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Major Article

Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance

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Background: We sought to determine the incidence of bacterial biofilm-based catheter-associated urinary tract infections, identify variables affecting biofilm formation, and identify etiologic bacterial pathogens and antibiotic-resistance patterns associated with biofilm-based catheter-associated urinary tract infections (CAUTIs) in our setup.

Methods: Patients who developed at least 2 symptoms of urinary tract infection after at least 2 days of indwelling urinary catheters were included. Urine was collected aseptically from catheter tubing and processed per standard microbiologic practices. Bacterial pathogens were identified on the basis of gram staining, colony morphology, and biochemical reactions. The detection of the biofilm was done using the tube adherence method. Drug susceptibility testing was done using the Kirby-Bauer disc diffusion method.

Findings: Biofilm was detected in 73.4% isolates, whereas 26.6% of isolates were nonbiofilm producers. Mean duration of catheterization after which biofilm was detected was 5.01 ± 1.31 days. A latex catheter was used in 69.5% of patients, whereas a silicone catheter was used in 30.4% of patients. *Escherichia coli* was found to be the most common pathogen isolated (52.3%), whereas *Enterobacter cloacae* exhibited the highest biofilm production (87.5%) among isolated pathogens. Among biofilm producers, the highest resistance was observed with ampicillin (100%). Fosfomycin exhibited the lowest resistance (17.2%). Significant association with biofilm was detected for gender, duration of catheterization, and type of catheter.

Conclusion: Biofilm-based CAUTI is an emerging problem. *E coli* was the most frequent isolate. High antibiotic resistance was observed in biofilm-producing strains. Using the variables affecting biofilm formation, tailored intervention strategies can be implemented to reduce biofilm-based CAUTIs.

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Urinary tract infections (UTIs) are the leading cause of health care-associated infections (HAIs), accounting for about 40% of all HAIs. Out of these, a large proportion, 80%, involve catheter-associated urinary tract infections (CAUTIs).[1] Because urinary stents or catheters are routinely used in urology practice and despite advances in design and materials used, UTIs remain among the major complications due to the contamination of such indwelling devices.[2] Twelve percent to 16% of adult hospital inpatients have an indwelling urinary catheter at some time during admission.[3] CAUTI is associated with high morbidity, high mortality, increased length of hospital stay, and increased cost of treatment.[4,5]

Biofilms are the sessile polymicrobial communities that adhere to biotic and abiotic surfaces and are encased within a self-produced extracellular polymeric matrix.[6] Biofilm is among the pathogens important virulent factors; it not only allows pathogen to escape host defenses but also enhances antimicrobial resistance due to slow penetration, resistant phenotype, and altered microenvironment.[7-9] Biofilms pose a serious threat because of their higher propensity to cause device-related infections that are not only difficult to treat but also often persistent and recurrent.[10]

The organisms that commonly contaminate indwelling urinary catheters and develop biofilm are *Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and other gram-negative organisms.[6,11]

With regard to resistance profile among biofilm-producing bacterial strains, carbapenems and fosfomycin are good therapeutic options. Biofilm-associated infections are often multidrug-resistant.[12]

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The urine of patients with indwelling catheters is the major site of isolation of resistant gram-negative organisms in both acute and long-term care facilities, including extended-spectrum β-lactamase–producing enterobacteriaceae[13] and carbapenem-resistant enterobacteriaceae.[14]

The rationale of our study was to determine the incidence of bacterial biofilm-based CAUTI. To identify variables affecting biofilm formation, identify etiologic bacterial pathogens, and antibiotic-resistance patterns associated with biofilm-based CAUTIs in our setup.

MATERIAL AND METHODS

This was a cross-sectional study conducted at the Department of Microbiology, Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi, Pakistan, July 2015-January 2017. A total of 1,070 urine specimens from 8 different departments of tertiary care hospitals of northern Pakistan were included in the study. CAUTI was defined using a combination of clinical signs and symptoms and laboratory criteria (Table 1).

