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Research Article

MODIFICATION OF URINARY CATHETERS USING ANTIMICROBIALS FROM *STREPTOMYCES SP. ABK 07* FOR URINARY TRACT INFECTION RESISTANCE

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ABSTRACT

Objective: The main aim of this study is to prevent biofilm formation by impregnating an antimicrobial on urinary catheter.

Methods: Catheter segments were immersed in the antimicrobial compound for impregnation. After 2 h, the segments were removed, sterilized and dried after which mechanical and antimicrobial properties of the catheter segments were determined. The shelf life of the impregnated segments was also ascertained as well as anti-biofilm assay. Spectral analysis (UV & FTIR) was also performed.

Results: Impregnation was achieved by immersing catheter segments in antimicrobial compound ensuring it does not affect the catheter texture. The impregnated antimicrobial catheters were able to prevent colonization by common uropathogens *Escherichia coli, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella,* and *Candida albicans* for up to 12 weeks. Antibiotic impregnation of the catheters did not affect the mechanical properties and did not render it as unfit for insertion. The antimicrobial-impregnated catheter offers a means of reducing biofilm formation and subsequently reducing the infection in long-term urinary catheter users. Spectral analysis was done by UV-Vis and FTIR.

Conclusion: Antibiotic impregnation of the catheters did not affect the mechanical properties and did not render it as unfit for insertion. The antimicrobial impregnated catheter offers a means of reducing biofilm formation and subsequently reducing the infection in long-term urinary catheter users.

Keywords: Biofilm, Catheter-associated urinary tract infections, Impregnation, Uropathogens.

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INTRODUCTION

Urethral catheters are used to drain urine from the urinary bladder, especially in the management of intractable urinary problems such as chronic urinary retention, in the treatment of hospitalized and bedridden patients, including those affected by stroke, aged and those with injured spinal cords [1]. Consequently, device-related infections are a major cause of clinical complications. Increased health-care costs due to prolonged hospital stay or revision surgery and treatment of new ailments are other effects. To some extent, this could affect productivity of an individual. Reports suggest that up to 80% of nosocomial infections attributed to catheter-associated urinary tract infections (CAUTIs).

Longer periods of catheter insertion are a major cause of CAUTIs pathogenesis in which the sources of causative pathogens are from the hands of health-care personnel and also from the patients' own flora, especially in the perineal area [2]. The migration of pathogens from the urine collection bags through both the inner and outer luminal surfaces of the catheter enters the urinary bladder and causes infection. During the migration of microorganisms along the luminal surface of the catheter, a number of interactions take place which determines the development of infection. The first step in biofilm formation is the adherence/attachment of free-floating microbes on the catheter surface, after which they form colonies and synthesize exopolymeric slime and form biofilm [3].

Naturally, bacteria grow in polymicrobial communities either as single species or coexist with other species to form a biofilm. Biofilm is an important virulence factor for pathogenic microbes [4]. These bacterial biofilms can develop on both biotic and abiotic surfaces [5]. The most common CAUTI pathogen is *Escherichia coli*, followed by *Proteus mirabilis*. *Klebsiella pneumonia, Enterococci,* and *Staphylococci* are less common, while *Pseudomonas aeruginosa* and *Candida* spp. may be observed in longer catheterized patients, especially after repeated courses of antibiotics [6]. This problem can be solved or controlled by the development of the modified catheter. The present study aims to modify a catheter using an antimicrobial compound from *Streptomyces ABK07* to inhibit biofilm formation by the pathogens causing UTI.

MATERIALS AND METHODS

Impregnation of antimicrobial on catheter

The antimicrobial obtained from *Streptomyces ABK07* [7] noted in previous published article was chosen for its activity against the CAUTI pathogens.

The antimicrobial was dissolved in methanol to give concentrations w/v of 0.2%. Latex Foley balloon catheter was cut into 2 mm segments and immersed in the solution for 2 h, during which the silicone swelled to almost double its volume. Then, the catheter pieces were removed and rinsed in absolute ethanol to remove residual antimicrobial compound after which they were left to dry at room temperature. After evaporation of the solvent, the catheters returned to their previous size, thus leaving the antimicrobial evenly distributed in the catheter pieces.

The catheter segments were then sterilized at 70°C for 15 min. The sterilization process did not affect the antimicrobial activity of the agent [8].

