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A STUDY OF BIOFILM FORMATION IN INDWELLING CATHETER DEVICES IN ICU & PICU OF SRM MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

MAHARAJA PANDIAN[1] AND SEKAR PASUPATHY[2]

[1]Department of Microbiology, SRM Medical College Hospital And Research Centre Kattankulthur, Kancheepuram District, Tamilnadu, India.
[2]Department of Pediatrics and Neonatology, SRM Medical college Hospital and Research Centre, Kattankulthur, Kancheepuram District, Tamilnadu, India.

ABSTRACT

Biofilm is defined as an association of organisms in which microbial cells attached to each other on a living or non-living surfaces. Biofilm can be a threat to human health by the growth of organisms on medical implants such as prosthesis, heart valves and urinary catheter leading to infections. Moreover biofilm shows an increased resistance to antimicrobial agents. The objective of the study is to isolate and identify the biofilm producing organisms and their antibiotic sensitivity pattern in patients with indwelling various catheter. This study was carried out from Jun 2009 to 2011 Dec at the Department of Pediatrics & Microbiology, SRM MCH & RC, Chennai. About 120 indwelling catheter tips were received from various clinically diagnosed patients by Brain heart infusion broth (BHI) and incubated for 24hrs at 37^0C. After 24hrs incubation, one loop-full of sample was taken from BHI broth and inoculated in Cysteine Lactose Electrolyte Deficient agar(CLED).The detection of biofilm using crystal violet stain tube method was performed and it was confirmed by colorimetric analysis according to the optical Density(OD)value. Maximum biofilm producers were formed in 75 catheters out of 120 specimens. *E.coli* was the predominant organisms producing biofilm in indwelling catheter among the 75 isolates .The present study also proved that *Staphylococcus aureus*, *Klebsiella sp*. *Enterococcus sp, Proteus mirabilis*, *Coagulase Nagative Staphyloccus* (CONS) and *Pseudomonas aeruginosa* produced biofilm formation on indwelling catheter. Antibiotic Sensitivity pattern showed the *E.coli* and *P.aeruginosa* were resistant to all antibiotic compared with other isolates. *E.coli* and *P.aeruginosa* also frequently isolated from various cases of indwelling catheter patients but both organisms cause major problem in hospital acquired infection and resistant to all antibiotics.

KEYWORDS: ***Biofilm, Indwelling catheter, BHI broth, CLED agar, E.coli, Test tube method, Hospital acquired infection.***

SEKAR PASUPATHY*

Department of Pediatrics and Neonatology, SRM Medical college Hospital and Research Centre, Kattankulthur, Kancheepuram District, Tamilnadu, India.

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INTRODUCTION

Biofilm is an association of organisms in which microbial cells attached to each other on a living or non-living surfaces. Biofilms may form on a wide variety of surfaces including living tissues, indwelling medical devices and natural aquatic system. The factors of Biofilms are structural heterogenecity, complex community interactions and are extracellular matrix of polymeric substances. Biofilm formation is a complex development process involving attachment immobilization on a surface and cell to cell interaction. Biofilms are important source of infection. Diseases due to bacterial biofilms are generally chronic and difficult to treat. Biofilms contribute to several serious diseases like acute ear infections in children and bacterial endocarditis. Biofilm plays an important role in water contamination, implant infections, ear infections and causes urinary tract infection (UTI).Biofilm on teeth causes yellow discoloration. Cells released from biofilms can enter into the blood stream and cause Septicemia[1]. Biofilm can defined as a community of microorganisms attached to wet moist surface. In 1964 Ralph Mitchell and Kevin Marshall studied the first stage of biofilm formation by bacteria in pure culture and afterwards the biofilms were developed. The different types of biofilms associated infections are as follows

1. Receiving artificial valve in heart.
2. Oral diseases caused by biofilm on the tooth surfaces.
3. Urinary tract infection caused by biofilm.
4. Respiratory infections and
5. ventilation associated pneumonia.
6. Other infections caused by biofilms are otitis media and ocular infections[2].

