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Original Article

Antibacterial Modification of Intravascular Catheter Surface for the Prevention of Catheter-Associated Infection

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ABSTRACT

Objective: Intravascular catheter-associated infection has been increasing hospitalization in post-surgery patients mainly due to microbial colonization of the catheter surface and formation of a superficial biofilm layer. The present study is aimed in developing an effective antibacterial device which can prevent colonization of organisms by modification of catheter. **Methods:** In the present study, the antibacterial activities of intravascular catheters are impregnated with third generation broad spectrum antibiotic cefixime under *in vitro* conditions. To enhance sustained drug release from the catheter surface, a carrier polyvinyl alcohol was added as a second layer. Biofilm forming abilities for the test cultures were initially determined using a standard Exit-site challenge test viz., Borosilicate tube assay and Microtitre plate assay .These test were performed to evaluate the biofilm production using biofilm index. **Results:** Qualitative and quantitative antibacterial activity tests were performed in modified catheters. It was observed that modified catheters could potentiality prevent the growth of test organisms. During the in vitro conditions it was observed that the growth and survival all the four high biofilm producers viz., *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and the moderate biofilm producer, *Proteus mirabilis* were prevented when exposed to drug-carrier coated catheters. These *in vitro* results suggest that the antibacterial drug-carrier coated catheters can potentially be used to combat catheter colonization and catheter-associated infections.

1.INTRODUCTION

Intravascular catheters are the most common assist devices utilized every year (Treter and Macedo, 2011). These catheters and its types are used for the administration of fluids, medications, parenteral nutrition, and blood products; to monitor hemodynamic status; and to provide hemodialysis (Donlan 2011). Due to these multiple functions, after exposure to body fluids the device becomes a suitable environment for the support of biofilm production and subsequent infection. Microbial adhesion to medical device surface is considered the base of the pathogenic mechanism of prevalent *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Treter and Macedo 2011). Donlan (2001) reported biofilm producers are highly associated with the increased risk of central line-associated bloodstream infections (CLABSI) which results in significant morbidity, mortality, and costs for health care delivery. World Health Organization (WHO) proposed several methods and guidelines for the prevention of CLABSI (Wisplinghoff *et al*., 2004). According to WHO and other

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agencies, antimicrobial resistance is now considered to be a global crisis; continued to increase and there was no exception regarding the organisms that cause CLABSI (Safdar and Maki 2004). Proportion of CLABSI caused by antimicrobial-resistant organisms, such as methicillin resistant gram-positive cocci and flucanozole-resistant *Candida* sp has been increasing at an alarming rate. Most predominant organisms responsible for nosocomial CLABSI are coagulase negative *Staphylococci*, *S. aureus*, *Enterococci*, *E. coli, Klebsiella* sp and *Candida* sp (Wisplinghoff *et al*., 2004). The systemic administration of antibiotics was employed for many years in an effort to inhibit bacterial colonization and encrustation; but antibiotic resistance of the organisms proved this method ineffective (Shaw *et al*., 2005). A promising alternative to systemic antibiotic administration against CLABSI is using local delivery of antibacterial agents from the surface coated catheters (Wu and Grainger 2006). Antibiotic-coated catheters (Cho *et al*., 2003), Silver-coated catheters (Srinivasan *et al*., 2006) and hydrogel-coated catheters (Sabbuba *et al.,* 2002), was used to modify the environment that surrounds the catheter for preventing the biofilm forming organisms (Rodrigues *et al*., 2007).

In the present study, a simple and cost-effective coating technique to form a cefixime-loaded layer of polyethylene glycol on the surface of intravascular catheter to prevent biofilm formation during short-term catheterization was studied. Poly (vinyl alcohol) (PVA) was applied on the top of poly (ethylene glycol) (PEG) coating to form a multilayer surface profile as a second layer carrier (Boccaccini *et al*., 2003). This was done to provide a rough surface texture on the catheter and also for sustained drug release. Furthermore the friction exerted by a catheter can cause irritation of mucosa and subsequent inflammation, PVA-coated catheter surface reduce this friction, reduces protein adsorption and exhibits better antibacterial activity against biofilm producers.

2. MATERIALS AND METHODS

About 20 different bacterial test cultures were isolated from the biological specimens collected from a diagnostic laboratory at Coimbatore, Tamil Nadu, India. The entire research work was carried out from September 2013 to July 2014.

