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PREVENTION OF BIOFILM FORMATION IN URINARY CATHETER BY ANTIBIOFILM AGENTS

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Abstract

The present study emphasize with investigation of microbial contamination in indwelling urinary catheters. The biofilm detection is carried out by three methods i.e. Tube Method, Tissue culture and Congo Red Agar Method. Prevention of biofilm formation in the urinary catheter by Pseudomonas aeruginosa was also determined by coating the catheter with some enzymes, gentamycin, EDTA, DNase enzyme, Antimicrobial drugs-Ceftazidimine and Ceftriaxone, some enzymes,gentamycin, EDTA and plant extract (Azadirachtaindica).Out of 20Urinary catheter sample,8 samples shows bacterial growth.It was found that 40% Urinary catheter gets contaminated during the course of catheterisation.Of these total 8 isolates biofilm formation was seen in 100% Pseudomonas species and Klebsiellap neumoniae and 50% Staphylococcus aureus. Ampicillin and Vancomycin (100%) were highly resistance against Klebsiellasp., Pseudomonas sp and S. aureus. while Ciprofloxacin and Penicillin G are sensitive against S. aureus. From the result obtained in different concentration of antibiofilm agent, we can interpret that biofilm production was completely prevented in 3% Ciprofloxacin, 3% EDTA,5% Tobramycin,5% Lysozyme and 5% α-amylase concentration. The 500µl Azadirachta indica plant extract was also capable of dispersing the biofilm formation in Pseudomonas sp, Staphylococcus aureus and Klebsiellapn eumoniae.

Keywords: Azadirachta indica, Bio-film, Enzymes, EDTA, Urinary catheter

INTRODUCTION

Urinary Tract Infections (UTI) account for an estimated 25-40% nosocomial infection, out of which 90% are associated with urinary catheter, called Catheter associated urinary tract infection (CAUTI). Catheter associated urinary tract infection (CAUTI) is one of the most frequently encountered health –care associated infections today (Ghanwate, et al., 2014). CAUTIs are directly related with the use of indwelling urinary catheters (Richards, et al., 2000; Krieger, et al., 983). Patient requiring an indwelling catheter are predisposed to the development of CAUTI by potentially pathogenic multidrug resistant organisms in the hospital setting (Donlan, 2001). Up to 25% of patients have an indwelling catheter placed at some time during their hospital stay.CAUTIs are associated with increased morbidity, mortality, length of hospital stay and cost. It has been estimated that one episode of nosocomial acquired UTI adds 1–3 days of extra hospital stay (Haley, et al., 1985).

Biofilm can cause significant problems in many areas, both in the medical settings and in the non-medical settings. The biofilms have a major medical significance as they decrease the susceptibility to the antimicrobial agents(Kokare, et al., 2009).The microbial biofilms pose a public health problem for the persons who require indwelling medical

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devices, as the microorganisms in the biofilms are difficult to treat with antimicrobial agents. Enzymes have been used and proven to be effective for the degradation of the multi structural EPS of the biofilms. The mode in which enzymes destroy the EPS is by degrading the physical integrity of the EPS (Xavier, et al., 2005). Another way to prevent the biofilm formation within a urinary catheter by using a broad spectrum Antimicrobial drugs-Ciprofloxacin and Tobramycin, Enzymes-Lysozyme and α-amylase, EDTA and plant extract(A.indica).Ciprofloxacin, Tobramycin and EDTA has a very important role to play as an 'antibiofilm' agent and therefore may have important implications for use in controlling biofilm in catheter(Percival, et al., 2009).In the present study, main aim was to see whether there is any change in the properties of P. aeruginosa, S. aureus, K. pneumoniae, E. faecalis biofilm formation at different concentration of Tobramycin,Ciprofloxacin,EDTA,Lysozyme, α-amylase and Plant extract.