A biofilm can be defined as an aggregation of bacteria, algae, fungi and protozoa enclosed in a matrix consisting of a mixture of polymeric compound. Polysaccharides are generally referred to as Extracellular Polymeric Substance (EPS). Biofilms are found everywhere and form micro cities[3]. Biofilm associated infectious disease has been an important area of clinical research. Increasing evidence suggests that many life threatening infections come out of biofilms either directly in the human host or indirectly via surgical instruments[4]. In a biofilm study, the common nosocomial infections are urinary tract infection , non healing chronic wound infections and respiratory infection. *E.coli* and *Pseudomonas sp* were the predominant organisms producing biofilm in indwelling catheter.Bacterial surface adhesions and presence of surfactants are certain factors which influence biofilm formation [5].

MATERIALS AND METHODS

Clinical specimens of catheter tips were received from patients admitted in SRM Hospital ICU & PICU over a period of 30 months. Institutional Ethical Clearance obtained with Number: 52/IEC on 2010.All these specimens were inoculated on appropriate culture media (CLED agar) and incubated for 24hrs at 37^0_C. After incubation microorganisms and its antibiogram were identified by standard protocol [6].

Tube method

A qualitative assessment of biofilm formation was determined using TSB glu. TSB glu (10µl)was inoculated with loop-full of microorganisms from overnight on the culture plates and incubated for 24hrs at 37^0C.The test tubes were decanted and washed in PBS(PH7.3)and dried test tubes were stained with 01% crystal violet.Excess stain was removed and the test tubes were washed with deionized water. Test tubes were then dried in inverted position and observed for biofilm formation. Biofilm formation was considered positive when a visible bio-film lined in the test tube wall and bottom of the tube. The biofilm formed in tube method which confirmed and graded by colorimetric analysis according to the optical Density(OD) value[7].

RESULT

The result indicated a strong biofilm formation by all the 56 bacterial isolates out of 75 biofilm specimens. It was observed that the bacterial isolates showed high mean absorbance that the optical density (OD) value greater than 0.28, indicates their strong adherence to the surface. Hence all the 56 bacterial isolates were considered as strong biofilm positive phenotype and 10 bacterial isolates were considered as moderate and 9 were weak (Table-2). Biofilm Production was investigated by the tube adherence test proposed by Christensen *et al* 1982[7]. A positive result was defined as the presence of a layer of stained material adhered to the inner wall and bottom of the tubes. The detection of biofilm by using crystal violet stain in test tube method was performed and it was confirmed by colorimetric analysis according to the optical Density(OD)value. Maximum biofilm

producers were formed in 75catheters out of 120(Table-2).Initial quantification assays using crystal violet stain were performed to evaluate the ability of bacterial isolates to form biofilm on test tube walls. When the test tubes were examined, a characteristic visible film lining the walls and bottom of the test tubes could be visualized. The results indicated a strong biofilm formation. A total 75 organisms were tested in our study. Biofilm producing bacteria: *E.coli* and *Pseudomonas aeruginosa* was the predominant organism producing biofilm in indwelling catheter. Apart from the present study, it was observed that *Staphylococcus aureus, Klebsiella sp, Enterococcus sp, Proteus mirabilis*, *Coagulase Negative Staphylococcus* (CONS) were also producing biofilm formation on indwelling catheter (Table-1). Maximum biofilm producers were 75 catheters tips out of 120 catheter specimens (Table- 1).Antibiotic sensitivity pattern showed the *E.coli* and *P.aeruginosa* were resistant to antibiotics (MRD) compared to other isolates(Table-3).Gram Positive biofilm producer were more resistant to rifampicin, ciprofloxacin, erythromycin and penicillin. All the gram(-) ve biofilm producers were more resistant to ciprofloxacin, ampicillin and amikacin.[8-10]

Table 1
Biofilm producing organisms in catheter tip samples

Biofilm - microorganisms	Number of isolates	(%)
E.coli	30	40
K.pneumoniae	8	10.6
P.aeruginosa	20	26.6
Staphylococcus aureus	10	13.3
P.mirabilus	1	1.3
Cons	3	3.9
Enterococcus sp	3	3.9
Total	**75**	**99.6**

Table 2
Biofilm formation by test tube method

Tube	Mean OD	Adherence	Biofilm - formation	n =75
1	>0.240	Strong	High	56
2	0.120-0.240	Moderately	Moderate	10
3	< 0.120	None	None(OR)Weak	9

