ORIGINAL ARTICLE

A Study of Biofilm Formation on Indwelling Devices by Multidrug Resistant Clinical Isolates of Staphylococci Species

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ABSTRACT

Background: The indwelling devices are the foreign body that increase the process of bio film formation in the body. The present study was carried out to detect bio film formation by Staphylococci spp. recovered from different indwelling devices received from hospitalized patients at Lahore General Hospital.

Methods: The present study was a cross sectional diagnostic study. All specimens obtained from the hospital were processed in the microbiology section of pathology department of Post Graduate Medical Institute. Staphylococci species were identified through routine microbiological and biochemical tests. Antimicrobial susceptibility was determined by modified Kirby-Bauer disc diffusion method. Staphylococcal isolates along with the controls were allowed to form bio film by Tube Method (qualitative) and Microtiter Plate Assay (quantitative). Data analysis was one by using SPSS 19.0.

Results: Bio film formation in the patients with more than one indwelling device was greater as compared to the patients with single device. Intravascular catheters were found more noteworthy for bio film formation. The possibility and strength of bio film formation increases with the number of days a medical device remains in the body (p-value<0.05).

Conclusion: The microorganisms with multi drug resistance showed very strong bio film formation. In the patients with indwelling devices, bio film formation is more pronounced with intravascular catheter and in the patients with more than one-inserted devices. Moreover, bio film formation is more pronounced if medical indwelling device is allowed to retain for more number of days inside the body.

Keywords: Biofilm formation, Medical devices, Intravascular catheter.

INTRODUCTION

Biofilms are small communities of microbes that are widely dispersed in nature, from water pipes to indwelling devices in hospital patients[(1)]. Biofilms are encased in secreted hydrated medium of extra cellular polymeric substance (EPS) containing microorganisms, irretrievably adhered to a substratum and to one another and show an transformed pattern of growth and gene transcription[(2,3)]. The stratum may be inert, non-living material or living tissue. The microbes forming the biofilms perform in a different way from plank tonic organisms and have the ability to resist antimicrobial drug concentrations. Biofilm formation is an active and self-motivated process. The biofilm formation on any solid surface such as medical indwelling devices can be segregated into various steps involving identification, attachment, micro colony formation, biofilm maturation and finally dispersion[(4)]. Several mechanisms are described that explain increased resistance to antimicrobial drugs because of biofilm formation. These are intrinsic and extrinsic. These intrinsic factors of biofilm production behave as physical barrier to the diffusion by the thick muddle of polysaccharide, penetration of nutrient to limited area and restricted oxygen supply inside biofilm has outcome of distorted metabolic activity and sluggish growth rate. Oxidative stress and other factors such as high osmolarity and sub-inhibitory antibiotic concentration are cause of increase drug resistance. Biofilm also exhibit resistance to phagocytic defences of the host[(5)]. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration of antimicrobials for the biofilm forming bacteria being upto100-1000 fold high than free-living bacteria and 150 - 3000 fold high to disinfectants. Extrinsic or induced resistance in biofilms is due to transcriptional induction by use of antimicrobial drugs[(6)]. Mutation frequency of bacteria in biofilm is significantly high as that of planktonic bacteria. This may be due to production of enzymes that degrade antimicrobials, low affinity of the drug targets and over expression

of efflux pumps[7]. The present study was planned to detect biofilm formation in Staphylococci spp. recovered from different clinical samples received from hospitalized patients of Lahore General Hospital, Lahore and to compare biofilm formation of Staphylococci isolated from patients with various inserted devices.

PATIENTS ANDMETHODS

Current study was a cross-sectional study. Various types of samples (blood, urine, pus, CSF, urinary catheters tips, endotracheal tube tips etc.) were collected from the indoor patients of Lahore General Hospital (LGH), were transported to Microbiology laboratory in Pathology Department of PGMI, Lahore for culture and sensitivity testing. After preliminary identification by viewing the morphology of colony, staining by Grams technique, performing tube catalase test, slide coagulase test confirmed with tube coagulasetest, Novobiocin sensitivity for the coagulase negative staphylococci and by spot inoculation on DNase agar. The antimicrobial sensitivity was found out for Penicillin (P), Cefoxitin (FOX), Erythromycin (E), Clindamycin (DA), Flouroquinolones (CIP), Doxycyclin (DO), Linezolid (LNZ), Gentamicin (GM) and Sulphmethaxazole (SXT) by modified Kirby-Bauer disc diffusion method and measured according to CLSI-2016 for the test and control organisms. Ninty four multi drug resistant Staphylococcal isolates along with the controls were tested for the formation of biofilm by two methods: Tube Method (qualitative)[8,9,10,11] and Microtitre Plate Assay (quantitative)[3,9,11,12].

