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Prevention of Biofilm formation in Urinary Catheter by coating Enzymes/ Gentamycin/ EDTA

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***Abstract*—**Urinary Tract Infections (UTI) account for an estimated 25-40% nosocomial infection, out of which 90% are associated with urinary catheter, called Catheter associated urinary tract infection (CAUTI). The microbial populations within CAUTI frequently develop as biofilms. In the present study, microbial contamination of indwelling urinary catheters was investigated. Biofilm forming ability of the isolates was determined by tissue culture plate method. Prevention of biofilm formation in the urinary catheter by *Pseudomonas aeruginosa* was also determined by coating the catheter with some enzymes, gentamycin and EDTA. It was found that 64% of the urinary catheters get contaminated during the course of catheterization. Of the total 6 isolates, biofilm formation was seen in 100% *Pseudomonas aeruginosa* and *E. coli*, 90% in *Enterococci*, 80% in *Klebsiella* and 66% in *S. aureus*. It was noted that the biofilm production by *Pseudomonas* was prolonged by 7 days in amylase, 8 days in protease, 6 days in lysozyme, 7days in gentamycin and 5 days in EDTA treated catheter.

***Keywords*—**CAUTI, biofilm, enzymes, EDTA, Pseudomonas.

I. Introduction

PATIENTS requiring an indwelling catheter are predisposed to the development of CAUTI by potentially pathogenic multidrug resistant organisms in the hospital setting. Clinical observations have established that the microbial populations within CAUTI frequently develop as biofilms, directly attaching to the surface of catheters [1] due to the secretion of sticky extracellular polymeric substances (EPS) that forms a biofilm matrix. Enzymes have been used and proven to be effective for the degradation of the multistructural EPS of the biofilms. The mode in which enzymes destroy the EPS is by degrading the physical integrity of the EPS [2]. Another way to prevent the biofilm formation within a urinary catheter is to impregnate catheters with a broad spectrum antimicrobial agent. Therefore, planktonic bacteria could be attacked before they colonize the catheter surface and develop into a biofilm. EDTA has a very important role to play as an 'antibiofilm' agent and therefore may have important implications for use in controlling biofilm in catheters [3]. Thus the present study was aimed at isolation and identification of bacteria from urinary catheter of patients, detection of their ability to form biofilm and *in vitro* examination of the ability of some enzymes, gentamycin and EDTA coated catheters to resist biofilm formation by *Pseudomonas aeruginosa*.

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II. Material and Methods

A. Isolation and Identification of Bacteria from Urinary Catheters

Total 100 Foley urinary catheters were collected aseptically from catheterized patients in sterile containers. Sections 1-2 cm and 3-4 cm from the catheter tip were cut, washed with sterile distilled water and suspended in Quarter strength Ringers solution (10 ml) in sterile test tubes. Sonication for 5 min at 35 kHz in a Transonic water bath and vortex mixing for 2 minute was used to remove and disrupt the colonizing biofilms. Loopful of the solution was inoculated on UTI chromogenic media (Hi Media make). After 24 hours of incubation, the resulting colonies were identified by standard methods.

B. Detection of Biofilm Formation by the Isolates

Biofilm formation of bacterial isolates from urinary catheters was determined by tissue culture plate method. This quantitative test described by Christensen *et al* [4] is considered the gold-standard method for biofilm detection [5]. Organisms isolated from fresh agar plates were inoculated in 10 mL of trypticase soy broth with 1% glucose. Broths were incubated at 37ºC for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture plates (Sigma-Aldrich, Costar, and USA) were filled with 200 µL of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37ºC for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm. OD value <0.120 was considered non biofilm forming, 0.120-

0.240 was considered moderate and >0.240 as strong biofilm producing.

C. The Coating of Urinary Catheters with Enzymes, Gentamycin and EDTA

Six new Foley urinary catheters were taken; one was kept as control (uncoated), and the others were coated with 1% sterile solution of amylase, protease, lysozyme, gentamycin and EDTA respectively. Coating was performed by filling the catheter lumen with the reagent tested. The complete procedure was carried out aseptically and the filled catheter was kept for 24 h, after which the solutions were decanted and the coated catheters were used for further experiment.

D. Bladder Model and Experimental Protocol

The model of the catheterized bladder has been described previously [6]. It consists of a glass chamber maintained at 37 ^{0}C by a water jacket. Each model was sterilized by autoclaving and then the coated urinary catheter was inserted into the chamber via an outlet in its base. Catheters were then attached to drainage tubes and bags. Sets of models were assembled and pooled human urine sterilized by filtration was pumped into the bladder chamber until it submerged the retention balloons of the catheters. The urine supply was then halted and models were inoculated with 1 ml 4 h broth culture (approx. 10^8 cfu) of strong biofilm producing *Pseudomonas aeruginosa* which was isolated from a urinary catheter. The models were left for 1 h to enable the organisms to become established in the residual urine. Fresh urine was then pumped into the chamber at 0.5 ml/ min and left to run for 9 days. After every 24 h 1-2 cm section of catheter tip from the drainage end was cut aseptically, flushed with PBS to remove planktonic bacteria and the cut sections were processed as above to detect whether the pseudomonas was able to establish itself in the form of biofilm.