Mechanical properties of the catheter and balloon

Mechanical performance of catheter shafts was assessed using an Instron 5985 as described by Fisher *et al.* [9]. Both the control and antimicrobial-impregnated catheters were immersed in artificial urine; with the following composition (g/l): $CaCl_2.2H_2$ O - 0.651; $MgCl_2.6H_2O$ - 0.651; NaCl - 4.6; Na_2SO4 - 2.3; KH_2PO4 - 2.8; KCl - 1.6; NH_4Cl - 1.0; Urea - 25.0; creatine – 1.1; Tryptic Soy Broth (TSB) - 10.0; pH - 5.8; and sterilized by sieving through 0.2 µm pore size filter for

30 days at 37°C. While the balloon is inflated, the catheter tips were inserted through an inverted funnel, a 1 kg weight was applied to stretch the balloon against the neck of the funnel. Volume retention by the balloons was tested by inflating them with 10.0 ml of 0.5 g/l methylene blue and placing on a white absorbent background for 30 days [10]. The tests were performed in three replicates of each analysis at room temperature.

Antimicrobial activity of the antibiotic coated segments

Bridge plate method [11]

Mueller-Hinton agar plates seeded with test pathogens. Two wells, 3 mm apart, were bored using the rear side of micropipette tips on the agar. The autoclaved antimicrobial-impregnated segments were placed on the bridge between the wells and gently pressed. The plates were then incubated at room temperature, and the zones of inhibition were measured after 24 h. The diameter of the zones of inhibition to the long axis of the catheter was measured. Unimpregnated catheter segments were used as controls.

Determination of shelf life

To check the activeness of the segments, the coated segments were stored at room temperature for 85 days and tested again for their antimicrobial activity.

Anti-biofilm assay

Biofilms were formed in synthetic urine composed as previously described**.** MHA medium supplemented with L-glutamine and buffered with morpholinepropanesulfonic acid containing 2 % glucose (w/v) with a final pH of 7.2 was used as a control for comparisons.

Two different *in vitro* models were used to assess biofilm growth and susceptibility to drugs. First, a microtiter plate-based model that utilizes polystyrene, flat bottom, and 96-microtiter plates as a substrate as previously described by Pierce *et al*. [12]. Cells were grown in nutrient broth medium overnight at 30°C, washed twice with sterile phosphate-buffered saline (phosphate buffer -10 nM; potassium chloride - 2.7 mM; sodium chloride - 137 mM; and pH 7.4) resuspended in MHA adjusted to McFarland 0.5. 100 µl of inoculum was added to each well of a 96-well microtiter plate. After 96 h of incubation at 37°C to allow for biofilm formation, the wells were washed 2 times with PBS to remove any non-adherent cells.

Second, biofilms were grown on silicon elastomer surfaces as described by Uppuluri *et al*. [13]**.** Briefly, the pathogenic strains were grown in MHA medium at 30°C overnight, washed in PBS, and diluted to a concentration of 5×10^6 cells/ml in MHA medium. The suspension was added to a sterile 24-well plate containing silicon elastomer squares. The inoculated plates were incubated at 37°C for 90 min for initial adhesion of cells. The squares were washed with PBS, transferred to fresh plates MHA medium, and incubated at 37°C for 96 h at 100 rpm to allow for biofilm formation. The degree of biofilm formation was estimated using XTT reduction assay [14]. For each assay, an aliquot of stock XTT was thawed, and 10 mM was added to a final concentration of 1 µM. 80 µl XTT-menadione solution was added to each prewashed biofilm and control wells. The microtiter plates were incubated in the dark at 37 °C for 2 h. Following incubation, 75 µl of XTT-menadione solution was transferred to the wells of a new microtiter plate, and the colorimetric change from XTT reduction was read in a microtiter plate reader at 490 nm.

Ultraviolet (UV) and Fourier transform-infrared (FTIR) spectral analysis

UV spectra of the antimicrobial compound were subjected to comparison of a general pattern, absorbance peaks and wavelength range. Each compound was determined in the UV region 200–400 nm using a Perkin Elmer Lambda 30 UV/Visual spectrophotometer.

After homogenization of the sample with KBR (1 mg of sample and 300 mg of KBr (IR grade) were mixed and a thin KBr disk was prepared). FTIR spectra of sample were analyzed. The FTIR spectrum of each compound was detected using SHIMADZU AUX 220 spectrometer in the range of 400–4000 cm^{-1} and plotted as intensity against wave number [15,16].

RESULTS

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 this study, catheter impregnation was achieved by dipping the segments into a solution which is by far simple, cheap, and easily done as no sophisticated equipment 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.

The primary testing of the processed catheter was performed by the bridge plate technique (Fig. 1) which showed antimicrobial activity. Uncoated catheter segments showed no activity. Catheters which had been coated using a concentration of the antimicrobial of 2 % w/v exhibited significant results.

