EVOLVULUS ALSINOIDES (VISHNU KRANTHI) EXTRACT COATING ON CATHETERS AGAINST URINARY TRACT INFECTION

S. Reshmika[1*] & B. Elayarajah[2]

[1]Bachelor of science in Microbiology, Department of Microbiology,Dr.N.G.P Arts and Science College, Coimbatore. India.

[2]Chief Scientist, Gram Positives research and development laboratory, Coimbatore, India.

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ABSTRACT: *Catheter Associated Urinary tract infection (CAUTI) is a nosocomial infection acquired by patients in the health care facilities. Bio-film is mainly responsible for causing Catheter Associated Urinary tract infection causing pathogens attribute their bio-film forming ability during catheterization. Increased resistance to drugs has led to search for a new antimicrobial compound from various sources. The effect of herbal plant (Vishnu kranthi) extract on urinary tract infection causing pathogens was determined. The surface colonizing capability of test bacteria, qualitative antibacterial assay of the herbal extract coated catheters and HET-CAM test for biocompatibility of coated catheter were evaluated. All the test organisms used in the research colonized the material surfaces between 24 to 48 hours. Among the test organisms Staphylococcus epidermidis and Escherichia coli colonized with in 24hours; Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus mirabilis colonized the catheter surface after 48 hours. No inhibitory zones were observed for all uncoated materials. Herbal extract coated catheters exhibited antibacterial zones but slightly less than that of the drug-carrier coated catheters. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative controls. Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed. ThusEvolvulusalsinoidesplant extract can be coated onto the catheters in treating the pathogens responsiblr foe UTI.*

Key Words: *Catheter Associated Urinary tract infection, Evolvulusalsinoides, Biofilm, Anti-bacterial activity, HET-CAM test*

Introduction

Catheter Associated Urinary tract infection (CAUTI) is one of the most common Nosocomial infection ,accounting for more than one million patients annually around the world those who are in acute care and extended care facilities . Indwelling catheters are the cause of this Infection .An indwelling catheters is a tube inserted into patients urethra .Infected catheters are mostly covered with a thick layer of bio-film often serves as source of recurrent infection. Prolonged use of same catheter highlighting risk of bio-film formation. Avoidance of CAUTI requires development of bio-film resistant catheter material.(Shakambrisrinath., 2003)

Bio-film is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Bio-films are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interaction and an extracellular matrix of polymeric substances.(Bendouh Z, *et al.,* 2006*).* Bio-film is ubiquitous strategies for preventing bio-film and bio-film associated infections include initial device contamination, Minimizing microbial cell attachment and Use of agents such as plant extract as antibiotic or anti-bio-filming agent.Bacterial adhesion is generally a prerequisite for the colonization process and thus represents an attractive target for development of bio-film preventive measure. An attractive approach is the use of agents that interfere with the ability of the bacteria to adhere to tissues of the host, since this is the initial stage of infectious process.

Using plant extract as anti-adhesive drugs coated on catheter significantly reduced or delayed biofilm formation by the isolated organism under ever condition examined, this offer an attractive measure for reducing or delaying bio-film associated infections.The Indian traditional medicinal plant (Vishnu kranthi) *Evolvulusalsinoides* common name Dwarf morning glory. It is mainly used as Nootropic, Nervine, psychostimulant and sedative agent. This was also seen to be effective in controlling many human pathogens contrary to synthetic drugs, antimicrobial activity of plant origin are not associated with many side effects and have an enormous therapeutic potential to treat many infectious diseases. Intake of oral drugs for Catheter associated urinary tract infection (CAUTI) can cause side effects or inflammation

(Anupama., 2012). Therefore, this study involves the effect of herbal plant (Vishnu kranthi) extract tested for its anti-biofilm activityagainst urinary tract infection causing pathogens was evaluated.

MATERIALS AND METHODS

Collection of Foley catheters and processing of the samples

The entire research work was carried out from December 2018 to January 2019 in GRAM POSITIVES – Research and Development Laboratory, Coimbatore, Tamil Nadu, India. The used catheters were brought to the laboratory and decontaminated in the autoclave. The catheter was cut into required size for all testing parameters and stored at 4ºC prior analysis.