MATERIALS AND METHODS

The 20 samples of Urinary Catheter associated sample (CAS) collected aseptically from catheterized patients from Government Medical hospital and Swastikhospital, Nagpur simply by swabbing in sterile sample bottle. The sample is incubated at 37^{o}C for 24 hours followed by inoculation on EMB, PIA, MSA, Enterococcus Isolation Agar respectively. The Gram staining of isolated bacteria was performed by method given by Grams,(1884)in which bacterial smear was dried and heat fix on the slide then flooded with Crystal Violet (Primary stain),Gram's Iodine (Mordant),95% Alcohol (Decolouriser) and Saffranin (Counter stain) and observe under the light microscope at 100Xusing oil immersion. The Biochemical Characteristics of the isolated bacterial strain was carried out by IMViC test i.e. (I-Indole Test, M-Methyl Red Test, Vi-Vogues Proskauer Test and C-Citrate test),Sugar fermentation test,Urease Test, Catalase Test, Oxidase Test and Triple Sugar Iron Test for classifying the bacterial depending on their biochemical character. For complete conformation of isolated bacteria and their Cultural Characteristics determination they are inoculated on using EMB, PIA,MSA and Enterococococcus Isolation Agar followed by incubation at 37^{o}C for 24 hrs respectively (Nisha Rajal, 2018).

The isolated bacteria are further characterised depending on their ability to form biofilm. The biofilm detection is carried out by three methods i.e. Tube Method(TM), Tissue culture Plate (TCP), and Congo Red Agar Method (CRA) given by Christensen,et al., (1985).The antibiotic susceptibility testing by using Mueller Hinton Agar for determination of susceptibility of microorganism to antimicrobial agents given by Kirby–Bauer,et al., (1966). The prevention of biofilm formation within a urinary catheter by using a broad spectrum antimicrobial drugs- Ciprofloxacin and Tobramycin, Enzymes-Lysozyme and α-amylase, EDTA and plant extract (Azadirachtaindica). It is carried out similarly by inoculation of bacteria forming biofilm on Brain Heart Infusion Media with 2% sucrose followed by treatment with 1%, 3% and 5% solution of EDTA, Tobramycin, Ciprofloxacin, α–amylase, Lysosome and Azadirachta indica respectively. The extraction of plant Azadirachtaindica was obtained from soxhlet apparatus using 90/10 (W/V) ethanol –water for 48hrs at 25^{o}C and the extract left at room temperature until the evaporation of solvent (Tarek, et al., 2012; Kodali, et al., 2013).

RESULTS AND DISSCUSSIONS

ISSN: 2278-4632
Vol-10 Issue-5 No. 16 May 2020

In this study, the Urinary catheter samples of Catheterised patient collected from two different hospitals i.e. Government Medical Hospital and Swastik Hospital, Nagpur.A total of 20 patients sample with indwelling urinary catheter were collected for the study.

Table 1:Results of morphological characters and Identified bacteria

Sr.no	Sample Collection No.	Identified Bacteria	Gram staining and shape	Motility
1.	CAS 01	*Pseudomonas aeruginosa*	Negative(Rod)	Motile
2.	CAS 09	*Klebsiella pneumonia*	Negative(Rod)	Non motile
3.	CAS 12	*Enterococcus facealis*	Positive(Cocci)	Non motile
4.	CAS 20	*Staphylococcus aureus*	Positive(Cooci)	Non motile
5.	CAS 25	*Enterococcus facealis*	Positive(Cooci)	Non motile
6.	CAS 32	*Klebsiella pneumonia*	Negative(Rod)	Non motile
7.	CAS 35	*Pseudomonas aeruginosa*	Negative(Rod)	Motile
8.	CAS 40	*Staphylococcus aureus*	Positive(Cocci)	Non motile

Where,CAS –Catheter associated sample

Table 2: Observation of Biochemical characteristics of isolated biofilm forming bacteria