2.1.Determining the surface colonizing capability of test bacteria on intravascular catheter materials

The surface colonizing capability of test bacteria on intravascular catheters (IVC) was determined using standard preliminary (Exit-Site challenge test) and confirmatory tests (microtitre plate biofilm assay and borosilicate tube biofilm assay). *Preliminary Exit-site challenge test* (Bayston *et al.,* 2009)

Exit-site challenge test was performed as the preliminary test. This test was used to identify the ability of specific test organism to grow on a type of biomedical materials used in the study. In this method, three-quarter strength of Iso-sensitest semi solid Agar was poured into a sterile boiling tube and allowed to solidify. The surface of the agar was then inoculated with 10µl of 18h test cultures (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The pre-measured size (length - 15mm) of IVCs were cut, sterilized and partially inserted into the Iso-sensitest semi-solid medium through the inoculated area and incubated at 37°C. Migrating ability of the test bacteria from the ''exit site'' down the material track i.e., outside of the materials were assessed visually up to 24-48 hours.

2.2. Assessing the biofilm forming capability of test bacteria on IVC using standard confirmatory test methods

The efficiency of test organisms to form biofilm on IVCs was confirmed after the preliminary exit-site screening test using two standard methods viz., Microtitre plate biofilm assay (Christensen *et al.,* 1985) and borosilicate tube biofilm assay (O'Toole *et al*., 1999). *Borosilicate tube biofilm assay* (O'Toole *et al*., 1999)

Biofilm formation assay in tubes were performed using the following protocol. Two millilitres of the inoculated TSB medium were incubated in borosilicate (BS) or polypropylene (PP) tubes for 24 and 48 h. For each condition, 4 tubes were processed. Two tubes were vortexed before determining the OD_{546} to estimate total cell biomass by using a spectrophotometer. The other 2 tubes were aspirated, washed with distilled water and stained with 2 ml of crystal violet (0.01%) for 30 min. The biofilm was visualized and classified as strong, moderate, weak or negative, and quantified by adding 2 ml of ethanol and measuring the OD_{546} of dissolved crystal violet. The OD values were considered as an index of bacteria adhering to surface and forming biofilms. Confirmatory test on biofilm formation using Microtitre plate biofilm assay (Christensen et al., 1985)

Bacterial attachment to an abiotic surface is assessed by measuring the stain taken up by adherent biomass in a 96-well plate format by means of microtitre biofilm assay. The test organisms were grown in 96-well microtitre plate for 48 hours. The wells were washed to remove any unbound test bacteria. Cells remaining adhered to the wells were subsequently stained with a dye that allowed visualization of the attachment pattern. Each of the test organisms were inoculated in a 5 ml culture broth and grown to stationary phase. Cultures were diluted at 1:100. Following this, 100 µl of each diluted cultures were pipetted into eight wells in a fresh microtitre plate. The plate were covered and incubated at optimal growth temperature for 24-48 hours. Four small

trays were set up in a series and 1 to 2 inches of tap water were added to the last three. The first tray was used to collect waste, while the others were used to wash the assay plates. Unbound bacteria if any were removed from each microtitre dish by briskly shaking the dish out over the waste tray. About 125 µl of 0.1% crystal violet solution was added to each well. Staining was done for 10 min at room temperature. The crystal violet solution was removed by shaking each microtitre dish out over the waste tray. The dishes were washed successively in each of the next two water trays and as much liquid as possible was shaken out after each wash. To remove any excess liquid, each microtitre dish was inverted and vigorously tapped on paper towels. The plates were allowed to air-dry. Added 200 µl of 95% ethanol to each stained well. The plates were covered to allow solubilization by incubating for 10 to 15 min at room temperature. The contents of each well were briefly mixed by pipetting. Following this, 125 µl of the crystal violet-ethanol solution was transferred from each well to a separate well in an optically clear flat-bottom 96-well plate. The optical density (OD) of each of these 125µl samples was measured at a wavelength of 500 to 600 nm. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader (Braun, Germany). Based on the OD value, the adherence of organism in the silicate tubes and titre plates were classified as mentioned in Table-1.

Table.1.