Soxhlet extraction of *Evolvulusalsinoides* (V i s h n u k r a n t h i)

Evolvulusalsinoides (Vishnukranthi) plant leaves were collected and shadow dried with in a temperature range of 37-40º C. The moisture content of the herb collected was reduced to less than 14 % with proper drying since most of the herbs have moisture content of 60-80% and cannot be stored without drying.Proper drying has to be carried out otherwise important compounds may get contaminated.After drying, the grinding was carried out to break down the leaves of the plant into very small units ranging from coarse fragments to fine powder.Extraction refers to separating the desired material by physical or chemical means with the aid of a solvent.Antimicrobial active substances were extracted from the plant by aqueous extraction method.The powde red plant material was extracted with water by adding 20g of her bal powder in 100 ml for 24h in Soxhlet extraction apparatus.

Determining the surface colonizing capability of test bacteria on Foley urinary catheter (UC) materials using Preliminary Exit-site challenge test (Baystone*t 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 18 hours test cultures (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The pre-measured size (length - 15mm) of Foley urinary catheter 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.

Preparation of antibacterial coatings of Foley urinary catheters (Boccaccini*et al*., 2003)

Antibacterial coatings on the urinary catheters were carried out using a standard slurry-dipping technique. The technique started with the preparation of stable slurry with specific amount of ofloxacin in the molten polyethylene glycol (PEG). Appropriate slurry temperature (40°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 the extract of ofloxacin(0.5g) in a glass vial. The mixture was heated at the range of 40 to 45°C in a water bath to obtain homogeneous slurry.

The resulting slurry was homogenized in a magnetic stirrer for 5 to 10min. Each piece of catheter (length - 6mm) was dip coated twice with intermittent drying (suspension coating method) in the drug-PEG slurry mixture. The dip-coating procedure was carried out in sterile glass beakers on a shaker (120 rpm) for 30 mins, with a drying period of about 15 mins 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 5mm of catheters outer diameters. After coating procedure, the catheter samples were stored at 4°C for upto 15 mins. 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 (carrier). 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. The drug and carrier coated catheter samples were thus mentioned as *drug-carrier coated* (*dcc*) materials; other few samples were coated with Vishnukranthi plant extracts (*dc*) to differentiate the antibacterial activity from *dcc* samples.

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

The method was performed for analysing the antibacterial activity of urinary catheter after slurry dip-coating with drug (ofloxacin) and carriers (PVA). In this qualitative method the pre-measured size (length- 6mm) of all sterilized materials were tested from each preparation [drug-carrier coated, Herbal extract coated and uncoated catheter samples]. 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 48 hours. The experiments were carried out in triplicate. Antibacterial activity was expressed as the diameter of the zone of inhibition.

Hen's Egg Test on the Chorioallantoic Membrane – CAM test

To study the allergic reactions or biocompatibility of drug-carrier coated catheter was placed on the surface of chorio-allantoic membrane (CAM) of embryonated chick eggs. A standard HET-CAM protocol was followed to detect the inflammatory reactions. HET-CAM (Hen's egg test-chorioallantoic membrane) method uses the vascular fetal membrane of chicken embryos. It is assumed that acute effects induced by a test substance and the small blood vessels and proteins of this soft tissue membrane are similar to effects induced by the same test substance in the skin of a treated rabbit. The membrane was evaluated for the development of irritant endpoints (vascular lysis, haemorrhage and coagulation) and qualitative assessments of the irritation potential of test substances are made.

Test substance preparation

Test samples were prepared carefully with one negative control (0.9% NaCl) and one positive control (0.1N NaOH).

Table 1. Sample numbers and its designation

S. No.	Sample number	Designation
1	Sample-1	Negative control (0.9% NaCl)
2	Sample-2	Positive control (0.1N NaOH)
3	Sample-3	Drug-carrier coated catheters

Preparation of Chorioallantoic membrane for experiment

Eggs from single hen was collected and observed for the presence of embryo using candle method. Defective eggs without embryo or cracks in the shell can be identified by this method. Eggs with good hard shell and developed embryo was selected and incubated in rotary egg incubator at 38.3 ± 0.2°C. After 9th day of incubation, the eggs were used for the experiment. About one square inch window was marked over the shell (above the air sac). The shell was carefully drilled and the window was removed without damaging the chorioallantoic membrane.

