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Research Article

DETECTION AND DISTRIBUTION OF BIOFILM PRODUCTION CAUSED BY UROPATHOGENIC *ESCHERICHIA COLI* IN TROPICAL CATHETERISED PATIENTS BY TUBE ADHERENCE METHOD

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Abstract:

The present study aimed to study the distribution and detection of biofilm production by uropathogens in tropical catheterised patients by tube adherence method. The study included 100 tropical catheterized patients admitted in Civil Hospital Ambala City. All the patients were interviewed and medical information was recorded in a pre-determined format. Samples were collected for the tests to identify the microorganisms in the isolates and also to detect the Biofilm product. The results suggested *E. coli* is most predominant uropathogen responsible for urinary tract infections in the catheterized patients in the present studied patient population. Out of these isolates, Resistant *E. coli* cases were found to be most abundant and strong Biofilm producer. Tube Adherence Method was found to be a reliable cost effective method for detection of Biofilm production in this patient population, especially when the causative organism is Resistant *E. coli*.

Keyword: *Escherichia coli*, Biofilm, Catheterised patients, Uropathogens, UTI.

Introduction

Urinary tract infection (UTI) is an extremely common clinical problem. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations.

Nevertheless, it is difficult to accurately assess the incidence of UTIs, because they are not reportable diseases

¹. Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder ². The armament of therapeutic agents available to treat bacterial infections today is

restricted to antibiotic developed specifically to kill or stop the growth of individual bacteria to survive and protect themselves against various environments, like antibiotic agents, microorganisms produce a barrier, biofilms³. According to a recent public announcement from National Institute of Health, “more than 60% of all microbial infections are caused by biofilm”^{4, 5}. A biofilm is a complex aggregations of microorganisms in which cells are adhere to each other and to abiotic or biotic surface. Most urinary tract pathogens are faecal in origin, but only aerobic and facultative aerobic species such as *Escherichia coli* or *Klebsiella pneumonia* possess the necessary attributes to colonize the urethra. Therefore Major biofilm producing bacteria in UTI’s is *E. coli* (52.18%), followed by *K. pneumonia* (23.91%), *Proteus* species (13.04%) and *Enterococcus species* (10.87%)^{6, 7}. Biofilms are an assembly of microbial cells formed by bacterial species that are irreversibly associated with a surface and enclosed in a matrix of polysaccharide and protein material^{8, 9}. This confers a number of advantages such as protection from antimicrobial agents, exchange of nutrients and exchange of genetic material¹⁰.

Biofilms are responsible for nosocomial infections and chronic infections¹¹. Biofilms may form on anatomical structures of the genitourinary tract and cause chronic urinary tract infection. This study was undertaken to study the prevalence of Biofilm in specimens collected from tropical catheterized patients by using Tube Adherence Method, which is sensitive, easy to perform and can be routinely employed. The present study aimed to detect the potential of biofilm formation by multidrug resistant Uropathogenic *Escherichia coli* strains in Tropical catheterized patients admitted in Civil Hospital Ambala City.

Materials and Methods

Sample Collection

A total of 100 clean catch urine samples from catheterized patients admitted in Civil Hospital Ambala City, presenting with complaints of urinary tract infection.

Microbiological processing

A single bacterium in uncentrifuged urine per oil immersion field in Gram smears and more than 5 White Blood Cells (WBCs) per HPF (High Power Field) in centrifuged urine was considered as an hallmark of UTI (Urinary Tract Infection). A set of symptoms including dysuria, frequency, incontinence, abdominal pain and suprapubic tenderness was also evaluated. The colony count in the urine sample was done qualitatively to get the results of confirmation of significant infection¹². The pathogens were identified using routine biochemical tests.

Detection of biofilm production

Tube Adherence Method was used for the detection of biofilm production.

Tube Method - A loopful of test organisms from overnight culture plates were inoculated in borosilicate glass tubes containing 10ml of Trypticase soy broth with 1% glucose. The tubes were then incubated at 37°C for 24 hours aerobically. After incubation, the tubes were decanted and washed with phosphate buffer saline at pH 7.3 and dried. Tubes were then stained with crystal violet (0.1%) for 15 minutes. The stain was decanted and the tubes were washed with de-ionised water and dried in inverted position.

Biofilm formation was considered positive when a visible film lined the walls and the bottom of the tube (Figure 1). Formation of a stained layer at the air-liquid interface was considered negative for biofilm formation. The amount of biofilm formed was scored as:

Table-1. Scoring of Biofilm production by Tube Adherence Method (TAM).

Score	Interpretation of Results
1	Weak or None biofilm production
2	Moderate biofilm production
3	Strong or High biofilm production