Classification of biofilm formation

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

Table adapted from Mathur *et al*., (2006)

2.3. Preparation of antibacterial drug and coating IVCs by slurry-dipping technique (Boccaccini *et al*., 2003)

Drug releasing intravascular catheters were made using a standard slurry-dipping technique. The technique started with the preparation of stable slurry with specific amount of Cefixime in the molten polyethylene glycol (PEG). Appropriate slurry temperature (37°C) was determined by an optimization process based on a trial-and-error approach to achieve optimum coating thickness, uniformity and stability of composite coating as well as adequate infiltration of drug particles into coating structure. PEG (2g) with a predefined molecular weight was mixed with Cefixime powder (0.5g) in a glass vial. The mixture was heated at the range of 60–70°C in a water bath to obtain homogeneous slurry. The resulting slurry was homogenized in a magnetic stirrer for 5 to 10 min. Each piece of catheter (length - 6mm) was dip-coated twice with intermittent drying (suspension coating method). The dip-coating procedure was carried out in sterile glass beakers on a shaker (120 rpm) for 30min, with a drying period of about 15 minutes between the two coating procedures, followed by drying at room temperature. All coating steps were carried out under strict aseptic conditions.

All samples were coated by a thickness of about 5% (mm) of catheters outer diameters. After coating procedure, the catheter samples were stored at 4°C for upto 15min. In order to increase drug loading and prevent excessive increase in catheter thickness, the coating process were repeated for replicates of each sample. Subsequently, in order to slow down the release rate of drug from PEG coating and mitigate the friction effect between catheter surface and mucosa, second coating layer was formed on the catheter surface. Polyvinyl alcohol (PVA) was dissolved in DMSO to acquire a 10 w/w% solution. PEG-coated samples were submerged into PVA solution three times for 1 min each. Thereafter, these samples were stored at 0°C or in a deep freezer to implement one freeze–thaw cycle and physically crosslink the samples. The coated catheters were left to dry on a clean bench for 1 week at room temperature to remove residual DMSO.

2.4. Assessing the qualitative antibacterial activity of dip-coated IVC materials (El-rehewy *et al*., 2009)

The method was performed for analysing the antibacterial activity of IVCs after slurry dip-coating with antibacterial drugs (Cefixime) and carriers (PVA). In this qualitative method the pre-measured size (length-6mm) of all sterilized IVC materials were tested from each preparation [*drug-carrier* coated and uncoated materials]. The materials were all rinsed twice in phosphate buffered saline (PBS) before testing to remove any surface accumulation of drug. All test materials were placed on the surface of Mueller-Hinton agar (MHA) plate which had previously been seeded with an overnight broth culture of the test organisms and incubated at 37°C for 24 to 48h. The experiments were carried out in triplicate. Antibacterial activity was expressed as the diameter of the zone of inhibition.

2.5. Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test (El-Rehewy *et al*., 2009)

Antibacterial activity of coated and uncoated IVC materials were quantitatively measured using standard

bacterial adherence test against test organisms. The coated materials were placed separately in a tube with 5 ml of each of the test bacteria and incubated at 37 °C for 18 h. During the incubation period the bacterial cells adhere on the surface. After incubation, the numbers of viable adherent cells were determined as follow: IVC materials were collected aseptically and washed in sterile normal saline twice to remove the non-adherent cells. The washed pieces were sonicated for 30 seconds to dislodge the sessile adherent. After sonication, serial dilution of the sonicated saline was made and the number of sessile bacteria was detected to determine the degree of adherence by viable count technique. Similar experimental set up was run in parallel for uncoated materials. The difference in number of adhered cells on coated and uncoated catheters was determined statistically using chi square analysis.

The percentage reduction of adhered organisms on the coated materials was determined using a standard percentage reduction formula.

$$\text{Bacterial reduction (\%)} = A - B/A \times 100$$

Where,

A = number of adhered organisms (in CFU) obtained from the uncoated materials

B = number of adhered organisms (in CFU) obtained from the coated materials

2.5. Statistical analysis of total viable bacteria on coated materials

Chi-square non parametric test using SPSS-9 for Windows 7 was used as a statistical tool to determine the effect of antibacterial drug on bacterial adherence. The hypothesis selected (H_0) was that "There is significant effect of antibacterial drug on the test organisms". The difference in the bacterial reduction percentage between the coated and uncoated materials were statistically calculated with $P<0.05$ considered significant.

