

**IMPREGNATION OF URINARY CATHETERS USING ANTIMICROBIALS FROM
Streptomyces sp BK18 FOR BIOFILM PREVENTION**

Angima Bichang'a Kingsley and R.Usha*

Department of Microbiology

Karpagam Academy Of Higher Education

Coimbatore

Tamil Nadu – 641021, India

ushaanbu09@gmail.com

Abstract

Microbial biofilms are a major impediment to the use of indwelling medical devices, causing device-related infections with high morbidity and mortality. Major efforts directed towards preventing this problem face difficulties because biofilms develop a mechanism to protect themselves effectively by producing a polysaccharide coating, reducing biofilm sensitivity to antimicrobial agents. Most techniques applied to fight biofilms have been primarily chemical. Impregnation of catheters with antimicrobials could be a lasting solution. . Antibiotic impregnation of the catheters did not affect the mechanical properties and did not render it as unfit for insertion.

Key words: *Biofilm, CAUTI, impregnation, uropathogens.*

Introduction

Indwelling urinary catheters are a major development in the management of hospitalized patients. Urethral catheters are used to drain urine from the urinary bladder especially in the management of intractable urinary problems¹. However, due to their prolonged use, risk factors related to microbial infections and biofilm formation result in higher morbidity and mortality rates among hospitalized patients, which causes an influx of medical costs.

Most urinary tract infection cases are due to indwelling medical catheters². Biofilms develop on the surfaces of medical implants and cause bacterial infections and sepsis. In patients with urinary catheters, infection rates increase with the duration of catheterization. This however, is predominant in patients who undergo long-term catheterization³. The U.S. National Institutes of Health estimates infection rates as high as 80% that are due to microbial biofilm⁴.

The magnitude of the biofilm problem in hospital set ups coupled with the financial aspects involved, massive investments of major efforts to develop novel anti-biofilm strategies have been stepped up. The idea has been to develop means for disruption of biofilm formation on the implants and total eradication of existing biofilms. This can be achieved by among others; targeting the exopolysaccharide matrix which is secreted by the microorganisms and in which developing colonies become encapsulated. The present study aims to modify a catheter by using an antimicrobial compound from *Streptomyces* BK18 to inhibit biofilm formation by the pathogens causing UTI.

Materials and Methods

Pathogens

P. aeruginosa, *C. albicans*, *E. coli*, *Proteus*

Impregnation of catheter

The antimicrobial obtained from *Streptomyces* BK18 dissolved in ethanol to give concentrations w/v of 0.2%. Catheter segments cut into 2mm were submerged in the solution overnight. The catheter pieces were removed and rinsed in absolute ethanol to remove residual antimicrobial

compound and left to dry at room temperature for 2 h and then sterilized at 70 °C for 15 min⁵.

Determination of shelf life

To determine the shelf life of the catheter segments, the impregnated segments were stored at room temperature for 3 months and periodically tested again for their antimicrobial activity. Unimpregnated segments were kept as negative control.

In vitro biofilm colonization

To determine *in vitro* biofilm colonization, a modified form of Kuhn's model of biofilm colonization was followed⁷, 1-cm-long segments of both Unimpregnated control catheters and impregnated catheters were tested in triplicate for inhibition of biofilm formation by MRSA, VRE, *P. aeruginosa* and *Candida albicans*. The catheter segments were submerged in artificial urine; with the following composition (g/l); {CaCl₂.2H₂O - 0.651; MgCl₂.6H₂O- 0.651; NaCl- 4.6; Na₂SO₄- 2.3; KH₂PO₄- 2.8; KCl- 1.6; NH₄Cl- 1.0; urea- 25.0; creatine- 1.1; Tryptic Soy Broth (TSB)-10.0; pH- 5.8 and sterilized by sieving through 0.2µm pore size filter for 30 days at 37 °C⁸ for 24 h at 37°C.

The artificial urine was then replaced with 5.0 x 10⁵ cells of various organisms in Mueller-Hinton broth, and the plates were incubated for a further 24 h after which, the microbial inoculum was discarded and segments were washed with shaking for 30 min in 1 ml of 0.9% sterile saline. The segments were then transferred into 5 ml of 0.9% saline and sonicated for 15 min at 40 kHz. After sonication, each sample was vortexed for 10 s, and 100 µl of liquid from each segment was serially diluted and spread onto Trypticase soy agar for quantitative culture of bacterial species and onto Sabouraud dextrose agar for yeast species. Plates were incubated inverted at 37°C for 24 h and then counted for colony growth. Experiments were repeated twice (n = 6 segments in total).

Results

Impregnation of catheter using antimicrobial

The most commonly used antimicrobials to impregnate catheters today contain minocycline and rifampin. However, since

minocycline and rifampin are also used therapeutically as antibiotics, concern exists that the use of catheters impregnated with these compounds may result in the development of bacterial resistance⁹.

Also emerging, are concerns for toxicity and hypersensitivity reactions arising due to these catheters. Reactions to topical and intra-urethral chlorhexidine have been reported in the United States of America¹⁰. In Japan, hypersensitivity to chlorhexidine catheters has been reported¹¹.

