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Prevention of Biofilm Formation in Urinary Catheters by Treatment with Antibiofilm Agents

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Abstract: ***Urinary Tract Infections (UTI) accounts for an estimated 25-40% nosocomial infection, out of which 90% are associated with urinary catheters, 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 catheters each with different antibiofilm agents like DNAse enzyme, antimicrobial drugs- Ceftazidime and Ceftriaxone, anticancer drug-Cisplatin and anticoagulant heparin. 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% of the Pseudomonas aeruginosa and E. coli isolates 90% in Enterococci, 80% in Klebsiella and 66% in S. aureus. It was noted that the biofilm production by Pseudomonas was prolonged by 14 days in Ceftazidime coated catheter, 8 days in Ceftriaxone and Cisplatin treated catheters, 6 days in heparin and 5 days in DNAse treated catheters.***

Keywords: CAUTI, biofilm, enzyme, Ceftazidime, Pseudomonas

1. 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. Ceftazidime has a very important role to play as an 'antibiofilm' agent and therefore may have important implications for use in controlling biofilm in catheters. 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 DNAse, Ceftazidime, Ceftriaxone, Cisplatin and Heparin coated catheters to resist biofilm formation by *Pseudomonas aeruginosa*.

2. Literature Survey

Frank DN *et al*, (2009) studied culture independent microbiological analysis of Foley urinary catheter biofilms. They identified *Staphylococcus epidermidis, Enterococcus faecalis, Pseudomonas spp, and Klebsiella spp* within the contaminated catheters.

Macleod SM *et al*, (2007) studied bacterial flora of catheter biofilms. *Proteus mirabilis* was recovered from nearly half of the catheters colonized by *Provedencia stuartii*. It was also found commonly in biofilms together with *Klebsiella pneumoniae* and *Enterococcus faecalis*. In contrast, *Proteus mirabilis* was recovered from only one of the 14 catheters colonized by *M. morganii* and none of the nine *Ent. cloacae* containing biofilms.

Goto T *et al*, (1999) investigated biofilm formation of *Pseudomonas aeruginosa* on the surface of a Teflon catheter and evaluated the bactericidal activity *in-vitro* and *in-vivo* of several classes of antibiotic alone or in combination with a macrolide antibiotic against *Pseudomonas aeruginosa*. The fluoroquinolone showed the most potent bactericidal activities against *Pseudomonas aeruginosa* biofilm both *in-vitro* and *in-vivo*.

Kunin CM *et al*, (1985) found that *E. coli* gradually colonizes the catheters over time but at all times was recovered in lower amounts than gram positive organisms and less often in males than in females. Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation was investigated by Czaczyc *et al*, (2007). The colonization of solid surfaces by microorganisms is a very complicated process that depends mostly on extracellular molecule production. The biosynthesis of EPS reflected not only the attachment and aggregation process but also provided an optimal environment for the exchange of genetic material between the cells. The comparative and comprehensive analysis of all documented data concerning EPS production can enable the development and effective control strategies for biofilms. In this review some of the basic concepts concerning the

biosynthesis of EPS and potential function of these compounds in biofilm development were discussed.

In vitro effect of silver on adherence of bacteria to urinary catheters was seen by Gabriel *et al*, (1995).They investigated strains of *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis,* and *Klebsiella pneumoniae*, mostly from complicated urinary tract infections, showed reduced adherence to silver-treated silicone or latex catheters as compared with latex or silicone catheters.

3. Material and Methods

3.1 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.

3.2 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* [9] is considered the gold-standard method for biofilm detection [11]. 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 to remove 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.

3.3 Coating of urinary catheters with DNAse, Ceftazidime, Ceftriaxone, Cisplatin and heparin

Six new Foley urinary catheters were taken; one was kept as control (uncoated), and the others were coated with 1% sterile solution of DNAse, Ceftazidime, Ceftriaxone, Cisplatin, and heparin 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.

3.4 Bladder model and experimental protocol

The model of the catheterized bladder has been described previously [12]. It consists of a glass chamber maintained at 37^0C 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.

4. Results and Discussion

4.1 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%), *Staphylococcus 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. Biofilm forming bacteria were isolated by Sonication, scraping and vortexing of biofilms by cutting the section (1-2 cm) from tip of catheter aseptically and colonies were identified by conventional microbiological methods. Macleod SM *et al*, (2007) isolated bacteria in a similar way.

4.2 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. 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). Parallel

results were observed by Barford *et al*, (2008) while studying culture-independent microbiological analysis of Foley urinary catheter biofilms. 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.