Tube Method: A loopful of all the test Staphylococcal spp. were allowed to form a liquid cultureinto trypticase soya broth. Test tube incubated at 37°C. After 24 hours of incubation, all the test tubes emptied and flushed by phosphate buffer saline, dried out, stained by crystal violet. Surplus stain was washed out with deionized water and dried in inverted position. In positive biofilm producer, visible stained biofilm seemed in the form of coating on around the wall and base of the tube. The results were scored visually as non-adherent/absent (0),weakly adherent (+), moderately adherent (++) and strongly adherent (+++).

Microtitre Plate Assay: Staphylococci were cultured in 10ml of the trypticase soya broth, allowed to incubate for 24hours at 37°C and diluted with fresh trypticase soya broth. Each and every individual wells of polystyrene tissue culture plates having the flat bottom with 96 well filled with of diluted liquid culture, incubated for 24 hours at 37°C. Wells washed with physiological saline. Fixation of biofilm forming bacteria was done with 99% methanol, emptied, and stained by crystal violet. Optical density (OD) of marked biofilm was measured by micro ELISA reader at 490 nm. All the test organisms were classified into following categories, depending upon the optical density of the test organism (OD) and the optical density of the negative control (OD_c). Non adherent ($OD \leq OD_c$), weakly adherent ($OD_c < OD \leq 2OD_c$), moderately adherent($2OD_c < OD \leq 4\ OD_c$) and strongly adherent($4OD_c < OD$).

RESULTS

The strength of biofilm increases by increasing the number of devices inserted in the body and also strength of biofilm increases by increasing the number of days, a device remain in the body. Out of 94 patients, 67 had been inserted with one device while 27 with two device, forming a sum of 121 devices in total. Figure 1 showed the effect of number of device on the Staphylococcal biofilm formation. Table 1 demonstrated the comparison of different devices with reference to biofilm formation by Tube method and Microtiter Plate Assay. For intravenous (IV) catheter. Figure 2 demonstrated that more number of days a medical device remain into the body, more are the chances to form strong biofilm and Microtiter Plate Assay proves better to document the biofilm formation.

Table 1: Comparison of biofilm formation from clinical specimen of the patients with various devices

Type of device	Tube Method N (%)	Microtiter plate assay N (%)	Total
IV catheter	76 (90.4)	81 (96.4)	84
Urinary catheter	11 (91.6)	12 (100)	12
CVP line	9 (100)	9 (100)	9
Prosthetic implants	6 (100)	6 (100)	6
Others	8 (80)	10 (100)	10

Figure 1: Effect of number of devices inserted in one patient at one time on Staphylococcal biofilm formation.

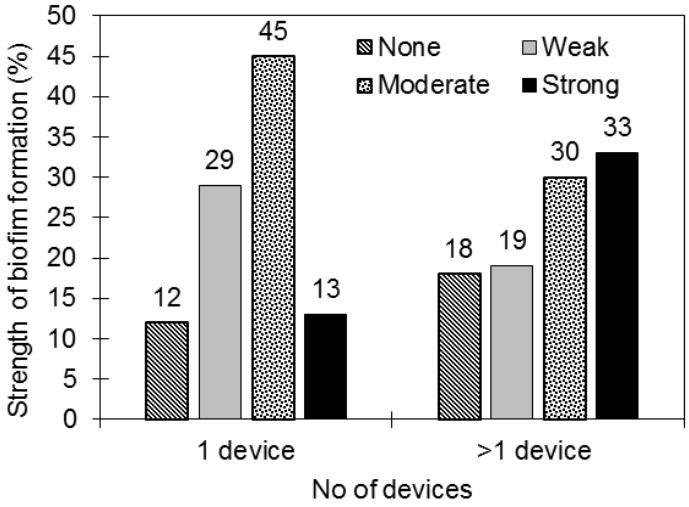
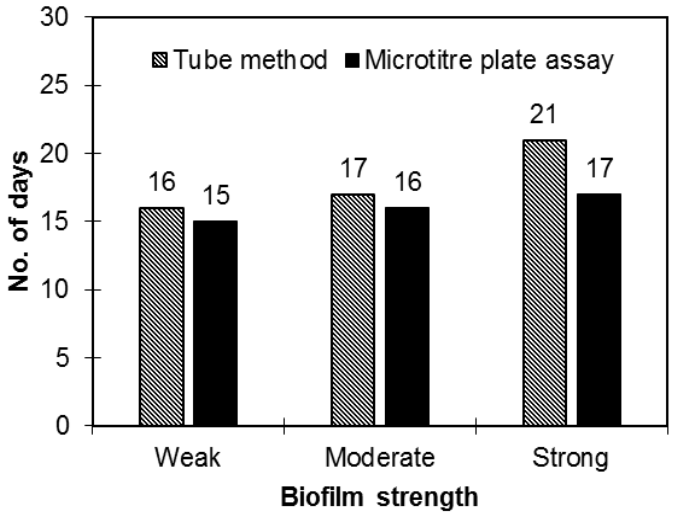


