ORIGINAL ARTICLE

An Investigation on the Detection and Distribution of Biofilm Production Caused Uropathogenic *Escherichia coli* in Tropical Catheterized Patients by Tube Adherence Method

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Abstract

Introduction: Infections of the urinary tract, commonly known as urinary tract infection (UTI), are very common and prevalent issue among patients in the general outdoor clinics. Moreover, association of biofilms further raises concerns in terms of the recurrence of UTI or resistance microorganisms. **Objective:** The objective of the present study includes the investigation of the distribution and detection of biofilm production by uropathogens in tropical catheterized patients by the tube adherence method (TAM). **Materials and Methods:** A total of 100 patients were included in the study. All the patients were tropical catheterized patients admitted in Civil Hospital, Ambala City. The patients were consulted, and the required necessary medical information was recorded in a pre-defined format for easy retrieval. Urine samples were collected and microorganisms identified alongside detected the biofilm production in the samples. **Results and Discussion:** The results demonstrated the prevalence of UTIs among the study patients and *Escherichia coli* had been detected as the most predominant uropathogen responsible for the infection in the catheterized patient. Another hallmark observation demonstrated that resistant *E. coli* cases were most abundant among the isolated and suggestive of strong producer of biofilm. The results also indicated that, as a cost-effective method, TAM is a reliable method for biofilm detection in the present studied patient population. **Conclusion:** This method had been demonstrated to most accurate when the causative organism is resistant *E. coli*.

Key words: Biofilm, catheterized patients, *Escherichia coli*, urinary tract infection, uropathogens

INTRODUCTION

Infections of the urinary tract, commonly known as urinary tract infection (UTI), are very common and prevalent issue among patients in the general outdoor clinics. The National Ambulatory Medical Care Survey (1997) and National Hospital Ambulatory Medical Care Survey indicated UTI as one of the most prevalent clinical problems accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations. It is also a matter of concern that most of the cases of UTI are not reported, and hence, it is not possible to state the accurate incidence of UTIs. In general, causative microorganism enters the urinary tract through the urethra and goes to the bladder for multiplication leading to UTIs.[1] Pharmacological interventions including various antibiotics are available to deal with this kind of infection. However, the formations of protective biofilm by certain microorganisms make the intervention more difficult and can lead to microbial resistance.[2] According to a recent public announcement from the National Institute of Health, "more than 60% of all microbial infections are caused by biofilm."[3,4] Biofilms are defined as "A biofilm is a complex aggregations of

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microorganisms in which cells are adhere to each other and to abiotic or biotic surface." In general, all the urinary tract pathogens are fecal in origin, but only aerobic and facultative aerobic species such as *Escherichia coli* or *Klebsiella pneumoniae* acquire the required attributes to colonize the urethra. Hence, as per studies, *E. coli* is the major biofilm producer in UTI cases accounting about 52.18% followed by *K. pneumoniae* (23.91%), Proteus species (13.04%), and *Enterococcus species* (10.87%).[5,6] Biofilm is the assemblies comprising of microbial cell bound to surface as a protective covering guarding the microbial cells. The biofilm is enclosed in a matrix of polysaccharide and protein material.[7,8]

The chronic UTIs are outcomes of these biofilm produced by microbial cells on the anatomical structures of the genitourinary tract. The biofilm is mostly stubborn leading to the chronic status of these infections. This present study was designed to investigate the prevalence of biofilm in the collected specimens from tropical catheterized patients using tube adherence method (TAM). TAM has been chosen because it is a cost-effective, reliable, routine, and sensitive method for biofilm detection. The study also aimed to investigate the potentiality of biofilm generation by resistant uropathogenic *E. coli* in tropical catheterized patients admitted in Civil Hospital, Ambala City.

MATERIALS AND METHODS

Collection of Samples

The study collected a total of 100 clean urine samples from 100 catheterized patients admitted in Civil Hospital, Ambala City. The patients from which samples collected are presented in the hospital with the complaints of UTI.

Microbiological Processing

UTI is considered positive when observed microscopically a single bacterium in uncentrifuged urine per oil immersion field in Gram smears and more than five white blood cells per high-power field in centrifuged urine. There are several hallmark sign and symptoms corresponding to UTI which include frequency, dysuria, abdominal pain, incontinence, and suprapubic tenderness. Qualitative screening in terms of colony counting has been performed in the urine samples for a confirmation of UTI.[9] For confirmation and identification of microorganism, other routine biochemical tests were performed.

Detection of Biofilm Production

There are several methods available for the detection of biofilms, and all these methods have their own advantages and disadvantages in different clinical setups. In this present study, we have adopted TAM for the detection of biofilm production.

TAM

In a borosilicate glass tube with 10 ml of trypticase soy broth and 1% glucose, loopful microorganism was inoculated from overnight culture plates. These tubes were then incubated in aerobic conditions at 37°C for 24 h. Once incubation is over, the tubes were decanted followed by washing with phosphate buffer saline at pH 7.3 and then subjected to drying. After washing, the tubes were subjected to crystal violet (0.1%) staining for about 15 min. The tubes, then, were decanted and washed with deionized water and dried in inverted position. This method detects the biofilm in the sample, and the test has been considered positive to biofilm when a clearly visible wall-lined and bottom-lined film was detected [Figure 1]. A stained layer at the air-liquid junction or interface denotes negative for biofilm production. The scoring for accounting the biofilm production can be summarized in Tables 1 to 4.