Sr. No	Sample No	Indole	MR	VP	Citrate	Urease	TSI		Catalase	Oxidase	Glucose		Lactose		Mannitol		Sucrose		Mannitol	
							Acid	Gas			Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
1.	CAS01	-ve	-ve	-ve	+ve	-ve	**-ve**	**-ve**	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
2.	CAS09	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3.	CAS12	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4.	CAS20	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5.	CAS25	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	CAS32	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7.	CAS35	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
8.	CAS40	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Identification of Biofilm Forming Bacteria:

The culture dependent examination of types of microorganism that colonised Foley Urinary was applied to study isolation and identification of biofilm forming bacteria from Urinary catheter.*Pseudomonasaeruginosa, Staphylococcus aureus, Klebsiellapneumoniae and Enterococci faecalis*were obtained from Urinary catheter samples of patients and all are the major cause of CAUTI (Table 1). Out of 20 Urinary catheter samples, 8 (40%) samples showed the growth of bacteria from which *P.aeruginosa* was isolated from 2 (10%) samples(CAS 01 and CAS35), *K.pneumoniae* was isolated from 2 (10%)samples (CAS 09 and CAS 32),*E.facealis* was isolated from 2(10%) samples (CAS 12 and CAS 25) and *S.aureus* was isolated from 2(10%) samples (CAS 20 and CAS 40) (Table 2).

Detection of Biofilm Formation:

Table 3: Results of detection of Biofilm formation

Sr.no	Name of microorganisms	Tube Method	Tissue Culture Plate Method	Congo Red Agar Method
1.	*P.aeruginosa*	+++	+++	+++
2.	*K.pneumoniae*	+++	+++	+++
3.	*E.facealis*	+	+	-
4.	*S.aureus*	++	++	+++
5.	*E.facealis*	-	-	-
6.	*K.pneumoniae*	+++	+++	+++
7.	*P.aeruginosa*	+++	+++	+++
8.	*S.aureus*	+	+	-

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Where, +++:Strong biofilm formation; ++:Moderate biofilm formation; +:Weak biofilm formation; -:No biofilm formation

Tube Method,Tissue Culture Plate Method and Congo Red Agar Method were performed and analysed for the biofilm formation.Depending upon the colour intensity of crystal violet remain adherent to the wall of Tube or 96 well plate they are classified as Strong biofilm former,Weak biofilm former and no biofilm former. Among 2 *P.aeruginosa* and 2 *K.pnuemoniae* isolates all produced biofilm (100%).In similar way 50% *S.aureus* produced biofilm.Of the 2 *E.facealis* isolates none could form biofilm (Table 3).

Antibiotic Susceptibility Testing:

Table4: Results of Antibiotic susceptibility test of isolated bacteria

Antibiotics	Zone of inhibition in mm							
	P.aeruginosa	*K.pnemoniae*	*E.facealis*	*S.aureus*	*E.facealis*	*K.pneumoniae*	*P.aeruginosa*	*S.aureus*
Ampicillin	No zone	No zone	No zone	No zone	No zone	No zone	No zone	No zone
Ciprofloxacin	12mm (R)	28mm (S)	-	30mm (S)	No zone	No zone	12mm (R)	8mm (S)
Erthromycin	-	-	No zone	-	12mm (R)	-	-	-
Gentamycin	No zone	17mm (S)	No zone	-	17mm (S)	10mm (R)	No zone	-
Methicillin	-	-	-	No zone	-	-	-	No zone
Penicillin G	-	-	-	38mm (S)	-	-	-	No zone
Tetracyclin	-	-	-	27mm (S)	-	-	-	No zone
Tobramycin	No zone	18mm (S)	-	-	-	16mm(I)	No zone	-
Vancomycin	No zone	No zone	16mm (I)	No zone	20mm (S)	No zone	No zone	10mm (R)

Where, S –Antibiotic is sensitive towards the organism, R-Antibiotic is resistant towards the organism

