Antibiotic Resistance Pattern of A Biofilm Forming Bacteria, Isolated From Implanted Catheters

Suraj Shinde*, Madhumati Patil**

*MSC Post Graduate Student, ** Associate Professor. Department of Microbiology, J.N. Medical College, KLE Academy of Higher Education And Research (Deemed-To-Be- University), Belagavi - 590010, Karnataka, India

Abstracts: Background & Objectives: Medical devices have become an essential part of modern health care system, but use of such devices have led to the adhesion of microorganisms on their surface and leading to the formation of biofilm. These biofilm act as a nidus for infection leading to device related infections. Microorganisms associated with biofilm formation are tolerant and resistant to antibiotics and host immune response, which increases the difficulties for the clinical treatment of biofilm infection. This study was done to know the prevalence of bacterial biofilm formation on the retrieved implants and the antibiotic resistance pattern among these biofilm forming isolates. Method: A total 148 retrieved catheter tips were subjected for culture. All the isolates were identified by standard biochemical reaction and antibiotic susceptibility testing was done as per CLSI guidelines. Detection of biofilm is done by using tissue culture plate method. Results: A total of 50 isolates are recovered from 148 catheter tips. Among these, 24(48%) were biofilm producers. S. aureus and S.epidermidis showed strong biofilm formation. A high antibiotic resistance pattern was seen among the biofilm producers when compared to non- biofilm producers. Conclusion: Bacterial biofilms are an important virulence factor associated with chronic nosocomial infection. Detection of biofilm forming organisms can help in appropriate antibiotic choice. Significant correlation between biofilm production and multidrug resistance was observed in our study. [Suraj S NJIRM 2017; 8(6):55-59]

Key Words: Biofilm, Catheters, Tissue culture plate method, Multidrug resistant organisms.

Author for correspondence: Madhumati Patil, Associate Professor, Department of Microbiology, J.N. Medical College, KLE Academy of Higher Education And Research (Deemed-To-Be-University), Belagavi-590010, Karnataka, India M: 9972615505 E-Mail: madhumatik2003@yahoo.co.in.

Introduction: The success of modern medicine is intimately attributed to the ever-increasing use of biomedical devices mainly used for vital functions management. After exposure to body fluids the device becomes an environment suitable to support biofilm growth and subsequent infection. Biofilm are microbial communities of surface attached cells embedded in a self produced extracellular polymeric matrix[1]. Due to their underlying disease conditions, hospitalized patients are highly susceptible to implant associated infections, such as catheter-related blood stream infections (CRBSI), catheter associated urinary tract infections (CAUTI), and ventilator associated pneumonia (VAP)[2]. Organisms primarily involved in biofilm formation consists of Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas. These organisms are either commensals on the skin or are nosocomial in origin.[3]

More than 60% of hospital-acquired infections worldwide are accredited to bacteria forming biofilms on medical devices [4]. Bacterial biofilm formation leads to chronic infections due to the increased tolerance to antibiotics and disinfectants, resistance to phagocytosis and to the human defense system. The decreased susceptibility to microbial agents within a biofilm arises from multiple factors, including physical impairment of diffusion of antimicrobial agents, reduced bacterial growth rates, and local alterations of the microenvironment that may impair activity of the antimicrobial agent.[5] Hence, antibiotic treatments are almost impossible to eradicate biofilm infections. In vitro and in vivo experiments demonstrated that the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for biofilm bacterial cells were usually much higher (approximately 10–1000 times) than the planktonic bacterial cells.[6] So, the implant associated infections lead to increased duration of hospital stay, increased cost and increased morbidity and mortality.

Here we have specifically analyzed catheters collected from patients who had no clinical infection in order to understand the threat constituted to patients, due to the presence of multiple-drug resistance properties in this group of biofilm isolates from these temporarily implanted medical devices.

Methods:

Place and duration of the study: The study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College and hospital, KLE

University, Belgaum from November 2012 to April 2013. Ethical clearance was obtained.

Selection of the isolates: A total of 148 catheter tips were sent to the microbiology laboratory in a sterile container. Catheter tips from urinary catheter, endotrachial tube, suction tube, central line, Ryle's tube, umbilical arhony catheter and long line received to the Microbiology laboratory were subjected for culture.