Understanding the pathogenesis of CAUTI is essential to designing preventative strategies. Any foreign body; such as a urinary catheter in the bladder, inevitably becomes colonized with sessile pathogens which could form a biofilm¹². An efficient method must be used for the incorporation of the antimicrobial to ensure that both the external and internal surfaces of Foley's latex catheters were uniformly incorporated with the antimicrobial agent. In an effort to limit biofilm the formation, strategies like making the catheter surface more hydrophilic¹³ with hydrogel coatings, impregnation of catheter with antimicrobial ointments or anesthetics and coating the catheters with antimicrobial agents like antibiotics nitrofurazone and rifampicin have been tried¹⁴.

In this study, catheter impregnation was achieved by submerging the segments into a solution which is by far simple, cheap and easily done as no sophisticated equipments are required. It also does not affect the texture of the catheter. The process of impregnating catheters with antimicrobial results in complete incorporation of antimicrobial on matrix without any major effect on its appearance and mechanical properties while providing a long lasting antimicrobial activity.

Catheters containing single antimicrobials failed to inhibit the growth of pathogens unlike the catheters with combinations of antimicrobials like rifampicin with clindamycin hydrochloride that were able to show activity¹⁵. Impregnation of catheter with a combination of minocycline-rifampin has been reported to produce a broad spectrum against Staphylococcal, gram negative bacillary and candida¹⁶. Chlorhexidine gluconate mostly used as an

antiseptic and has a broad spectrum activity. Sherertz and group demonstrated the efficacy of coating catheters with chlorhexidine both *in vitro* and *in vivo*¹⁷. In randomized clinical trials, Maki *et al.*,¹⁸ reported that the catheters coated with triiododecylmethyl ammonium chloride (TDMAC) and chlorhexidine gluconate and silver sulfadiazine (CH-SS) decreased the risk of catheter-related septicemia by more than 4 times.

Shelf life of the coated catheter

Pathogen	Day of failure
<i>E. coli</i>	85
<i>P. mirabilis</i>	64
<i>P. aeruginosa</i>	80
<i>C. albicans</i>	61

Table 1: shelf life of impregnated catheter

Most CAUTIs are caused by enterobacteria (*E. coli*, *K. pneumoniae*, *P. mirabilis*) with minor incidences due to *P. aeruginosa* or *C. albicans*¹⁹. *S. aureus* is also a UTI pathogen associated with the long term urinary catheters²⁰. The test catheters were able to delay the colonization by these CAUTI pathogens by up to about 12 weeks (table 1). The antimicrobial activity showed a long duration of activity. At 41 days, the antimicrobial inhibited *E. coli* while *P. aeruginosa* was only inhibited up to the 40th day. *C. albicans* was not inhibited beyond the 31st day.

These results contradict with those recorded by Cho *et al.* who dipped their catheters in a mixture of gentamicin and poly (ethylene-co-vinyl acetate) but reported a gentamicin release for only 1 week²¹ and those by Rafienia *et al.* who reported 12 days' release from catheters dipped in a mixture of gentamicin-copolymer²² (Table 1).

In vitro biofilm colonization

The risk of CAUTI begins at the point of catheter insertion. The pathogens form a biofilm on the surface of the catheter, which provides a safe-haven for the pathogens. Biofilm is formed by irreversible attachment of microorganisms to the surface of the catheter, producing a matrix of extracellular

polymeric substances (EPS) which protects the microorganism through either inhibiting the diffusion of antimicrobials into the matrix or by causing reduced growth rates for the microorganisms hence affecting the killing capacity of antimicrobials, or completely inhibiting antimicrobial uptake².

In vitro adherence of impregnated catheters showed a significant reduction in the viable biofilm colony counts of the pathogens as compared to those with uncoated control. The p values (control/ impregnated catheters) for *E. coli* was $p=0.006$; *P. aeruginosa* was $P=0.003$; For *Proteus*, $P=0.005$; and *C. albicans* $p=0.002$.

For Raad et al.,¹⁶; *In vitro* biofilm adherence of various microorganisms to different antimicrobials coating CVC surfaces after 24 h of biofilm formation, P values for MRSA and *P. aeruginosa* were as follows: control versus M/R CVC, $P=0.005$; control versus CHX-M/R CVC, $P=0.003$; control versus CHX/SS CVC, $P=0.004$; CHX-M/R CVC versus M/R CVC, $P=0.03$; and CHX-M/R CVC versus CHX/SS CVC, $P=NS$. P values for *C. albicans* and *C. glabrata* were as follows: control versus M/R CVC, $P=NS$; control versus CHX-M/R CVC, $P=0.003$; control versus CHX/SS CVC, $P=0.003$; CHX-M/R CVC versus M/R CVC, $P=0.003$; and CHX-M/R CVC versus CHX/SS CVC, $P=0.003$.

Biofilm quantification assay

The antimicrobial compound from *Streptomyces sp.* BK 18 was able to reduce biofilm formation by the uropathogens. Biofilm was well established in the untreated samples as compared to the treated sample. The *Streptomyces sp.* BK 18 extract was able to inhibit biofilm formation (Figure 1).