Invitro antimicrobial susceptibility testing of isolates were carried out using Kirby Bauer's disc diffusion method according to the Clinical and Laboratory Standard Institute on Muller-Hinton agar (CLSI,2012).The Gram –ve isolates i.e.*P.aeruginosa*and *K.pneumoniae*(Sample no. CAS01,CAS35,CAS09 and CAS32) were highly resistance to Ampicillin,Ciprofloxacin,Gentamicin,Tobramycin and Vancomycin. While the Gram+ve isolates i.e. *S.aureus and E.faecalis*(Sample no. CAS12,CAS20,CAS25, CAS40) were highly resistance to Ampicillin, Methicillin,Vancomycin and highly sensitive to Ciprofloxacin and Penicillin G (Table 4).

Tobramycin, Ciprofloxacin, EDTA, Lysozyme, α-amylase and *Azadirachtaindica*(plant extract) activity on biofilm formation:

Table 5: Results of Tobramycin, Ciprofloxacin, EDTA, Lysozyme,α-amylase and plant extract activity on biofilm formation

Name of m.o's	*P.aeruginosa*-(A)			*K.pneumoniae*-(A)			*S.aureus*			*K.pneumoniae*-(B)			*P.aeruginosa*-(B)		
Antibiofilm agent name/conc.	1%	3 %	5 %	1%	3 %	5 %	1 %	3 %	5 %	1 %	3 %	5%	1%	3 %	5%
Tobramycin	+++	++	-	++	+	-	-	-	-	-	-	-	+++	++	-
Ciprofloxacin	++	-	-	-	-	-	-	-	-	-	-	-	++	-	-
α-amylase	+++	++	-	++	-	-	-	-	-	++	+	+	+++	++	-
Lysozyme	+++	++	-	++	++	-	-	-	-	++	+	-	+++	++	-
EDTA	++	-	-	++	-	-	-	-	-	++	-	-	++	-	-
Plant Extract	+			-			-			++			-		

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Vol-10 Issue-5 No. 16 May 2020

To inhibit the biofilm formation on Urinary catheter which ultimately leads to catheter associated urinary tract infection (CAUTI), the catheter must be impregnated with antibiofilm agents (drugs, enzymes, plant extract). Here it was found that Tobramycin, Ciprofloxacin, α-amylase, Lysozyme, EDTA,*Azadirachtaindica* affect the biofilm formation at various different concentration. In present study three different concentration are used 1%, 3% and 5%.In the present study, Ciprofloxacin and EDTA proved to be most effective against the biofilm formation.Ciprofloxacin is a broad spectrum antibiotic of fluoroquinolone class. It is active against both Gram negative and Gram positive bacteria. EDTA solution cause dissolution of Calcium and magnesium salts that gets deposited on the catheter and reduce crystalline biofilm. Biofilm is composed of bacteria and EPS. In the present work, it was observed that the leaves of *A.indica* plays important role in dispersion of biofilm (Table 5).

Thus from the present study it can be concluded that the pretreatment of Urinary catheter with Tobramycin, Ciprofloxacin, α-amylase,Lysozyme and *A.indica* plant extract may prolong the contamination and subsequently biofilm formation by organism causing CAUTI.

SUMMARY

In the present study, we investigated microbial contamination within indwelling Urinary catheter. Biofilm forming ability of the isolates was determined by Tube Method, Tissue Culture Plate Method & Congo Red Agar Method. Antimicrobial susceptibility was done by Kirby Bauer's disc diffusion method. Prevention of biofilm formation was determined using Tube Method by using 1% ,3% and 5% solution of Tobramycin, Ciprofloxacin, α-amylase,Lysozyme,EDTA and*Azadirachtaindica* (plant extract).Out of 20Urinary catheter sample,8 samples shows bacterial growth.It was found that 40% Urinary catheter gets contaminated during the course of catheterisation .Of the total 8 isolates biofilm formation was seen in 100% *Pseudomonas Sp.* and *Klebsiellapneumoniae* and 50% *S,aureus*. Ampicillin &Vancomycin (100%) were highly resistance against *KlebsiellaSp.,Pseudomonas sp.*and*S.aureus*.While Ciprofloxacin and Penicillin G are sensitive against *S.aureus*. From the result obtained in different concentration of antibiofilm agent,we can interpret that biofilm production was completely prevented in 3% Ciprofloxacin, 3% EDTA,5% Tobramycin,5% Lysozyme and 5% α-amylase concentration. The 500µl *A.indica* was also capable of dispersing the biofilm formation in*Pseudomonas Sp.,S.aureus*and *K.pneumoniae.*