Figure 2: Relationship between biofilm strength with number of days of device insertion by Tube Method and Microtiter Plate Assay (n=94)



DISCUSSION

Biofilm is formed by the microorganisms that adhere on any foreign surface such as urinary catheter, endotracheal tube, implanted plate and joint material and prosthetic valves etc. Intravascular catheters and cannulas are most frequently used among various medical devices and also have tremendous ability to form biofilm[13,14]. Intra vascular catheters are used for the fluid replacement therapy in general hospital setups. By talking of critically ill patient, intra vascular catheters are used to administer fluids and antimicrobial agents and also for the management of total parentalnutrition[15].

The intensive cares of health careunits use numerous medical devices for intervention and treatment of the patients[16]. Contamination and colonization of these devices with the microorganisms, leading to biofilm formation is reported in various studies[17,18]. According to a study performed in Turkey, patients admitted in the intensive care unit comprised 25% of the hospital infections, and this infection rate is higher in the developing countries than developed countries, varying 4.4% to 88.9%[19].

A study was performed in Algeria and documented that more than 60% of the infections were due to formation of biofilm on the medical devices by the microorganisms[17]. Another study conducted in Baylor College of Medicine, Houston, Texas, about the infections related to the medical devices. It was stated that the presence of medical device or any foreign body minimizes the number of microorganisms required to initiate and develop infection in the body. Furthermore, the combination of increasing age of the subjects and more number of devices inserted in body markedly increases the rate of infective complications related to indwelling medical devices[20]. According to a study conducted in 2014 at Microbial Biofilm Laboratory, Rome, Italy, biofilm may form on the extraluminal or in the intraluminal surface of the intravascular catheters in case of catheter related blood stream infections (CRBSI). Extraluminal biofilm formation was the cause of sepsis, for the intravascular catheters that were implanted for one week or less period. And intraluminal formation of biofilm in the intravascular catheters was the cause of infections that are implanted for more than one week. The findings of the above mentioned study were in consistent with our study findings in which the biofilm formation was observed at 15th days (average) of catheter insertion in case of weak biofilm formation. Whereas moderate and strong biofilm formation take 16 and 17 days of catheter insertion by the microtitre plate assay[21]. Biofilm formation on various devices was effected by number of days of insertion in the body. Short-term catheterization (<10 days) causes infection by biofilm formation on the external surface, while the catheters inserted for long term (>10 days) impose clinical infection by formation of biofilm on the internal surface (Donlan, 2001). Donlan also commented that short-term urinary catheterization (7 days) is responsible for 10% - 50% of urinary tract infections while long term catheterization (>28 days) caused infections in almost every patient. The findings of Donlan were also in accordance to our study in which the adherence of biofilm formation increases with the increasing number of days of device insertion[22]. Another study was conducted by the same author in 2011, on biofilm infections related to medical indwelling devices at the Division of Healthcare Quality Promotion, Centre for Disease Control and Prevention, Atlanta, Georgia. His research findings demonstrated that biofilm on the medical devices including intravascular catheters form as early as 3 days but increase with number of days of device insertion on external and after that on the internal surface[23-25]. His findings were in agreement with our study findings. Another similar type of study performed in Italy on the hindrance and management of biofilm linked medical devices infections, duration of catheterization and the anatomical site of insertion of central venous catheter were the important factors causing device related infections[26-28]. The impact of duration of insertion of device with biofilm formation was in consistent to the findings of our study.

CONCLUSION

Biofilm formation is one of the several discovered mechanisms of drug resistance. The microorganism with multi drug resistance show very strong biofilm formation. In the patients with indwelling devices, biofilm formation is more pronounced with intravascular catheter and in the patients with more than one inserted devices. Moreover, biofilm formation is more pronounced if medical indwelling device is allowed to retain for more number of days inside the body.

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