All catheter tips were directly cultured by roll plate method, catheter tip was rolled over the surface of 5% sheep blood agar plate and incubated at 37^0 c and bacterial count of 15 or more colonies were considered positive. Then the tip was placed in 10 ml of Brain Heart Infusion broth, incubated for 2hrs at 37^0 c and then vortexed for 15sec. Broth was subcultured on blood agar, chocolate agar and Mac conkey agar. [7] (Himedia, Mumbai, India)

Among 148 catheter tips, growth was seen in 50 samples. These clinical isolates were identified by standard microbiological procedures (Gram staining, colony morphology, catalase test cytochrome oxidase test and biochemical reactions). Then the isolates were subjected to biofilm detection by Tissue Culture Plate method and antibiotic sensitivity testing by Kirby- Bauer disc diffusion method.

Tissue Culture Plate method (TCP): This is a quantitative test described by Christensen et al. [8]is considered the gold-standard method for biofilm detection. Organisms isolated from fresh agar plates were inoculated in 10 ml of Trypticase soy broth (TSA) with 1% glucose. Broths were incubated at 37^oC for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well flat bottom polystyrene tissue culture treated plates (Sigma-Aldrich, Costar, and USA) were filled with 200 µL of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37^oC for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm[9]. The experiment was performed in triplicate. The interpretation of biofilm production was done according to the criteria of Stepanovic et al, [1] (Table 1). Reference strain of positive biofilm producer Staphylococcus epidermidis ATCC 35984 and Staphylococcus epidermidis ATCC 12228 (non biofilm producer) were used as control.

Table 1: Interpretation of biofilm production

Average OD value	Biofilm production
≤ ODC / ODC < ~ ≤ 2x ODC	Non /weak
2x ODC < ~ ≤4x ODC	Moderate
> 4x ODC	Strong

Optical density cut-off value (ODc) =average OD of negative control + 3x standard deviation (SD) of negative control.

Antibiotic sensitivity testing: Antibiotic susceptibility test for Gram positive and Gram negative biofilm producers was performed by using the Kirby-Bauer disc diffusion techniques according to CLSI guidelines [11]. Antibiotic discs were used depending on the type of microorganism and on the type of specimen (ampicillin 10 µg, cotrimoxazole 25 µg, ciprofloxacin 5 µg, aztreonam 30 µg, meropenem 15 µg, cefoperazone-sulbactam 105 µg, chloramphenicol, vancomycin 30 µg, erythromycin 15 µg, amoxicillin-clavulanic acid 20/10 µg, oxacillin 1 µg, linezolid 30 µg, penicillin 10 units, gentamicin 10 µg). The results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI). [11]

Result: The total number of retrieved implants was 148. Out of these, major were endotracheal tube tips 39(26%), 37(25%) were peripheral venous catheters and 24(16%) were urinary catheters (Table-2). Staphylococcus aureus was a major isolate 8 (16%) followed by Klebsiella pneumonia 7(14%)(Table-3). Among 50 isolates, 24 (48%) were biofilm forming. Staphylococcus aureus and Staphylococcus epidermidis showed strong bofilm production isolated from central venous line and endotracheal tube.

A high antibiotic resistance pattern was seen in biofilm producers. Tables 4 and 5 show the antimicrobial resistance pattern of Gram positive and Gram negative biofilm producing bacteria in this study, respectively. Gram positive biofilm producer were

more resistant to penicillin, rifampicin, cefoxitin, ciprofloxacin, erythromycin and cotrimoxazole than non biofilm producer. All Staphylococci were methicillin resistant (MRSA) tested by using cefoxitin disc and they were only sensitive to vancomycin, linezolid and teicoplanin. All Gram negative biofilm producers were more resistant to ampicillin, ciprofloxacin, cotrimoxazole, gentamycin, amikacin, ceftazidime and pipercillin and tazobactum as compared to non biofilm producers. Only 5 (20%) Gram negative biofilm producing bacteria were resistant to meropenem.