III. Results

A. Isolates from Urinary Catheters

Out of 100 catheters , 64 were found to be contaminated with microorganism from which *Pseudomonas aeruginosa* was isolated from 32 catheters (50%), *Enterococci* from 20 catheters (31%), *E. coli* from 16 catheters (25%), *S. aureus* from 12 catheters (18%), *Klebsiella* from 10 catheters (15%) and *Candida* from 6 catheters (9%). The percent of *Pseudomonas aeruginosa* was highest among all isolates.

B. Biofilm Formation by the Isolates

Among 32 *Pseudomonas aeruginosa* and 16 *E. coli* isolates, all produced biofilm (100%). In a similar way 90% *Enterococci*, 66.6% *S. aureus* and 80% *Klebsiella* produced biofilm (Figure 1). Of the 6 Candida isolates none could form biofilm.

C. Effect of Enzymes, Gentamycin and EDTA Coating on Catheters

It was observed that biofilm was produced by *P. aeruginosa* in just 2 days in the control catheter (uncoated). But it was very interesting to note that biofilm formation was prolonged for 7 days in amylase, 8 days in protease, 6 days in lysozyme, 7days in gentamycin and 5 days in EDTA treated catheters (Table-I)

TABLE I
MEAN TIME OF BIOFILM FORMATION IN TREATED CATHETERS

Days / Catheter	1	2	3	4	5	6	7	8	9
Control	-	-	+	+	+	+	+	+	+
Amylase	-	-	-	-	-	-	-	+	+
Protease	-	-	-	-	-	-	-	-	+
lysozyme	-	-	-	-	-	-	+	+	+
Gentamycin	-	-	-	-	-	-		+	+
EDTA	-	-	-	-	-	+	+	+	+

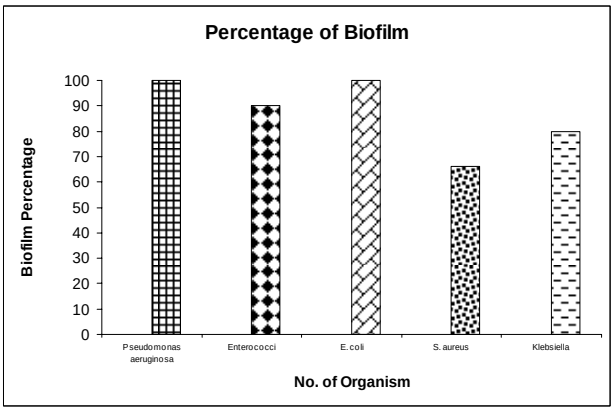

Fig. 1 Percentage of Biofilm Formation by the Isolates

IV. Discussion

In the present study, culture dependent examination of the types of microorganisms that colonized Foley urinary catheter was applied to study isolation and identification of biofilm forming bacteria from urinary catheter. *P. aeruginosa, E. coli, S. aureus, Klebsiella, Enterococci* and *Candida* were obtained from urinary catheters of patient and all are the major cause of CAUTI. All the isolated bacteria are the normal flora of human intestine voided in the faeces and are also present on the perianal region from where they may migrate to the genitals exterior and contaminate the catheter tip while inserting, if proper cleaning of the genitals or urethra is not done. From the tip of the catheter these bacteria may migrates to the lumen and establish them in the form of a biofilm. Another reason may be due to the open drainage system; bacteria may travel up to the catheter opposite to the direction of urine flow and produce the biofilm. Similar explanation was also given by Stamm *et al*, (1991).Similar results were observed by Barford *et al*, (2008) while studying culture-independent microbiological analysis of Foley urinary catheter biofilms. Biofilm formation was seen in 100% isolates of *P. aeruginosa* and *E. coli*. Employing an *in-vitro* model of catheter associated infection, Goto *et al*, (1999) investigated biofilm formation of *P. aeruginosa* on the surface of a Teflon catheter in artificial urine.

In the present investigation it was observed that biofilm formation was not inhibited but prolonged for more than 6 days in enzyme treated catheters. Mollobella *et al*, (2010) studied that the removal of biofilms by *Pseudomonas fluorescence* with protease and amylase enzymes is difficult. Protease enzymes were capable of destroying the EPS. The amylase enzymes were also effective but less effective as compared to protease for the degradation of *P. fluorescence* biofilms. They studied the removal of formed biofilm but the present investigation states about the prevention of biofilm formation.

In the present study it was noted that the biofilm production by Pseudomonas was prolonged by 5 days in EDTA treated catheter. The results shown by Percival *et al* stated that the mean time of blockage of the control catheters was 45 h for saline, 57 h for water and 67 h for those exposed to daily instillations of the EDTA solution. *Pseudomonas aeruginosa* form urease which hydrolyses urea to ammonia and carbon dioxide leading to an alkaline pH in urine and catheter biofilm due to which magnesium and phosphate crystals are formed resulting in the formation of the crystalline biofilm. But EDTA solution cause dissolution of the calcium and magnesium salts that gets deposited on the catheters and reduce the crystalline biofilm. Gentamycin being an antibiotic was also able to prolong biofilm formation. Thus from the present study it can be concluded that pretreatment of urinary catheters with amylase, protease, lysozyme, gentamycin or EDTA may prolong the contamination and subsequently biofilm formation by organism causing CAUTI.

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