



Research Article

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ANTIBACTERIAL DRUG AND CARRIER LOADED CATHETERS TO RETARD DEVICE ASSOCIATED BACTERIAL INFECTIONS

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ABSTRACT

In this research work, the intravascular catheter surface was modified with antibacterial coatings for the prevention of bacterial biofilm formation. Surface was coated with two synergistic drug combinations (Moxifloxacin-Ornidazole) grafted with a biodegradable carrier (DL-lactic acid). The carrier acts like bridging agent between catheter surface and drugs; it also allows the drug mixtures to get released from the surface very stable. So that the concentrations of the drugs would get released at sustained rate with an initial drug burst concentrations. To evaluate the biofilm producing capability of the test organisms, Microtitre plate assay was employed. The qualitative antibacterial activity of the coated catheters was evaluated against the test organisms (*Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*). Good and evident inhibitory zones around the coated catheters were found potential in preventing the growth of biofilm producers. In conclusion, the obtained results revealed that the catheter surface modified with antibacterial coatings have prevented catheter-associated biofilm producing organisms.

Keywords: Intravascular, Biofilm, Moxifloxacin, Ornidazole, DL-lactic acid,

INTRODUCTION

Eradicating the device-associated biofilm forming organisms and its associated proteins are highly complicated in healthcare sectors¹. It was reported that the biofilm producers are highly (1000-fold) resistant than free-floating organisms². The reason for their resistivity was described critically due to different complex factors like expression of specific genes that codes for drug resistance and biofilm formation⁵.

Biofilm tolerance is of major clinical importance because more than 60% of the bacterial infections currently treated by physicians in the developed world are considered to involve biofilm formation⁶. The diseases like cystic fibrosis, endocarditis, device-related infections, and ventilator-associated pneumonia are considerably complicated by the formation of these biofilms, which are resistant to most current antimicrobial therapies⁷.

Certain bacteria, such as *Pseudomonas aeruginosa*, can exist as biofilms on a variety of surfaces, including tissues and medical devices. Here, the bacteria cluster together with an extracellular matrix that often includes exopolysaccharide (EPS), proteins, and DNA⁸, thereby providing an effective protection barrier against host immune responses and antibiotics. Each of these exopolysaccharides has been found to be involved in biofilm formation⁹.

Staphylococcus epidermidis was reported as a representative biofilm producing organism in causing medical device-related infections¹⁰. The onset of infection and the clinical complication depends on the type of catheter and its insertion site¹¹. Bacteriuria is highly associated with *S. Epidermidis* in case of repeated use of urinary catheters¹², and prosthesis-related infection in joint arthroplasty cases¹³. Biofilm formation by this organism was considered as multifactorial process solely dependent upon PIA expression^{14,15}. PIA, a linear β -1,6-linked glucosaminoglycan coupled with N-acetylglucosamine residues identified as the

significant component which allows the biofilm producer for intercellular adhesion^{16,17}.

Biofilm expression due to the action of PIA is influenced by different factors, very importantly the use of several antibiotics¹⁸. The structural genes required for the PIA expression is encoded in icaR locus operon (icaADBC genes)¹⁹. The icaR gene encodes a transcriptional repressor which was involved in the ica operon expression and biofilmformation²⁰.

Based on these perspectives, an antibacterial coating method was employed using two different set of drugs with a grafting carrier on the intravascular catheter surface. The drugs are selected based on their similar mode of action on inhibiting DNA replicative enzymes of biofilm producing organisms.

MATERIALS AND METHODS

Five biofilm producing organisms (*Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) were obtained from a diagnostic laboratory at Coimbatore, Tamil Nadu, India. All laboratory work was carried out in the Department of Microbiology Laboratory, Sree Narayana Guru College, Coimbatore, Tamil Nadu, India.

Evaluating the biofilm production of test organisms (Microtitre plate method)²¹

The evaluation was done using the method described by Christensen et al. (1985)²¹. In brief, all the test organisms (*Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) were allowed to grow in a 96-well microtitre plate for 24h. All the wells were rinsed to remove unbound cells. A dye solution was prepared (0.1% crystal violet solution) and 125 μ l of this standard was added to each well and incubated. The dye was removed by

washing successively with sterile distilled water. About 200µl of 95% ethanol was added to remove the bound dyes from each stained well. The wells were covered and incubated for 60min at 28°C. The bound dyes were removed and added onto a fresh titre plate. The colour intensity obtained was measured as optical density at a wavelength of 600nm using ELISA reader. In Table-1, the classification of biofilm index was presented.

Anti-biofilm coating of intra vascular catheter materials using antibiotics²²

Anti-biofilm coating of intravascular catheter materials was carried out under strict aseptic conditions using a drug combination; each being from a different group. In brief, a known concentration Moxifloxacin (fluoroquinolone drug) and ornidazole (nitroimidazole drug) was selected and added with molten polyethylene glycol (PEG). PEG was used for a drug-slurry preparation and the resulting slurry was homogenized in a magnetic stirrer. The catheter samples were coated twice with intermittent drying by dipping inside the drug and polymer slurry mixtures. Following this protocol, the catheter samples were immersed into a carrier solution (DL- lactic acid was used as a drug-carrier molecule). All the samples were stored under freezing conditions and finally dried at room temperature prior testing.