Table: 2 Number of catheter tips showing the growth

Catheter tips	With growth	Without growth	Total
Endotracheal tip	15(38.4)	24(2)	39(26.35)
Peripheral venous catheter tip	9(24.3)	28(75.6)	3(26)
urinary Catheter tip	10(41.6)	14(58.3)	24(16.2)
Center line tip	6(24)	19(76)	25(16.8)
Suction tip	5(55.5)	4(44.5)	9(6.08)
Umbilical Arhony catheter tip	5(33.7)	9(64.29)	14(9.45)
Total	50(33.7)	98(66.2)	148

Table 3: Screening of clinical bacterial isolates for Biofilm formation by Tissue culture plate method (TCP)

Organisms isolated	Biofilm production by TCP	Non-Biofilm formingI solates	Total
Staphyolcoccus aureus	6 (75%)	2(25%)	8 (16%)
klebsiella pneumonia	3(42.8%)	4(57.1%)	7(14%)
Enterobacter cloacae	2(40%)	3(60%)	5 (10%)
Pseudomonas aeuriginosa	3(60%)	2(40%)	5 (10%)
Staphylococcus epidermidis	3(75%)	1(25%)	4 (8%)
Acenetobacter baumanii	2(50%)	2(50%)	4 (8%)
Burkholderia cepaciae	2(33.3%)	4(66.6%)	6 (12%)
Cryseobacterium meningosepticum	1(33.3%)	2(66.6%)	3 (6%)
Enterococcus species	0	3(100%)	3 (6%)
Escherichea coli	1(50%)	1(50%)	2 (4%)
Staphylococcus hemolyticus	1(50%)	1(50%)	2 (4%)
Citerobacter diversus	0	1(100%)	1 (2%)
Total	24(48%)	26(62%)	50(100%)

Table 4: Resistance pattern of Gram positive biofilm producers in comparison with non-biofilm producers

Antimicrobial agent	Biofilm producer gram positive organisms (%)	Non- Biofilm producer gram positive organisms (%)
Ampicillin	62	57
Ciprofloxacin	57	45
Cotramaxazole	70	62
Penicillin	100	100
Gentamycin	76	74
Rifampacin	90	42
Tetracyclin	53	46
Vancomycin	08	00

Table 5: Resistance pattern of Gram negative biofilm producers in comparison with non-biofilm producers

Antimicrobial Agent	Biofilm producer gram negative organisms %	Non- Biofilm producer gram negative organisms %
Ampicillin	92	73
Ciprofloxacin	53	45
Cotramaxazole	100	95
Ceftazidime	84	82
Gentamycin	76	74
Amikacin	61	82
Piperacillin-Tazobactum	53	46
Meropenem	20	12

Discussion: Microbial biofilms may pose a public health problem for persons requiring indwelling medical devices. Indwelling devices are becoming increasingly frequent in medical practice and are applied to more than 25% of hospitalized patients. It has been estimated that about 65 per cent of the hospital acquired infections are associated with biofilm formation [12, 13]. These infections are 10 to 1000 times more difficult to eliminate with an otherwise successful treatment.[14,15] The microorganisms

involved in biofilm formation are difficult or impossible to treat with antimicrobial agents and detachment of these from the device may result in infection. [16] Biofilms are an ideal site for plasmid exchange in bacteria and provide the necessary environment for induced antibiotic resistance development, specifically, when we consider that many of the catheterized patients may be receiving antibiotics. [17]

In our study, 48% of the tested organisms have shown the potential to make biofilms. Biofilm formation is detected by In vitro Tissue culture plate method, which is a quantitative test and is considered as the gold-standard method for biofilm detection.

We found that gram-positive cocci, S.epidermidis and S. aureus (75%) were involved in large number in production of biofilm. These organisms form ubiquity as skin flora and their adherence to IMD's surface is well documented. Ammendolia et al. [18] and Bose et al.[19] also reported involvement of S.aureus in biofilm production. In this study, majority of the biofilm producers were isolated from Endotrachial tube catheter tip 39(26.35%) followed by peripheral venous catheter tips 37(25 %).