Treating the CAM using the samples Treatment of Eggs with Test Substances

Catheter samples and filter paper discs impregnated in sodium chloride solution (negative control) and sodium hydroxide solution (positive control) was used in the experiment. The samples were kept over the developing chorioallantoic membrane and observed for the irritation endpoints to develop with in 300seconds.

Observations

During the incubation time (300 seconds), the morphological change over the CAM was recorded. Three types of endpoints were noted viz., haemorrhage, coagulation and vascular lysis.

Irritation Score (IS) calculation – IS [B] analysis method

The time taken for the development of each endpoints, hyperemia, hemorrhage and coagulation was substituted in the standard formula as per IS [B] analysis method. The time values assigned/obtained to each endpoint were totalled to give an overall IS value for the test substance. An IS score could be calculated using the following general formula,

$$\left(\left(\frac{(301-\textit{Hemorrhage time})}{300}\right)\times 5\right)+\left(\left(\frac{(301-\textit{Lysis time})}{300}\right)\times 7\right)+\left(\left(\frac{(301-\textit{Coagulation time})}{300}\right)\times 9\right)$$

Where, *Hemorrhage time* = time (in seconds) of the first appearance of blood hemorrhages, *Lysis time* = time (in seconds) of the first appearance of vessel lysis

Coagulation time = time (in seconds) of first appearance of protein coagulation

In Table 2 the final IS value ranged from 0 (for test substances that do not induce development of any of the observed endpoints) to 21 (for test substances that induce development of all three endpoints

within 5min of application of the test substance) was presented. The relationship between scores and category of irritation was tabulated below.

Table 2. Relationship of scores with category of irritation

Scores on HET-CAM	Category of irritation
0 – 0.9	No irritation
1 – 4.9	Weak or slight irritation
5 – 8.9	Moderate irritation
9 – 21	Strong or severe irritation

From Cazedey et al

RESULTS AND DISCUSSION

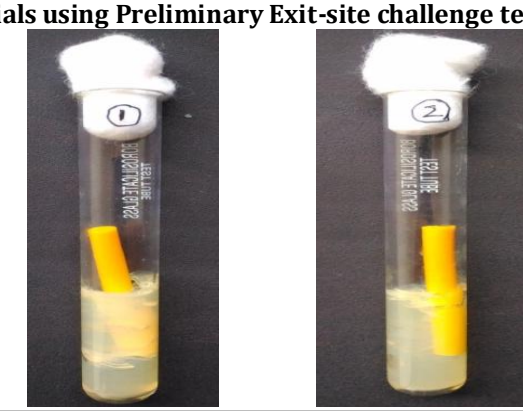
Urinary catheter and stent-associated infections are difficult to be treated with antibiotics and there is a need to change catheters (Quesada and Light, 1993). Catheters are manufactured from silicone or from latex; these materials provide attractive, unprotected sites for bacterial attachment. In addition, irregular surfaces left by the manufacturing process, particularly around eye-holes, can trap cells from an infected urine flows through the catheter (Stickler *et al*., 2003).

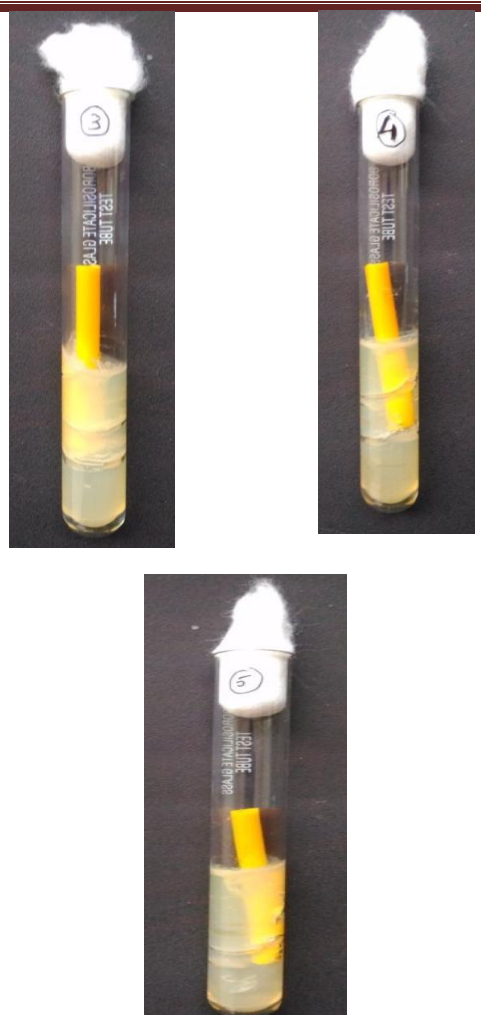
Biofilm is a microbial lifestyle, in which the microbes attach to these surfaces, allowing this community to survive in hostile environments (Murugan*et al*., 2011). Biofilms produced by urease-positive bacteria such as *Proteus mirabilis*, pose particular threats to the health of catheterized patients. As urease generates ammonia and creates alkaline conditions under which crystalline biofilms develop rapidly and block the urine flow from bladder resulting in urinary retention, painful distension of bladder, reflux of infected urine to kidneys, pyelonephritis and septicaemia (Elayarajah *et al*., 2011). The simplest way to prevent biofilm formation is to impregnate catheters with a broad-spectrum antimicrobial agent that elutes into the surrounding environment and attack plank tonic bacteria in the vicinity of the device before they colonize the surface and adopt biofilm-resistant phenotype (Danese, 2002).