According to these results, the impregnation procedure had no effect on the mechanical properties of the catheters. The antimicrobial compound had no notable effects on the catheter segments.

Despite a load of 70 N being applied at 100 mm/min, causing an elongation of 100%, no fracture was observed. Statistically differences between control and impregnated catheters in their unused state were insignificant (two-tailed homoscedastic Student's t-test) with regard to load, tensile strength, and modulus or between control and impregnated catheters after soaking in artificial urine. The control and impregnated balloons, before and after immersion in the synthetic urine, passed the test. No leakage was seen from either the control or impregnated balloons. The mechanical performance of the catheter and balloon was not adversely affected by the impregnation of the antimicrobials onto the catheter and balloons. The surface texture of the catheter and balloons remained intact.

The activity of the antibiotic-impregnated catheters against Gram-negative pathogens, as measured by zones of inhibition and recorded as *E. coli* 19 mm, *P. aeruginosa* 17 mm, *Klebsiella* 16.7 mm, *Proteus* 13.8 mm, *Staphylococcus aureus* 10 mm, and *Candida albicans* 9.4 mm.

The test catheters were able to delay the colonization by these CAUTI pathogens by up to about 12 weeks. The antimicrobial activity showed a long duration of activity. At 85 days, the antimicrobial inhibited *E. coli* while *P. aeruginosa* was only inhibited up to the 80th day. *C. albicans* was not inhibited beyond the 61st day (Table 1).

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

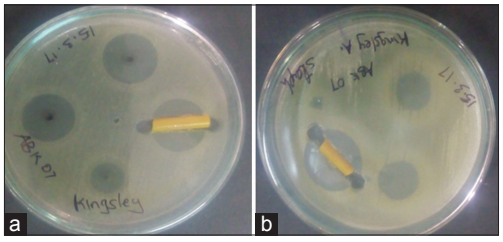

Fig. 1: (a) ***Escherichia coli,*** **(b)** ***Staphylococcus aureus***

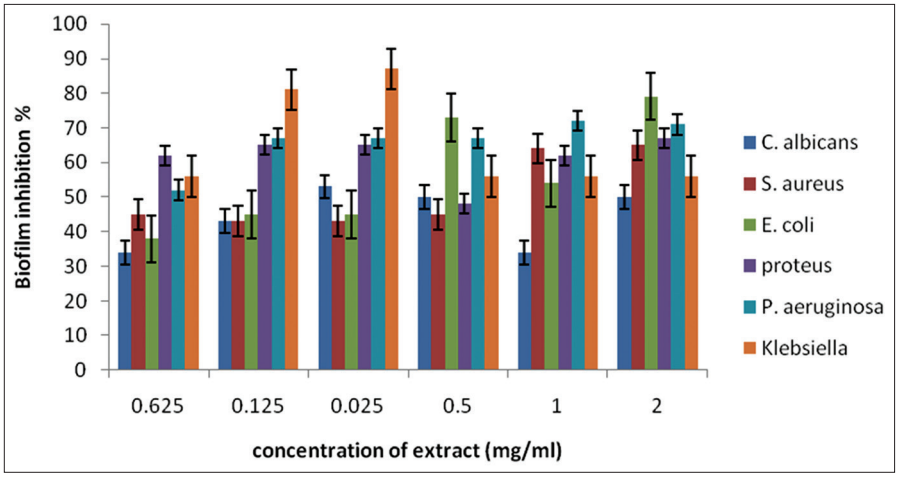

Figure 2: Biofilm inhibition under different concentrations

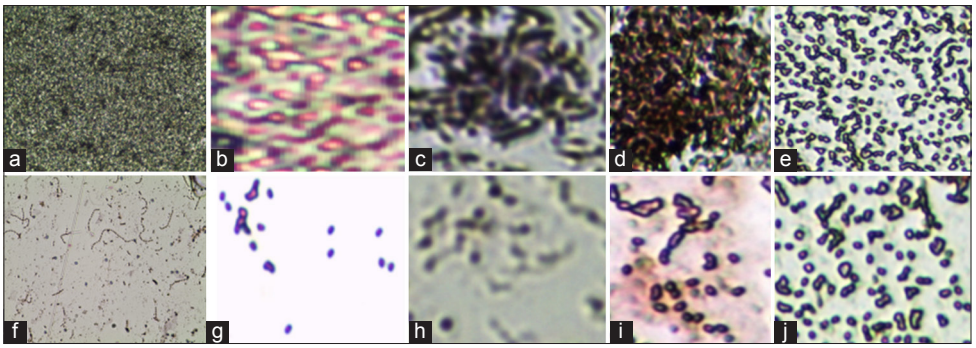

Fig. 3: Visualization of biofilm under phase contrast microscope. Untreated biofilm: (a) *S. aureus*, (b) *E. coli*, (c) *Klebsiella*, (d) *P. aeruginosa*, (e) *Proteus*, treated biofilm: (f) *S. aureus* (g) *E. coli*, (h) *Klebsiella*, (I) *P. aeruginosa*, (J) *Proteus*