Evaluating the qualitative antibacterial activity of coated IVC materials²³

Antibacterial activity of all the coated catheter samples was qualitatively evaluated. Coated catheters were sterilized and placed over Mueller-Hinton agar (MHA) surface which was seeded with test organisms. All the plates were incubated at 37°C for 48h. Qualitative antibacterial activity of the coated catheters was identified by the inhibitory zones.

Bio-statistical analysis to determine the effect of antibacterial drug on bacterial growth

A non-parametric test (Chi-square) was used as a bio-statistical tool to determine the effect of antibacterial drug on bacterial growth. The hypothesis selected (H_0) was that "There is significant effect of antibacterial drug on the test organisms". The difference in the size of bacterial inhibitory zones between the antibacterial drug coated and uncoated catheter materials were statistically calculated with $P < 0.05$ considering significant.

RESULTS AND DISCUSSION

Ability of the test organism to produce biofilm

Microtitre plate method was employed to evaluate the ability of each test organism to produce biofilm. The biofilm production of each organism was compared with the standard index value (Table 1) and presented in Table 2. These standard biofilm index values were earlier reported in Christensen *et al.*, (1985). When compared to the standard values in Table 1, four organisms [*S. epidermidis* (0.252), *S. aureus* (0.292), *E. coli* (0.293) and *Pseudomonas aeruginosa* (0.265)] showed OD values greater than 0.240. Hence, these organisms were considered as high biofilm producers. *Proteus mirabilis* showed moderate biofilm index value of 0.195. Difference in the biofilm production was correlated with the obtained OD values of crystal violet dye that

was absorbed by the organism in the microtitre well. In Fig-2, the image showed the difference in the colour intensities of each organism that emphasizes the biofilm production.

According to Hassan *et al.*²⁴ the microtitre plate test is a more quantitative and reliable method for the identification of biofilm producing bacterial species when compared to other similar type of methods (Congo Red agar method and Borosilicate tube method). The researchers also recommended using this method as a common screening method for detection of biofilm producers in diagnostic laboratories. During their analysis, similar differentiation in biofilm production by the organisms was observed. Among the 110 clinical isolates, the researchers found 22.7% of high biofilm producers, 41% of moderate and 36.3% of weak biofilm producers. In another study carried out by Donlon²⁵ similar type of biofilm production was observed from the urinary catheters.

Qualitative antibacterial activity of coated catheter materials

The inhibitory zone measured in millimetres for moxifloxacin-ornidazole drug coated catheter materials against each test organisms were presented in Table-3. The antibacterial drugs and the carrier coated catheter samples (DCC) showed significant inhibitory zones. The zones were ranged from 14mm to 30mm against all the test organisms. Whereas, no inhibitory zones was observed for the uncoated materials. The drug and carrier coated samples showed maximum inhibitory zones of 30mm and 29mm against *Acinetobacter baumannii* and *Escherichia coli* respectively. Other biofilm producers, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* also showed significant inhibitory zones measuring 25mm, 24mm and 26mm respectively. In Fig. 1a, 1b, 1c, 1d and 1e, the qualitative antibacterial activity indicating the clear inhibitory zones around the drug-carrier coated IVCs against the test organisms were presented.

The rationale of combination of moxifloxacin-ornidazole was based on the fact that quinolones and imidazole derivatives act synergistically. This could be due to action of drugs on different sites on the bacterial DNA. A primary effect of quinolones is inhibition of bacterial DNA synthesis in the presence of competent RNA and protein synthesis²⁶. According to Bharadwaj *et al.*²⁷ fluoroquinolone and nitroimidazole drugs in combination inhibits the activity of one of the A-subunits of the bacterial enzyme DNA gyrase, which is responsible for the negative super coiling of DNA, an essential conformation for DNA replication in the intact cell. Fluoroquinolone inhibits the activity of one of the A-subunits of the bacterial enzyme DNA gyrase, which is responsible for the negative supercoiling of DNA, an essential conformation for DNA replication in the intact cell. This inhibition of DNA gyrase activity is lethal to the bacterial cells. The 5-nitro group of imidazole undergoes reductive transformation to an active intermediate, which then exerts an inhibitory or lethal effect against DNA. Not only is DNA synthesis inhibited but the reduced metabolite also causes a loss of the helical structure of DNA with subsequent DNA strand breakage. Other reduction-oxidation processes within anaerobic organisms may also be inhibited, which also contribute to cell death. It is primarily active against obligate anaerobic microorganism. Under strictly anaerobic conditions, it also has an effect on facultative anaerobic organisms²⁸.