Determining the surface colonizing capability of test bacteria on urinary catheter (UC) materials using Preliminary Exit-site challenge test

In this present study the surface colonizing ability of test bacteria on the UC sample materials was investigated using exit-site challenge test. All the test organisms used in the research colonized the material surfaces between 24 to 48 hours. Among the test organisms *Staphylococcus epidermidis* and *Escherichia coli* colonized with in 24hours; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* colonized the catheter surface after 48 hours (Fig-1).In Fig-1, of the preliminary exit-site challenge test, the migration or growth of the test organism around the materials after incubation was indicated by tracking of bacteria along the abluminal surface. 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. Baystone*t 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 Baystone*t al.,* (2009) showed surface colonization of methicillin resistant *Staphylococcus aureus* (MRSA) on the CSF silicone shunts surface. Two significant biofilm producing organisms, *Staphylococcus epidermidis* and *Escherichia coli* produced biofilm within 24 hours in the present study. The obtained results were thus considered as the preliminary test to determine the surface colonizing ability of the test organisms.

Fig-1: Determining the surface colonizing capability of test bacteria on urinary catheter (UC) materials using Preliminary Exit-site challenge test



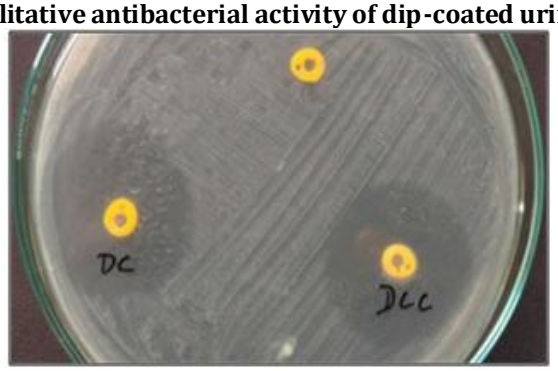


1. Staphylococcus epidermidis 2. Escherichia coli 3.Pseudomonas aeruginosa 4.Klebsiella pneumoniae 5. Proteus mirabilis

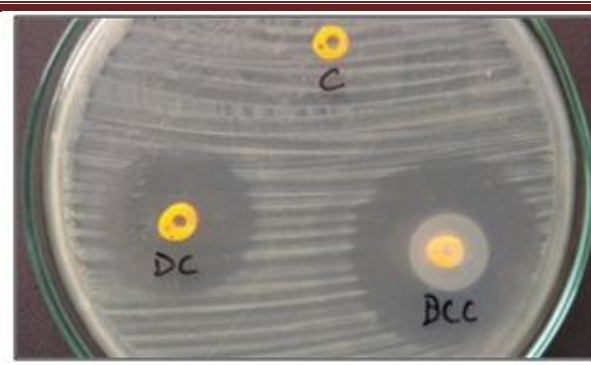
Assessing the qualitative antibacterial activity of dip-coated urinary catheter materials

The diffusing ability of the herbal extractcoated 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 millimetreswas calculated. In Table-1, the antibacterial activity of *drug-carrier* coated catheters and herbal extract coated catheters for all the test organisms was presented. No inhibitory zones were observed for all uncoated materials. In contrast, all the *drug-carrier* coated catheter sample materials showed significant inhibitory zones ranged from 29mm to 33mm against the high biofilm producers. Herbal extract coated catheters exhibited antibacterial zones but slightly less than that of the drug-carrier coated catheters. The antibacterial activity with the clear inhibitory zones around the coated materials against the biofilm producing test cultures was presented in Fig-2.

