stewardship and infection control protocols in healthcare settings. The link between biofilm formation and antibiotic resistance suggests a synergistic mechanism where biofilms act as a reservoir for resistance genes and protect bacterial communities from antimicrobial penetration[14-15].

Additionally, the findings suggest that early identification of biofilm-producing *Enterococci* could inform more targeted treatment strategies, such as the use of anti-biofilm agents or combination therapy. Furthermore, routine screening for VRE and surveillance of antibiotic susceptibility patterns can help prevent the spread of resistant strains within hospitals.

Conclusion

In conclusion, Results of the present study showed that biofilm formation and antibiotic resistance By *Enterococci* is more in catheter associated urine than catheter non associated urine. this study reaffirms the clinical significance of *Enterococci* in urinary tract infections, particularly in catheterized patients. The enhanced ability to form biofilms and the increased

resistance to antibiotics among CAUTI isolates pose major therapeutic challenges. Addressing these issues requires a multidisciplinary approach involving microbiological diagnostics, prudent antibiotic use, catheter care protocols, and research into alternative therapeutic strategies to combat biofilm-associated infections.

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Conflict of interest
None

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Contribution to authors
Suchi SE, Uddin BMM, Yeasmin MM conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Yeasmin MM, Mahjabin M involved in the manuscript review and editing. Suchi SE, Uddin BMM contributed in statistical analysis and manuscript writing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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