Overall the result reported in the study showed that Ciprofloxacin and EDTA (3%) were proven to be effective for the degradationof multistructural EPS of the biofilm in low concentration.

REFERENCES

1) Ghanwate A Niraj , Thakare V P,Bhise R P, TaydeSwapnil,(2014)''*Prevention of Biofilm Formation in Urinary Catheters by Treatment with Antibiofilm Agents*''.Int. J. Biol., Biomol., Agr., F. and Biot.Engi.**6**:10
2) Richards M. J, Edwards J. R, Culver D. H, andGaynes R. P, (2000) ''*Nosocomial infections in combined medical-surgical intensive care units in the United States*'', Infect Control HospEpidemiol ,**21:**8, 510-515.
3) Krieger J. N, Kaiser D. L., & Wenzel R. P,(1983) ''*Nosocomial urinary tract infections: secular trends, treatment and economics in a university hospital'',*J Urol ,**130**:1,102-106.
4) Trautner B. W. and Darouiche R.O, (2004),*''Role of biofilm in catheter associated urinary tract infection''.* Am J Infect Control, **32**: 177-183.
5) Haley R., Culver D., White J., Morgan W. M., andEmori T. G, (1985)''*The nationwide nosocomial infection rate: A new need for vital statistics*''..*Am J Epidemiol*, **9**:8
6) Kokare C. R., Chakraborty S. , Khopade N. A. and Mahadik R. K. (2009) "*Biofilm :Importance and Application''*. Indian Journal of Biotechnology, **8**: 159-168.
7) Xavier J.B., Picioreanu C., Rani S.A., Van Loosdrecht M.C. and Stewart P.S., (2005) "*Biofilm control strategies based on enzymic disruption of the extracellular polymeric substance matrix – a modeling study*". Microbiol, 151 :3817-32.
8) Percival S.L., Sabbuba N.A., Kite P., Stickler D.J., (2009) "*The effect of EDTA instillations on the rate of development of encrustation and biofilms in Foley catheters*",Urol Res, **37**: 205-209.
9) Gram, C. (1884). "*Gram Stain Protocol*", Fosrtschr. *Med.* **2:**185.
10) http://microbeonline.com/gram-staining-principle-procedure-results10.09.2017
11) Christensen G.D., Simpson W.A., Younger J.A.,Baddour L.M., Barrett F.F.,Melton D.M. (1985) ''*Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices*'',J ClinMicrobio,**22**: 996-1006.
12) Bauer A.W., Kirby W.M.,Sherris J.C.,Turck M. (1966) "*Antibiotic susceptibility testing by a standardized single disk method*", American Journal of Clinical Pathology, **45** (4): 493–6.
13) Tarek El-Banna, Fatma Ibrahim Sonbol, Ahmed Ahmad Abd El-Aziz, Amal Abo-Kamar and DoaaWaheedSeif-Eldin,(2012) ''*Effect of the Combination of Salicylate with Aminoglycosides on Bacterial Adhesion to Urinary Catheters*'',Intl. R. J. of Pharmaceuticals,**02(**2): 39-4.
14) KodaliVidyaPrabhakar, Abraham Peele Karlapudi, MadhuriKotam, Rohini Krishna Kota, TejaswiniPunati, RajendraBabuByri,(2013)''*Plant extract as a antibiofilm agent*'',Int. J. Pharm. Sci. Rev. Res., **21**(1):325-328.