3. RESULTS AND DISCUSSION

3.1. Determining the surface colonizing capability of test bacteria on intravascular catheter materials

3.1.1. Preliminary Exit-Site challenge test

Bayston *et al.,* (2009) reported that the most frequent routes of catheter associated infection are from the skin exit site, the tissue tunnel associated with the catheter and the catheter lumen. Similar exit-site challenge model under *in vitro* condition used by Bayston *et al.,* (2009) showed surface colonization of methicillin resistant *Staphylococcus aureus* (MRSA) on the CSF silicone shunts surface. In this present study the surface colonizing ability of test bacteria on the IVC sample materials was investigated using exit-site challenge test. Migration or growth of the test organism around the materials after incubation was indicated by tracking of bacteria along the abluminal surface. All the test organisms used in the research colonized the material surfaces between 24h to 48h. Among the test organisms *Staphylococcus epidermidis* and *Staphylococcus aureus* colonized with in 24h; *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis* colonized the catheter surface after 48 hours. According to Bayston's concept, the inoculated site was considered to be as skin exit-site and migration and growth of the organisms along the media surface was considered to be as the tissue tunnel and tissue surroundings. So the obtained results were considered as the preliminary test to determine the surface colonizing ability of the test organisms.

3.2. Assessing the biofilm forming capability of test bacteria on IVC using standard confirmatory test methods

3.2.1. Borosilicate tube biofilm assay

In the borosilicate tube biofilm assay, strong and moderate biofilm formation by test organisms was observed. In Table-2, the optical density (OD) values and biofilm index of the test organisms were tabulated. The test organisms considered as strong biofilm producers in this assay were *S. epidermidis* (0.24), *S. aureus* (0.25), *E. coli* (0.24) and *Pseudomonas aeruginosa* (0.22). All the four test cultures showed the OD values >0.240. Moderate biofilm formation was observed for *Proteus mirabilis* (0.18). In Fig.1, the amount of crystal-violet absorbed by the test organisms in the bottom of the tubes was clearly presented along with the control samples. Control samples showed weak biofilm index (<0.120) when compared to that of the bacterial samples.

Table.2.
Screening test bacteria for biofilm formation by borosilicate tube assay

Test Bacteria	Biofilm formation (OD 570_{nm})	Biofilm index
Control C_1 (Crystal violet)	0.09	Weak
Control C_2 (Nutrient broth)	0.10	Weak
S. epidermidis	0.24	High
S. aureus	0.25	High
E. coli	0.24	High
P. mirabilis	0.18	Moderate
P. aeruginosa	0.24	High

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

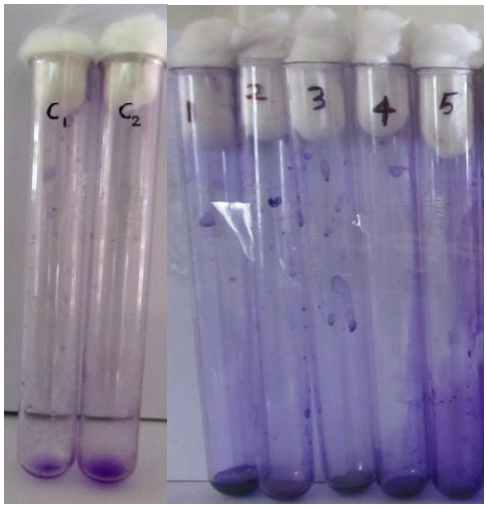

Fig. 1: Assessing the biofilm forming capability of test bacteria on IVC using Borosilicate tube biofilm assay

C_1 - Control (Crystal violet), C_2 - Control (Nutrient broth), 1. Staphylococcus epidermidis 2.Staphylococcus aureus 3.Escherichia coli 4. Proteus mirabilis 5.Pseudomonas aeruginosa

3.3. Confirmatory test on biofilm formation using Microtitre plate biofilm assay (MTP)

The observations of MTP biofilm assay was recorded in Table-3. When compared to borosilicate tube assay, similar biofilm index for all the test organisms were observed in MTP assay. The optical density (OD) values and biofilm index of the test organisms were tabulated based on the biofilm classification described by Christensen *et al*., (1985). The test organisms considered as strong biofilm producers in MTP assay were *S. epidermidis* (0.252), *S. aureus* (0.292), *E. coli* (0.294) and *Pseudomonas aeruginosa* (0.264). These organisms showed OD values >0.240. Moderate biofilm formation was observed during the MTP assay for *Proteus mirabilis* (0.195). The differences in OD values were due to the amount of crystal-violet (dye) absorbed by the test organisms in the microtitre well. The high and moderate biofilm producers were identified by the colour intensities formed in the microtitre plates .Appropriate control was mantained.