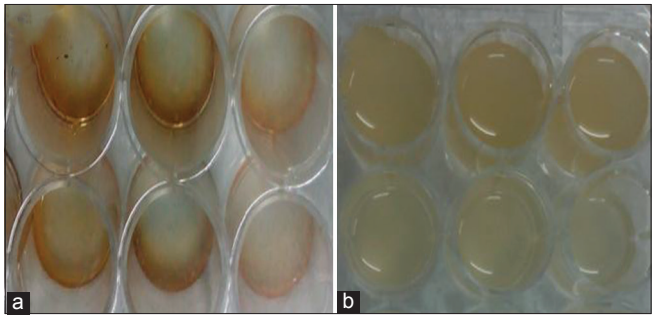

Fig. 4: MTP assay in microtiter plate, (a): Untreated samples, (b) treated samples

Table 1: Shelf life of impregnated catheter

Pathogen	Day of failure
E. coli	85
S. aureus	72
K. pneumonia	60
P. mirabilis	64
P. aeruginosa	80
C. albicans	61

E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis, P. aeruginosa: Pseudomonas aeruginosa, C. albicans: Candida albicans

The antibiotic compound from *Streptomyces sp*. ABK 07 was able to reduce biofilm formation by the pathogens (Fig. 3). Biofilm was well established in the untreated samples as compared to the treated sample. The *Streptomyces sp*. ABK 07 extract was able to distort biofilm formation (Fig. 4).

The antimicrobial compound was characterized by FTIR. Infrared spectra exhibited absorption at 3402 and 3398 cm^{-1}, indicating a hydroxyl group and absorption at 1655 and 1631 cm^{-1} indicated a double bond of polygenic compound while absorption at 1665, 1636, and 1631 cm^{-1} indicated a hydrocarbon chassis (Fig. 5). A bioactive region was detected on the TLC plate; Rf 0.70. The bioactive compound exhibited a maximum UV absorption at 217. These strains produced either a broad-spectrum antimicrobial compound or several compounds with different activities.

DISCUSSION

Bacterial biofilm is formed by initial bacterial attachment which is reversible and is guided by physical forces like Van der Waals forces, and hydrophobic interactions biofilm is also formed by subsequent irreversible adhesion mediated by molecular reactions between bacterial surface structures and substratum, leading to the formation of irreversible biofilms. Furthermore, the ability of UTI pathogens to form biofilm on inanimate surfaces contributes immensely to antimicrobial resistance as well as impending destruction of the biofilm once formed [16].

In an effort to limit biofilm the formation, strategies like making the catheter surface more hydrophilic [17] with hydrogel coatings, impregnation of the catheter with antimicrobial ointments or anesthetics, and coating the catheters with antimicrobial agents such as antibiotics nitrofurazone and rifampicin have been tried [18].

The antibiotic also inhibited *S. aureus* and also *C. albicans.* Antimicrobial coated catheters have the capacity to decrease the risk of pathogen colonization and subsequently reduce the levels of infection [19].

According to Nooupur *et al.* [20], while isolate A1 showed antibacterial activity against both Gram-negative and Gram-positive bacteria. It showed maximum zone of inhibition against *E. coli* (20 mm) followed by *P.*