Table 3
Antibiogram for Gram + Ve & -Ve organisms

Microorganisms	Sensitive	Intermittent	Resistant
E.coli	Gentamicin,Amikacin, Ertapenam, Imipenam, Ofloxacin, Meropenam	-	Cefalosporins, Imipenem were found to be resistant
K.pneumoniae	Colistin Meropenam	Imipenem	Penicillin G, Gentamicin
P.aeruginosa	Ertapenam, Imipenam	-	Ciprofloxacin, Ampicillin and Amikacin
Staphylococcus aureus	Teicoplanin Vancomycin	-	Erythromycin, Penicillin G, Co-trimoxazole, Cefotaxime, Clindamycin, Gentamicin
P.mirabilus	Ofloxacin, Meropenam	Gentamicin	Cefalosporins, Imipenem
Cons	Linezolid,Teicoplanin Vancomycin	-	Amikacin,Ciprofloxacin, Erythromycin, Penicillin G, Co-trimoxazole, Cefotaxime, Clindamycin, Gentamicin and Ofloxacin.
Enterococcus sp	Linezolid,Vancomycin Teicoplanin	-	Ciprofloxacin, Erythromycin, Penicillin G, Amikacin

DISCUSSION

The patient who needs medical devices suffers from the public health problem due to microbial biofilm production in recent times. The microorganisms in biofilm attached with medical device may cause infection in patients. Many workers have hypothesized the mechanism for biofilm formation and the role of Extracellular polymeric substance(EPS) which has been identified for the formation of biofilm. Adhesion of bacteria to metallic and non metallic surface is facilitated by the quality and quantity of extracellular polymeric substance produced by the bacteria. In the present study, infectious organisms were isolated from indwelling catheter. There is a strong evidence about the prevalence of catheter and other surgical related infections. Rodney *et al*.,(2002)[11] revealed that organisms were found in large number of environments and medical devices which caused human infection and this supports our present study.The present study proved *E.coli, Staphylococcus aureus, Klebsiella sp*. *Enterococcus sp, Pseudomonas aeruginosa*, *Proteus mirabilis*, *Coagulase Negative Staphylococci* (CONS) produces biofilm formation on indwelling catheter (Table-1) *E.coli* and *Pseudomonas aeruginosa* found to be the major cause of infection in our study and this was supported by Stover *et al* ., (2000)[12] who stated that *Pseudomonas aeruginosa* is a ubiquitous environmental bacterium and is one of the top three causes of opportunistic human infections. Also Morten *et al*., (2001)[13,14] stated that *Pseudomonas aeruginosa* was a causative agent for chronic Cystic Fibrosis (CF) infections.Initial quantification assays using crystal violet stain were performed to evaluate the ability of bacterial isolates to form biofilm on test tube walls. In the present study, when the test tubes were examined, a characteristic visible film lining the walls and bottom of the tubes could be visualized.

CONCLUSION

The prevention and treatment of Biofilm associated diseases (BAD) is an inter disciplinary approach involving Microbiology, Medicine, Gene technology, Biochemistry and Bioinformatics. The treatment is very challenging against the organisms producing biofilm. The formation of biofilm is an important step in pathogenesis which may due to acute and chronic bacterial infections produces infections of the body related foreign substances. Eradication of biofilms are very difficulty with usual antimicrobial agents. Potential antimicrobial resistance mechanisms have been developed by bacterial biofilms. This resistance mechanisms may act simultaneously and in some cases with other growing parts also. The key factor involved in the development of new therapeutic strategies is an understanding of the mechanisms of biofilm associated antimicrobial resistant .*E.coli* and *P.aeruginosa* are frequently isolated from various cases of indwelling catheter patients. It causes major problem in hospital acquired infection and resistant to all antibiotics.The future treatments may be focusing on inhibition of genes involved in cell attachment and biofilm formation. Biofilm are responsible for loss of billions of Rupees in hospital equipment damages occur in every year. So it is very essential for preventing and controlling of biofilm formation in medical devices.Virulence and pathogenicity of microorganisms is often enhanced if it is grown in biofilm. New and innovative antimicrobial strategies are therefore required to control biofilm formation and development.

CONFLICT OF INTEREST

Conflict of interest declared none.

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