Mathur *et al*., (2006) reported similar findings as observed in the present study .Out of 152 clinical isolates 88 were identified as biofilm producers. Their results showed that, 57.8 % displayed a biofilm-positive phenotype and the strains were further classified as weak 22 (14.47 %), moderate 66 (39.4 %) and 70 (46.0 %) isolates as high biofilm producers. This method was found to be most sensitive, accurate and reproducible screening method for the detection of biofilm formation. The method has the advantage of being a quantitative model to study the adherence of organism on biomedical devices. In this test, even though some of the test organisms were proved to as weak or moderate biofilm producers, still due to their clinical complications and pathogenicity in the medical sciences, it made curious to proceed for further analysis.

Table.3.

Screening test bacteria for biofilm formation by MTP method

Test Bacteria	Biofilm formation (OD 570_{nm})	Biofilm index
Control C_1 (Crystal violet)	0.08	Weak
Control C_2 (Nutrient broth)	0.09	Weak
S. epidermidis	0.252	High
S. aureus	0.292	High
E. coli	0.294	High
P. mirabilis	0.195	Moderate
P. aeruginosa	0.264	High

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

3.4. Assessing the qualitative antibacterial activity of dip-coated IVC materials

The diffusing ability of the antibacterial drugs from the *drug-carrier* coated IVC materials to retard the growth of test bacteria seeded on MHA plate was calculated based on the zone of inhibition. The zone of inhibition measured in millimetres for each drug-carrier combinations (tested in triplicates) was calculated to obtain the mean value. In Table-4, reveal the antibacterial activity of *drug-carrier* coated materials for all the test organisms. No inhibitory zones were observed for all uncoated materials. In contrast, all the *drug-carrier* coated IVC sample materials showed significant inhibitory zones ranged from 31.3mm to 39.6mm against all the test organisms.

Table.4.

Assessing the qualitative antibacterial activity of dip-coated IVC materials

S. No	Sample	Organisms	Zone of Inhibition (mm)	
			dcc IVCs	uc IVCs
1	Intravascular Catheter	S. epidermidis	38.3	0
2		S. aureus	39.6	0
3		E. coli	32.6	0
4		P. mirabilis	32.3	0
5		P. aeruginosa	31.9	0

dcc: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

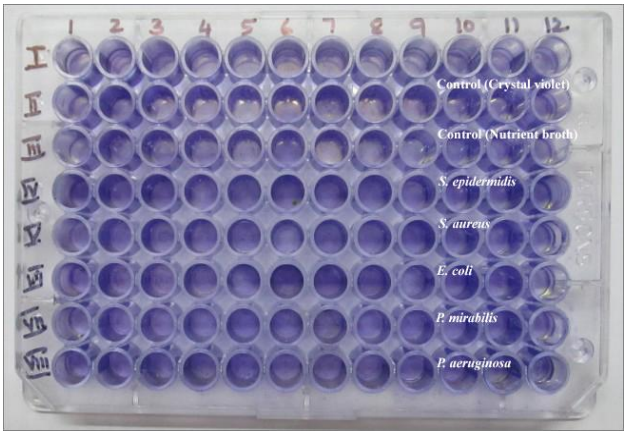

Fig. 2: Assessing the biofilm forming capability of test bacteria on IVC using Microtitre plate biofilm assay

Modified IVC samples showed maximum inhibitory zones of 39.6mm and 38.3mm against *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively. Other high and moderate biofilm producers, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Proteus mirabilis* also showed significant inhibitory zones measuring 32.6mm, 31.9mm and 32.3mm respectively. In Fig.3a- 3d indicates the antibacterial activity with the clear inhibitory zones around the *drug-carrier* coated IVCs against the high biofilm producing test cultures was presented. The drug coated with the carrier in IVC materials was cefixime, a cephalosporin. Cefixime is a beta-lactamase stable third generation cephalosporin, which is a semi synthetic compound and was the first orally active and effective antibiotic with longest half-life (Rafal'skii *et al*., 2011). In the present study, the modified catheter samples showed significant antibacterial activity against all test cultures. In support of this Memon *et al* (1997) reported that, Cefixime has very significant biological properties, as it exhibits potent antibacterial activity against a varied range of different strains of bacteria. Investigation of Rafal'skii *et al*., (2011) revealed that antibacterial activity of Cefixime is due to aminothiazole ring and the R-oxy amino group present on the side chain at the 7-position in its chemical structure; which inhibits peptidoglycan synthesis in the bacterial cell wall (Petri, 2006).