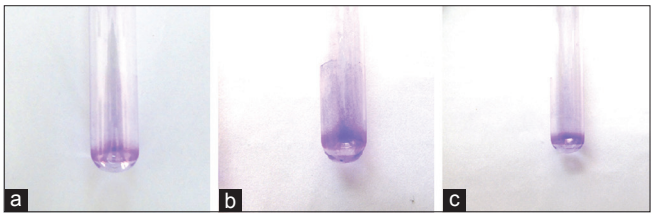

Figure 1: Biofilm detection depiction by tube adherence method. (a) Control, (b) high positive and (c) low positive

Table 1: TAM scoring of biofilm production by the samples

Score	Interpretation of results
1	Weak or no biofilm production
2	Moderate biofilm production
3	Strong or high biofilm production

TAM: Tube adherence method

Table 2: Set of signs and symptoms among the studied patient pool

Symptoms	Distribution
Burning micturition	43
Abdominal pain	27
Fever	24
Anuria/k stone or a tumour	15
Difficulty	17
Frequency	22
Dysuria	14
Hematuria	10
Urgency	21
Renal stone	9

Table 3: Microorganism identified in the isolates and distribution

Organism	Isolates (%)
Resistant *E. coli*	36 (36)
E. coli	29 (29)
K. pneumoniae	22 (22)
P. aeruginosa	5 (5)
Enterobacter spp.	5 (5)
S. aureus	3 (3)
Total	100 (100)

E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus

Table 4: Organism wise distribution of biofilm production

Organism	Total isolates	Biofilm producers (%)
Resistant *E. coli*	36	27 (75)
E. coli	29	-
K. pneumoniae	22	-
P. aeruginosa	5	-
Enterobacter spp.	5	-
S. aureus	3	-
Total	100	27

E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus

RESULTS AND DISCUSSION

The study investigated and screened 100 urine samples from patients diagnosed with UTI. Around 80 (80%) samples were analyzed and found to be positive for Gram-negative organisms and rest 20 (20%) samples were positive as Gram-positive organisms. The results revealed that *E. coli* is mostly predominant bacterial strain in the pool of 100 samples. The other predominant strain detected and identified following *E. coli* is *K. pneumoniae.* In another aspect, *Enterococcus faecalis* was found to be the most abundant Gram-positive organism found. The results demonstrated significant efficacy of the TAM in detecting biofilm in the samples. The results showed that 40 cases were detected by this method as positive for biofilm of total 100 collected samples. This indicated this method as a reliable and sensitive one. Maximum biofilm was detected in isolates which were positive for *E. coli,* followed by *Enterobacter* spp. and *K. pneumoniae*. This revealed clearly that *E. coli* is the most abundant biofilm.

Biofilms are the hallmark factor associated with increased bacterial resistance leading to treatment failure. They cause a serious problem issue for public health due to the increased resistance of biofilm-associated organisms to antimicrobial agents and the perspective of these organisms to root infections in patients with indwelling medical devices. Biofilms protect the bacteria from the destruction effect of antimicrobial agents. Bacteria tend to survive at very high concentration of antimicrobial drugs when associated with biofilms. A biofilm-associated bacterium survives antimicrobial agents at concentrations of 1000–1500 times higher than those needed to eradicate them normally without biofilm. Biofilms are also actively associated and linked to many bloodstream infections and UTIs. In spite of good and excellent aseptic precautions, around 50% of catheterized patients develop bacteremia in the 1st 10–14 days of catheterization.

The present study dealt with 100 specimens and analyzed, of which Gram-negative organisms were found to be the predominant isolates of the total growth. More than 50% of the isolates were found to be of *E. coli* isolates, of which 36% was identified as resistant *E. coli* isolates and 29% as *E. coli* isolates. This has been followed by other isolates including *K. pneumoniae* and *Pseudomonas aeruginosa*. The results of the present study demonstrated the same trend as suggested by previous studies in the same line of research. Several studies involving Indian population also identified *E. coli* as the predominant uropathogen followed by *K. pneumoniae*.

The maximum biofilm production was seen in resistant *E. coli* isolates. Some previous studies showed *Enterococcus* spp. as the principal biofilm producer. The study by Praharaj *et al*. 2013 found 53% of *Enterococcus* spp. isolates to be biofilm producers. In the present study, of 32% isolates of resistant *E. coli*, 23% demonstrated biofilm production. This is probably because, in the present study, the samples analyzed were from tropical patient where *E. coli* have been considered as the main causative organism associated with UTIs.

CONCLUSION

UTIs are very common nowadays and the number of patients requiring a hospital stay are found to be increasing with the increasing cases of treatment failure or relapse due to the growing incidence of antibiotic resistance. The results of the present study have justified the current scenario indicating the higher number of resistant *E. coli* cases and instances observed. The most probable culprit and observed cause were found to the biofilm production by the bacteria resulting in bacterial resistance and treatment failure. Biofilms were found to be the major culprit in this scenario causing the increased trend in recurrent UTIs, leading to increased morbidity in the patient, increased duration of hospital stay, and increased economic burden on the patients. Mostly patients in outpatient department diagnosed with uncomplicated UTI were rarely subjected to biofilm detection which over time paved these uncomplicated UTI cases into complicated one due to the presence of biofilms. Therefore, the possible role of biofilms in making complicated UTI cases

needs to be studied well in detail so that necessary measures can be taken in time to eradicate the uropathogens in complete to avoid any relapses. TAM came handy in this regard which is a method with good reproducibility and good specificity in biofilm detection. This method can be exercised routinely in the microbiology laboratory to detect biofilm production, especially when the causative organism is resistant *E. coli.*

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