Fig-2: Assessing the qualitative antibacterial activity of dip-coated urinary catheter materials



Staphylococcus epidermidis



Escherichia coli

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

S. No.	Organisms	Zone of inhibition (mm)		
		UC	DC	DCC
1	*Staphylococcus epidermidis*	0	27	29
2	*Escherichia coli*	0	25	33

UC: Uncoated DC: Vishnukranthi coated DCC: Drug-carrier coated

In qualitative antibacterial activity, the herbal extract coated catheters recorded strongest inhibition against both Gram-Negative and Gram-Positive organisms. The different cell wall susceptibility amongst bacteria may be the key contributor to various inhibitory concentrations of drugs. Gram-Positive bacteria are often found to be more susceptible to antibacterial compounds than the Gram negative bacteria (Fennel *et al*., 2009). It is well known that the outer membrane present only in the Gram negative bacteria play an important role as an effective barrier. Although Gram positive bacteria lack of outer membrane, the thicker cell wall consist of few peptidoglycan layers could act as functional barrier thus hinder the penetration of antimicrobial compound into the bacterial cell (Tian*et al*., 2004). The bioactive compounds present in the herbal extract were responsible for antibacterial activity.

Hen's Egg Test on the Chorioallantoic Membrane: Histological Evaluation of CAM

The test sample (Herbal extract coated) does not develop any irritant end points, revealing that they are biocompatible. When compared with the negative control (sodium chloride) samples, no irritation points were observed. And the positive control (NaOH) samples revealed different irritant end points as described earlier. Irritation score was evaluated by the time in seconds consumed for the endpoints developed. Using the standard formula mentioned above (methods) end points was calculated. The mean value of time for the development of haemorrhage, hyperemia and coagulation were identified as 5.8, 6.7 and 6.8 respectively (Table-2).

Table 4: Comparative evaluation of irritation scores for test materials, negative control and positive control by HET-CAM test

Materials on CAM	Endpoint development			Irritation score[1]
	Haemorrhage	Hyperemia	Coagulation	
Sample-1 (Negative control)	0	0	0	0
Sample-2 (Positive control)	5.8	6.7	6.8	19.3[2]
Sample-3 (Vishnukranthi coated)	0	0	0	0

[1]Irritation score calculated as described by IS [B] analysis, [2]Irritation category – Severe irritation

The naked eye observations of three endpoints like haemorrhage hyperemia and coagulation was presented in Fig-3. The CAM exposed with Negative control (0.9 % NaCl) samples showed well developed blood vessels and nucleated epithelial cells without any irritation endpoints; in Fig-4 these observations were clearly evident. Irritation end points for test material implanted CAM samples were compared with the interpretations of positive and negative control samples. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative

controls (Fig-5). Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed.

Fig-3: Positive control CAM sample

All three irritation types like lysis of blood vessels, haemorrhage and coagulation on the chorioallantoic membrane of chick embryo was observed for the positive control (NaOH) coated sample

Fig-4: Negative control CAM sample

No Irritation types on the membrane of chick embryo was observed for the sample coated with negative control Sodium chloride

Fig-5: CAM after exposed coated catheter samples

No Irritant endpoints were observed for coated catheter samples thus indicating the biocompatible properties of the herbal extract used in the research.

Conclusion

All the test organisms used in the research colonized the material surfaces between 24 to 48 hours. Among the test organisms *Staphylococcus epidermidis* and *Escherichia coli* colonized with in 24hours; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* colonized the catheter surface after 48

hours. No inhibitory zones were observed for all uncoated materials. Herbal extract coated catheters exhibited antibacterial zones but slightly less than that of the drug-carrier coated catheters. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative controls. Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed. Thus*Evolvulusalsinoidesplant extract can be coated onto the catheters in treating the pathogens responsiblr foe UTI.*

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13. Biofilms are communities of microbes attached to surfaces, which can be found in medical, industrial and natural settings. In fact, life in a biofilm probably represents the predominate mode of growth for microbes in most environments.cited by:672.