The assay was done so as to determine the concentration at which the extract showed a significant inhibition to biofilm formation. The highest average biofilm inhibition percentage was observed at 2 mg/ml (Figure 2).

Statistical methods.

CFU were compared by the Kruskal-Wallis test for each organism. If a significant result was detected for the test, we used Wilcoxon rank sum tests for pairwise comparisons. For the study of durability of prolonged biofilm inhibition, two-way nonparametric analysis of variance (ANOVA) was applied.

Conclusion

Nosocomial infections as a consequence of long term intravascular catheterization result in morbidity and mortality. Catheter modification by the antimicrobial impregnation indeed inhibited biofilm formation. Subsequently, this results showed the biofilm inhibition is possible with biological compounds from *Streptomyces sp.* The *Streptomyces sp.* BK 18 extract was able to distort biofilm formation. The reduction of biofilm could be attributed to antibacterial compounds.

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Fig 1: Biofilm quantification

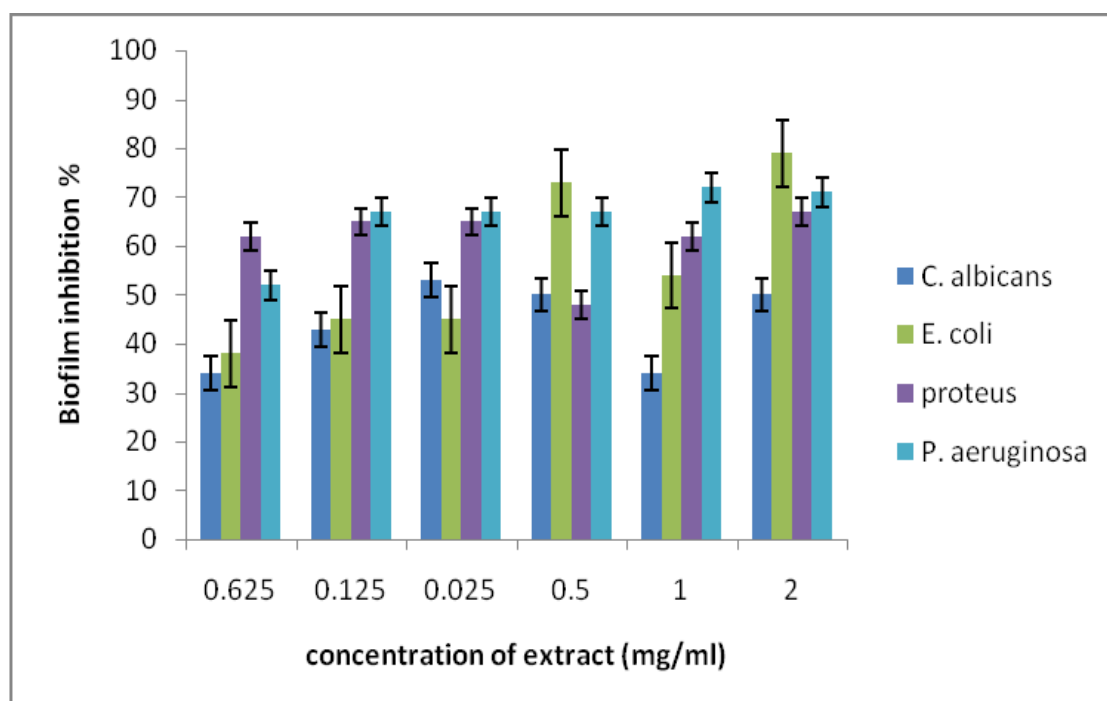
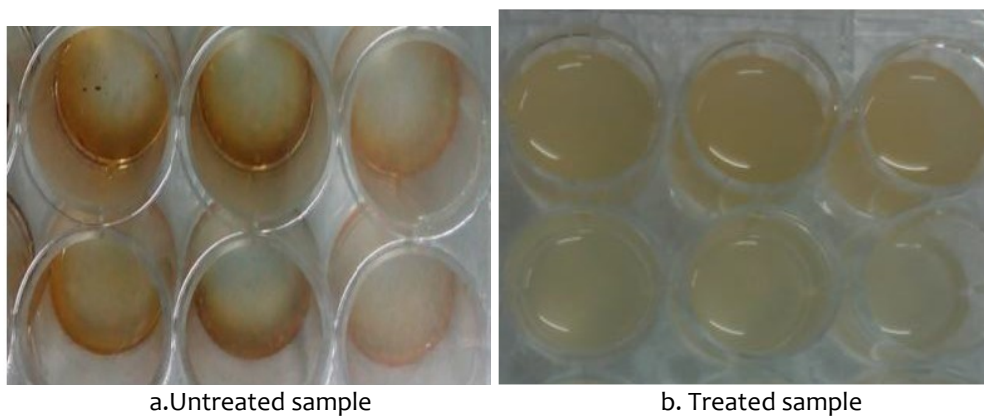


Fig 2: Biofilm inhibition percentage