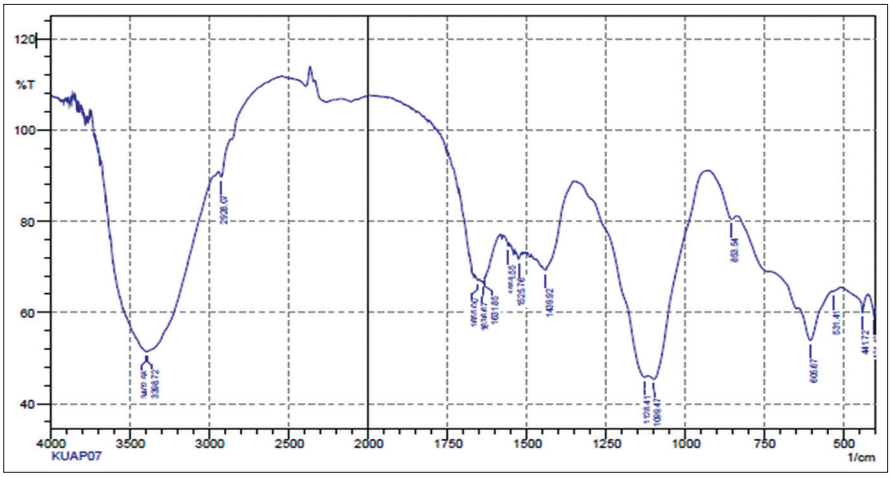

Fig. 5: Fourier transform-infrared analysis of antimicrobial compound

aeruginosa (18 mm), *B. subtilis* (17 mm), *Klebsiella* (5 mm), and *P. vulgaris* (13 mm) while isolate A3 showed a broad spectrum of antimicrobial activity against both Gram-positive, Gram-negative, and fungal pathogens.

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 activity [11]. Impregnation of catheter with a combination of minocycline-rifampin has been reported to produce a broad spectrum against *Staphylococcal*, Gram-negative bacillary, and *Candida* [21]. Chlorhexidine gluconate mostly used as an antiseptic and has a broad-spectrum activity.

Sherertz and group [22] demonstrated the efficacy of coating catheters with chlorhexidine both *in vitro* and *in vivo*. In randomized clinical trials, Maki *et al*. [2] reported that the catheters coated with tri-iododecyclemethyl ammonium chloride and chlorhexidine gluconate and silver sulfadiazine decreased the risk of catheter-related septicemia by more than 4 times.

Vaudaux *et al.* [23] noted that silver is an effective antimicrobial agent active in a wide variety of materials including glass and polymers. The mechanism of antibacterial activity of silver is due to the silver ions. The ions bind to the electron donor groups in biological molecules and cause defects in bacterial cell wall. The silver ions also bind to bacterial DNA, thus affecting permeability and bacterial metabolism at all levels. Hertrick *et al*. [24] indicated that polymers that release silver have strong antibacterial property, act as reservoirs of silver, and are capable of releasing silver ions for extended periods and for this production of an effective zone of inhibition is necessary.

Most CAUTIs are caused by enterobacteria (*E. coli, K. pneumoniae,* and *P. mirabilis*) with minor incidences due to *P. aeruginosa* or *C. albicans* [25]. *S. aureus* is also a UTI pathogen associated with the long-term urinary catheters [26]. These results contradict with those recorded by Cho *et al*. [27], who dipped their catheters in a mixture of gentamicin and poly (ethylene-co-vinyl acetate) but reported a gentamicin release for only 1 week and those by Rafienia *et al*. [28], who reported 12 days' release from catheters dipped in a mixture of gentamicin - copolymer. Yahiaoui *et al.* [29] illustrated that cinnamaldehyde and eugenol in cinnamon oil inhibited the growth of bacterial isolates. Similar results were recorded by Augustine *et al.* [15] for their ethyl acetate extract of *S. albidoflavus* PU23 whose absorption at 3296 and 1031.8 cm^{-1} indicated hydroxyl groups and absorption at 1639 cm^{-1} indicated a double bonding. Maximum absorbance peaks ranged between 215 and 270 nm. The characteristics of absorption peaks indicate a highly polyene nature compound. The spectral data in this research are consistent with those obtained by Saadoun *et al.* [30]. Mangrove actinomycetes showed the highest antimicrobial activity that can be exploited for various medical purposes [31].

Maleki and Mashinchian [32] reported that spectrum of ethyl acetate extracts of G614 C1 exhibited absorption at 3411 cm^{-1}, which indicated hydroxyl groups, the absorption at 2856 and 2915 cm^{-1} indicated hydrocarbon chassis, and the absorption at 1649 cm^{-1} indicating a double bond of polygenic compound. Noopur *et al*. [20] reported the peaks of isolate A3; between 600 and 800 cm^{-1} showing alkyl halide, 1080–1360 cm^{-1} amine group, 1400 and 1600 cm^{-1} aromatic group, 1670–1820 cm^{-1} carbonyl group, and peaks between 1800 and 1830 cm^{-1} showing anhydride group.

CONCLUSION

Conventionally available antimicrobial coated urinary catheters have been shown not to be effective in laboratory. Furthermore, there is no coated catheter available for either short-term or long-term catheter users, thus leaving them with the problem of reinfection. The results of this research suggest that the antimicrobial-impregnated catheters might reduce CAUTI in both short-term and long-term catheter usage.

Further studies on the antimicrobial catheter will include an *in vivo* evaluation of the impregnated catheters.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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