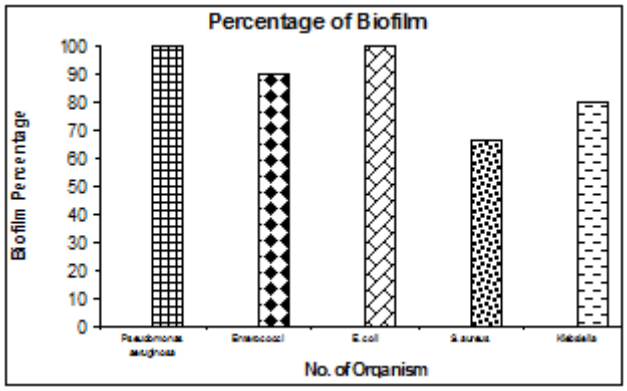

Figure 1: Percentage of Biofilm formation by the isolates

4.3 Effect of Ceftazidime, Ceftriaxone, Cisplatin, Heparin and DNAse 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 14 days in Ceftazidime, 8 days in Ceftriaxone and Cisplatin, 6 days in heparin and 5 days in DNAse treated catheters. Riedl *et al*, (2002) studied, the inhibition of urethral stent encrustation by heparin coating and found that in contrast to uncoated polyurethane stents, heparin coated urethral stents did not show any organic (biofilms) or inorganic (crystals) deposits after being in situ for up to 6 weeks and effectively inhibited the encrustation process. Williams G J *et al*, (2004) studied the bacterial biofilm formation on urological devices and heparin coating as preventive strategy. Their result showed that urologic devices are protected against encrustation and biofilm formation for a longer period of time i.e. for 6-12 months.

In the present study Ceftazidime proved to be most effective against the biofilm information by *Pseudomonas aeruginosa.* It is a semi synthetic broad spectrum cephalosporin and used especially for pseudomonas and other gram negative infections in debilitated patients. EPS consists of water, proteins, polysaccharides and extracellular DNA (eDNA). eDNA, being the longest molecule in EPS, connects the different EPS components and therewith holds an adhering biofilm together and assists in facilitating adhesion, aggregation and maintenance of biofilm structure. DNAse enzyme works by involving in endonucleolytic cleavage of extracellular DNA to 5' phosphodinuclear and 5' phosphor-oligonucoltide end products. Thus in the present investigation DNAse coated catheter also prolonged the formation of biofilm for 5 days.

4. Conclusion

All the antibiofilm agents used in the present investigation makes an ideal coating for biomaterial implants and devices, known to fail due to biofilm formation with disastrous consequences for patients and high costs to the healthcare system. With threatening increase in antibiotic resistance, these coatings may provide a timely, potent new approach to biofilm prevention on biomaterial implants.

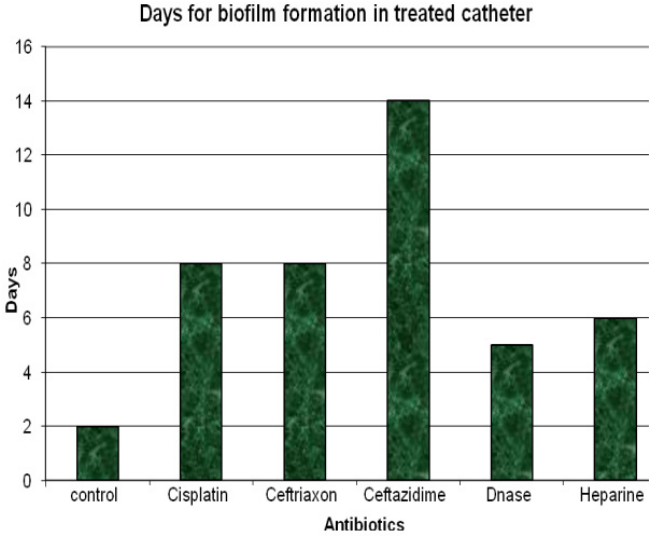

Figure 2: Days for Biofilm formation in treated catheters

5. Future Scope

More research has to be done on the parameters of intraluminal coating of the catheters. (e.g., duration of coating the catheter). Furthermore, the usefulness of different types of antibiofilm agents, and their optimum concentration remain to be defined. Much more research is needed to expose additional and/or novel antibiotic - induced factors in biofilms. The multifactorial nature of biofilm antibiotic resistance has hindered identification of these pathways, and much remains to be elucidated about induced factors in biofilm resistance. Discovery of these unknown factors will lead to new and better treatments for biofilm related infections.

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