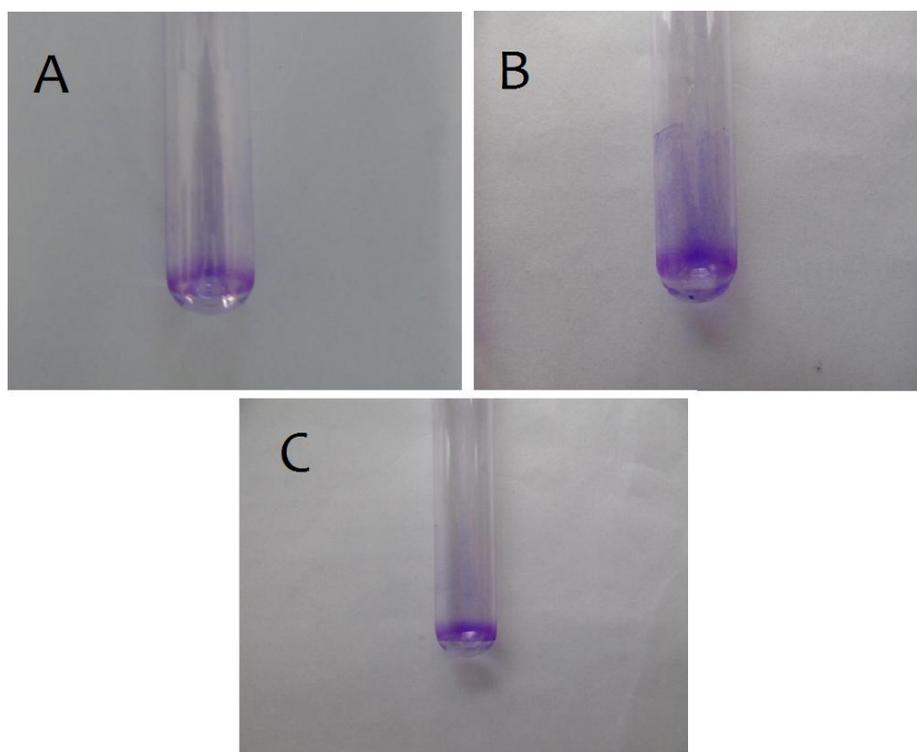


Figure 1. Detection of Biofilm production by Tube Adherence Method. A. Control, B. High Positive and C. Low Positive.

Results and Discussion

A total of 100 urine specimens from patients admitted with UTI were analyzed. Gram negative organisms were isolated from 78 (78 %) specimens and Gram positive growth was seen in 22 (22 %) specimens. *Escherichia coli* was the commonest organism isolated followed by *Klebsiella pneumoniae*. Among Gram positive organisms, *Enterococcus faecalis* was the predominant isolate. Out of 100 cases, 42 (42 %) isolates showed biofilm formation by the Tube Adherence Method. Maximum biofilm production was shown by *Escherichia coli* in isolates followed by *Klebsiella pneumoniae* and *Enterobacter spp.*

Table-2. Symptoms distribution among hundred studies patient.

Symptoms	Distribution
Burning Micturation	52
Abdomeninal pain	36
Fever	33
Anuria/k stone or tumour	14
Difficulty	16
Frequency	31
Dysuria	13
Haematuria	9
Urgency	30
Renal stone	8

Biofilms pose a serious problem for public health because of increased resistance of biofilm associated organisms to anti-microbial agents and the potential of these organisms to cause infections in patients with in-dwelling medical devices. Bacteria in a biofilm survive antimicrobial agents at concentrations 1000-1500 times higher than those needed to eradicate their planktonic counterparts. Many bloodstream infections and urinary tract infections are associated with biofilm formation. Despite good aseptic precautions, around 50% of catheterized patients develop bacteriuria in the first 10-14 days of catheterization. Of the 100 specimens analyzed, Gram negative organisms were the predominant isolates of the total growth. *Escherichia coli* were isolated from more than half the urine specimens followed by *Klebsiella pneumoniae* and *Pseudomonas*

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aeruginosa. Out of the total *Escherichia coli* isolates, 32 % accounted for Resistant *Escherichia coli*. Previous studies in the same line of research also in accordance with the present results. Indian studies also showed *Escherichia coli* and *Klebsiella pneumoniae* as the predominant uro-pathogens. The same results were found in this present study also. The maximum biofilm production was seen in Resistant *Escherichia 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, out of 32 % isolates of Resistant *Escherichia 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.

Table-3. Microorganism identified in the isolates and distribution.

Organism	Isolates	Percent
Resistant <i>Escherichia coli</i>	32	32
<i>Escherichia coli</i>	28	28
<i>Klebsiella pneumoniae</i>	25	25
<i>Pseudomonas aeruginosa</i>	7	7
<i>Enterobacter spp.</i>	4	4
<i>Staphylococcus aureus</i>	4	4
Total	100	100

Table-4. Organism wise distribution of Biofilm production.

Organism	Total Isolates	Biofilm Producers	Percent
Resistant <i>Escherichia coli</i>	32	23	71.88 %
<i>Escherichia coli</i>	28	-	-
<i>Klebsiella pneumoniae</i>	25	-	-
<i>Pseudomonas aeruginosa</i>	7	-	-
<i>Enterobacter spp.</i>	4	-	-
<i>Staphylococcus aureus</i>	4	-	-
Total	100	23	-

Conclusion

In the conclusion, biofilms are a major cause of recurrent urinary tract infections (UTIs), which leads to increased morbidity in the patient, increased duration of hospital stay and increased economic burden on the patients. Uncomplicated UTI has rarely been studied for biofilm formation over the entire spectrum of uropathogens. The role of biofilms in conversion of uncomplicated UTI to chronic UTI due to partial clearance of the infection needs to be studied in greater detail. Tube Adherence method (TAM) is a method with good reproducibility and good specificity. This method can be used routinely in the microbiology laboratory to detect biofilm formation especially when the causative organism is Resistant *E. coli*.

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