Table 1: Classification of biofilm formation

Mean OD values	Biofilm formation	Biofilm index
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<0.120	Nil	Non / weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

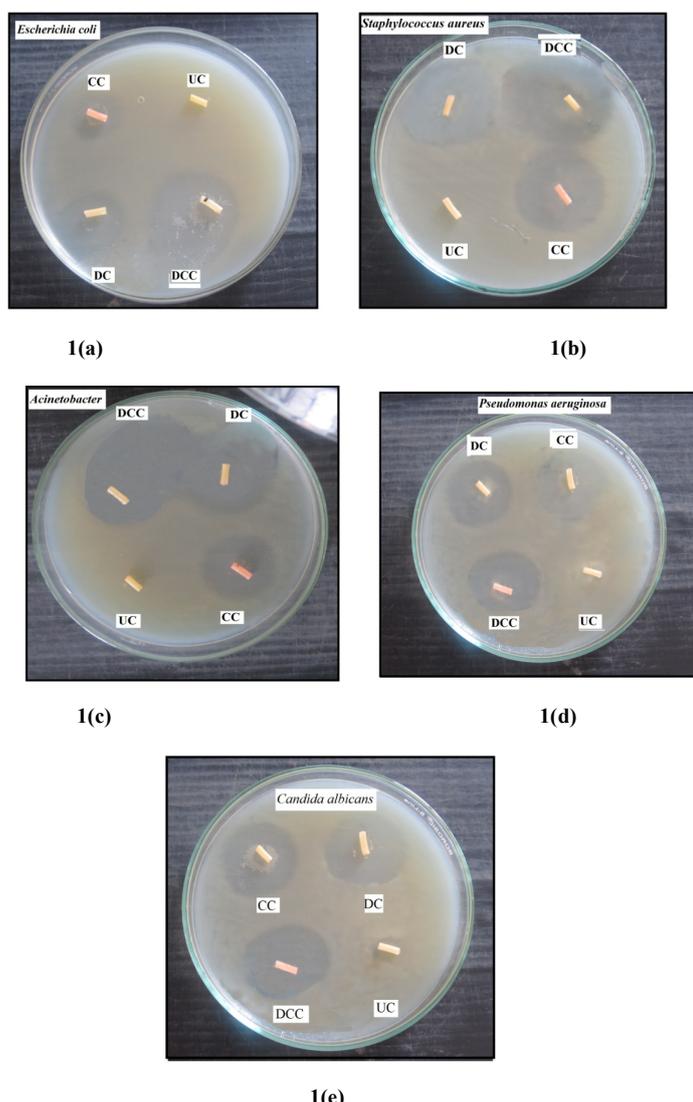
Table 2: Screening test bacteria for biofilm formation by MTP method

Test Bacteria	Biofilm formation (OD 570nm)	Biofilm index
Control C ₁ (Crystal violet)	0.08	weak
Control C ₂ (Nutrient broth)	0.09	weak
<i>S. epidermidis</i> (ATCC 35984)	0.252	High
<i>S. aureus</i> (ATCC 29213)	0.292	High
<i>E. coli</i> (ATCC 43894)	0.293	High
<i>P. mirabilis</i> (ATCC 49565)	0.195	Moderate
<i>P. aeruginosa</i> (ATCC 700829)	0.265	High

Biofilm Index - <0.120: Weak, 0.120-0.240: Moderate, >0.240: High

Table 3: Assessing the qualitative antibacterial activity of dip-coated materials

Sl. No	Sample	Zone of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Acinetobacter baumannii</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
1	Uncoated catheter (UC)	0	0	0	0	0
2	Drug coated (DC)	18	22	23	21	24
3	Carrier coated (CC)	14	20	19	22	21
4	Drug carrier coated (DCC)	29	25	30	24	26



1(e)

Figure 1: Assessing the qualitative antibacterial activity of dip-coated materials

Bio-statistical determination of the effect of antibacterial drug on bacterial growth

The effect of antibacterial drug against the bacterial growth was determined using a non-parametric chi-square bio-statistical test method. The difference in the size of bacterial inhibitory zones

between the antibacterial drug coated and uncoated catheter materials was selected and statistically calculated with $P < 0.05$ considering significant. The coated materials showed more inhibitory zones than the uncoated materials. During the statistical analysis, the calculated value was found significantly less than the table value; hence the designed hypothesis was accepted. The statistical survey of the research proved the qualitative antibacterial activity of the drug coated catheter materials.

CONCLUSION

Intra vascular catheter associated infection accounts for approximately 35% of infections in the health-care center. Several reports reasoned for the infections as the surface colonization of catheters by biofilm producing organisms. The present research was aimed to prevent the biofilm organisms by surface modification of the catheters by coating with two types of drugs and a carrier molecule. The antibacterial activity of the coated materials proved the anti-biofilm properties of the drug and carrier mixtures against the test organisms. To conclude, the results showed the anti-biofilm activity of drug and carrier combinations in the presence of a polymer, PEG. The biocompatibility of the antibacterial drug coated materials can be studied as future perspective in order to know the accurate concentrations essential to prevent the biofilm formation without any tissue toxicity.

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