Study on antibacterial activity of modified drug coated biomaterials exhibit that, the increase in antibacterial activity was due to the synergistic behaviour of drugs and carrier in combination rather than individual effect (El-rehewy *et al*., 2009). Further it was reported that the Ciprofloxacin/N-acetylcysteine coated polyurethane vascular catheters produce more inhibitory zones for *P. vulgaris, P. aeruginosa, S. aureus* and *C. freundii*. The measured zone of inhibition was between 15–20 mm.This status holds good for the present study also with respect to biofilm producing *S. epidermidis, S. aureus, E. coli* and *P. mirabilis* using drug-carrier combinations. Matl *et al*., (2008) reported, gentamicin-teicoplanin/DL-lactic acid coated PTFE prosthesis inhibited the biofilm producing *S. aureus* (ATCC 49230) at a greater level than a prosthesis coated without a Dl-lactic acid. Similarly, Bayston *et al*., (2009) inhibited the biofilm producing methicillin resistant *S. aureus* and *E. coli* on the rifampicin and trimethoprim surface coated peritoneal dialysis catheters.

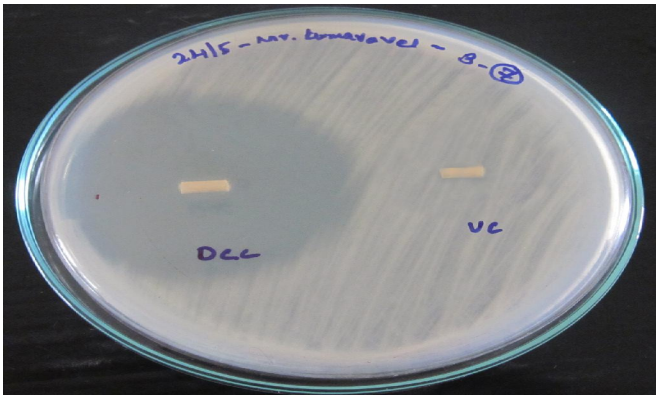

Fig. 3: Assessing the qualitative antibacterial activity of dip-coated IVC materials(*Fig. 3a: Staphylococcus epidermidis*)
dcc: Drug-carrier coated catheter samples, uc: Uncoated catheter samples

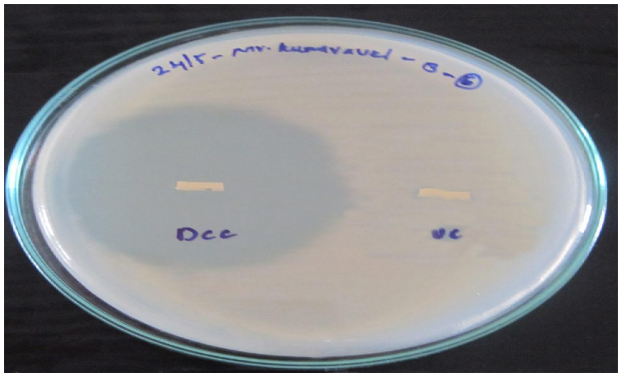

Fig. 3b: Staphylococcus aureus. *dcc*: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

Fig. 3b: Escherichia coli. *dcc*: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

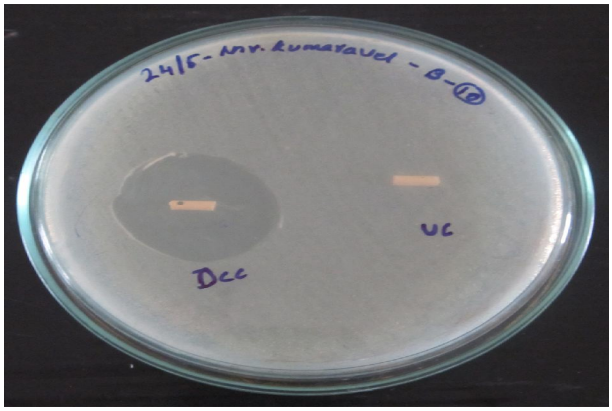

Fig. 3d: Pseudomonas aeruginosa. *dcc*: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