All catheterized patients irrespective of gender and age between 12 and 70 years who met the criteria of CAUTI were included in the study. Noncatheterized patients, those already diagnosed with a UTI, those who were immunosuppressed, and those taking antibiotic prophylaxis before catheterization were excluded. Permission from the Institutional Ethical Committee was taken. Hospital identity number, age, gender, symptoms, duration of catheterization after which biofilm was detected, and type of catheter were recorded. Urine samples were collected aseptically from catheter tubing and transported to a laboratory without any delay. Samples were taken on day zero; that is, the day when a catheter was inserted to rule out previous UTI. Urine cultures were repeated in all patients who developed symptoms of UTI, like fever (>38°C), suprapubic tenderness, costovertebral angle pain, or tenderness after 48 hours of catheterization.

Urinalysis

Urinalysis was done using Urinalysis Hybrid (FUS-2000, DIRUI, Changchun, China) to detect pyuria (urine specimen with ≥10 white blood cells/mm^3 unspun urine or >5 white blood cells/high power field spun urine) and nitrite. Gram staining of unspun urine was done to detect microorganisms.

Culture technique

Each urine specimen was inoculated on cysteine lactose electrolyte-deficient media (Oxoid CM0301; ThermoScientific, Waltham, MA), Blood agar (Oxoid CM0055), and Mac Conkey agar (Oxoid CM0007) with 1 μL calibrated disposable loop. Culture plates were inoculated aerobically at 37°C for 24 hours. Next day, plates were checked for growth of any pathogen. In case of significant growth on media; that is, ≥10^3 and <10^5 CFU/mL, the isolates were identified on the basis of colony morphology; Gram staining; and biochemical reactions like catalase, coagulase, and dNAase, in case of gram-positive organisms and applying analytical profile index strips, (BioMerieux, Marcy-l'Étoile, France) in case of gram-negative organisms. If there was no growth on the first day, plates were re-inoculated for another 24 hours.

Table 1
Catheter-associated urinary tract infection (CAUTI) criteria

All patients who had an indwelling urinary catheter in place for >2 calendar days, with day of device placement being day 1, and catheter was in place on the date of event
And
At least 1 of the following signs or symptoms: fever (>38°C); suprapubic tenderness, costovertebral angle pain, or tenderness
And
At least 1 of the following findings: positive nitrite, pyuria (urine specimen with ≥10 white blood cells/mm^3 unspun urine or >5 white blood cells/high power field spun urine) or microorganisms seen on Gram stain of unspun urine.
And
A positive urine culture of ≥10^3 and <10^5 CFU/mL and with no more than 2 species of microorganisms.

Detection of biofilm formation

The detection of the biofilm was done by the tube adherence method described by Christensen et al.[15] About 10 mL Oxoid Brain Heart Infusion Broth (ThermoScientific) was inoculated with a loopful of microorganisms from overnight culture plates and incubated at 37°C for 48 hours. Then the supernatants were discarded and glass tubes were stained with 0.1% safranin solution, washed with distilled water 3 times, and dried. Slime formation was considered positive when a visible film lined the inner wall of the tube. Ring formation at the liquid air interface was considered as negative. Tests were repeated 3 times (Fig 1). The tube adherence method can be used as a general screening method for detection of biofilm.[16] Easy application, low cost, reliability, excellent sensitivity, and specificity are major advantages of this technique.[17] The pitfall of this test is that quantitative analysis of biofilm cannot be performed.

Figure 1 shows positive and negative tests of biofilm detection. Biofilm formation is indicated by an arrow.

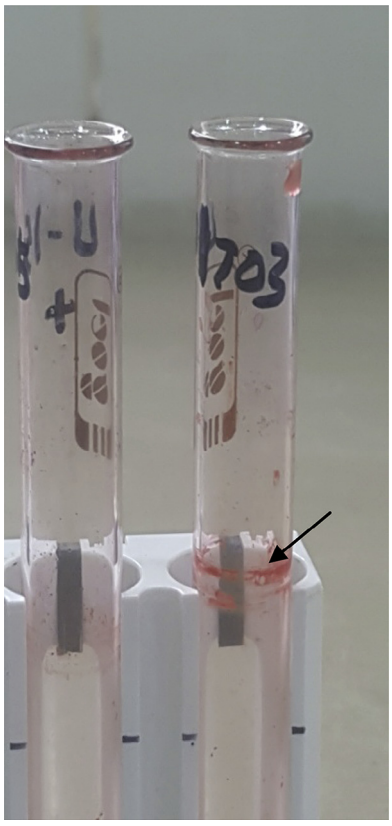

Fig 1. Biofilm detection by the tube adherence method. Arrow points out area of adherence.

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