3.5. Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test

Anti-adherent activity for each coated IVC sample materials were analysed using bacterial adherence test. The anti-adherent activity was calculated by bacterial reduction percentage. The anti-adherent activity of Modified IVC materials against the test organisms was concentration dependent as the reductive effect of drugs and carriers was in the range of 96.5% to 100% (Table-5). Bacterial reduction percentage calculated from the CFU of the antibacterial drug coated materials against the test organisms was measured. Maximum bacterial reduction percentage was observed for two high biofilm producers, *S. epidermidis* (100%) and *S. aureus* 100%). Interestingly, maximum antibacterial activity was observed for both of these test cultures during the qualitative assay. Other cultures also showed significant reduction percentage. *E. coli, P. aeruginosa* and *P. mirabilis* were reduced upto 96.5%, 98.5% and 97% respectively.

Table.5.

Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test

S. No	Sample	Organisms	Bacterial reduction (%)	
			dcc IVCs	uc IVCs
1	Intravascular Catheter	S. epidermidis	100	0
2		S. aureus	100	0
3		E. coli	96.5	0
4		P. mirabilis	97.0	0
5		P. aeruginosa	98.5	0

dcc: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

Reid *et al*., (1994) reported that bacterial adherence test with biofilm producing bacteria was reduced to more than 99% for drug coated implants that Pre-incubation of catheters with 50 and 100µg/ml concentrations of ciprofloxacin resulted in 99% reduction in the number of adherent bacteria in comparison to uncoated materials. Liedberg and Lundeberg, (1990) analysed that the silver coated urinary catheters prevents bacteria adherence and reduces the amount of catheter associated urinary tract infections. Gollwitzer *et al*., (2003) reported that the Poly DL-Lactic acid-coated implants significantly reduced adhesion of viable Staphylococci compared with bare K-wires made from either titanium or stainless steel alloy. In the present study the strong biofilm producers, *S. epidermidis* and *S. aureus* were significantly reduced to 100% when exposed to drug and carrier combinations.

3.6. Statistical analysis of total viable bacteria on coated materials

Using chi-square statistical analysis, the effect of antibacterial drug on bacterial adherence was determined. The difference in bacterial reduction percentage of *drug-carrier coated* (*dcc*) and *uncoated* (uc) materials were taken as the experimental design. The hypothesis selected was "There is significant effect of antibacterial drug on the test organisms". The difference in the bacterial reduction percentage between the *dcc* materials and the *uc* materials were statistically calculated with $P<0.05$ considering significant. For all the data, the calculated value was less than the table value. In Table-6, the calculated value of each *dcc* materials tested against all the test bacteria was presented. Since the calculated value was less than the table value, the assigned hypothesis could be accepted. The *dcc* materials showed more bacterial reduction percentage than the *uc* materials. The statistical survey of the research proved the quantitative antibacterial activity of the *dcc* materials.

Table.6.

Statistical analysis on anti-adherent activity of IVC materials

Test Cultures	Significant comparisons	Calculated value	Table value	Chi square test
S. epidermidis	dcc vs uc	3.77	14.06	P < 0.05
S. aureus	dcc vs uc	4.26	14.06	P < 0.05
E. coli	dcc vs uc	4.89	14.06	P < 0.05
P. mirabilis	dcc vs uc	4.93	14.06	P < 0.05
P. aeruginosa	dcc vs uc	5.65	14.06	P < 0.05

Calculated value is less than the Table value.Table showing significant difference in the reduction percentage between *Drug-carrier coated* (*dcc*) materials and the *uncoated* (*cc*) materials were observed ($P < 0.05$)

CONCLUSION

Catheter-associated infection is the most common site of healthcare-associated infection, accounting for more than 30 % of infections reported by acute care hospitals. Prevention of CLABSI is one of the significant scientific efforts. In this study one such approach was reported to prevent the catheter-associated infection for short and long-term catheterization. Qualitative and quantitative antibacterial activity of drug-carrier coated catheters showed promising results against all the varied test cultures used in the study. Thus the obtained results showed that combination of an antibiotic and a biocompatible polymer can hold promise for the chance of suppression of CLABSI. Drug release analysis from the catheter surface and the biocompatibility of the surface modified catheters need to be assessed for the approval in human trails.

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