In our study, antibiotic susceptibility pattern of biofilm producing organisms was obtained. The clinically relevant observation was high resistance of biofilm producers to commonly used antibiotics. This observation was also mentioned in another study (Donlan and Costerton, 2002).[20] We have seen that Gram positive biofilm producers showed 100% sensitivity to vancomycin and among the Gram negative bacteria, most of them were sensitive to broad spectrum antibiotics like meropenem and imipenem. High resistance could be attributed to the biofilm producing ability of the isolates. Current antibiotics have classically been developed to treat infections involving planktonic bacterial populations and are typically ineffective in the eradication of bacteria in biofilm leading to persistent infections. The wide difference in resistance rates in these bacterial isolates may be attributed to injudicious and inappropriate use of antibiotics and further biofilm formation complicates the resistance problem.

Novel therapeutic solutions other than the conventional antibiotic therapies are in urgent need. Replacing the old, biofilm-laden catheters before antibiotic treatment is a sensible option. [21] Strategies have to be devised to control and prevent nosocomial infections associated with the use of implants in clinical practice. There are many recent research in discovery of alternative approaches to prevent or treat biofilms. Current anti-biofilm technologies includes the use of small molecules and enzymes which will inhibit or disrupt biofilm formation. Another group of anti-biofilm technologies focuses on modifying the biomaterials used in medical devices to make them resistant to biofilm formation.[22] The antibiotic policy need to be changed at regular intervals to prevent the development of resistant pathogens that leads to medical device related complications. Also newer microbiological techniques need to be developed to identify biofilm based infection.

Conclusion: In conclusion bacteria colonise and develop biofilm in the indwelling catheters, which needs to be taken care. Antibiotic treatment alone is often inadequate to overcome biofilm infections. Biofilm forming bacteria showed a higher drug resistance when compared to non biofilm forming bacteria. A knowledge of the antibiotic susceptibility of the organisms isolated from the devices helps to formulate an antibiotic policy. This also avoids un-necessary use of broad-spectrum empirical antibiotics and prevents emergence of drug resistant bacterial strains.

References:

1. Halebeedu PP, Kumar GV, Gopal S. Revamping the role of biofilm regulating operons in device-associated Staphylococci and Pseudomonas aeruginosa. Indian J Med Microbiol 2014;32:112-23
2. Singhai M, Malik A, Shahid M, Malik MA, Goyal R. A study on device-related infections with special reference to biofilm production and antibiotic resistance. J Global Infect Dis 2012;4:193-8
3. Von Eiff C, Jansen B, Kohnen W, Becker K. Infections Associated with Medical Devices: Pathogenesis, Management and Prophylaxis. Drugs 2005;376-9.
4. Pradeep Kumar SS, Easwer HV, Maya Nandkumar A. Multiple Drug Resistant Bacterial Biofilms on Implanted Catheters - A Reservoir of Infection. JAPI 2013; 61:18-23.
5. Niveditha P S, Umadevi S, Shailesh Kumar, Selvaraj. Antiobiotic resistance pattern of biofilm-

-forming uropathogens isolated from catheterized patients. AMJ 2012;(5)7:344-348.
6. Wu H, Moser C, Wang H.Z, Hoiby N, Song Z. J. Strategies for combating bacterial biofilm infections. International Journal of Oral Science 2015; 7: 1–7.
7. Raad II, Sabbagh MF, Rand KH, Sherertz RJ. Quantitative tip culture method and diagnosis of the central venous catheter-related infections. Diagn Microbiol Infect Dis 2008;26:333-7.
8. Christensen GD, Simpson WA, Yonger JJ, Baddor LM, Barrett FF, Melton DM, Beachey EH: Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 1985;22:996-1006.
9. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011; 15(4):305-311.
10. Stepanovic S, Vukovi D, Hola V et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by Staphylococci. APMIS 2007; 115:891-9.
11. Clinical and Laboratory Standards Institute (CLSI) 2011; Performance Standards for Antimicrobial Susceptibility Testing; Twenty first Informational Supplement. M100-S21; 31(1).
12. Rodney M. Donlan. Biofilms and Device-Associated Infections. Emerging Infectious Diseases 2001;7(2):277-281.
13. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;28 :318-22.
14. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9 : 34-9.
15. Nickel JC, Ruseska I, Wright JB, Costerton JW. Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 1985; 27 : 619-24.
16. Rodney M. Donlan. Biofilm Formation: A Clinically Relevant Microbiological Process. Clinical Infectious Diseases 2001; 33:1387–92.
17. Ghigo JM. Natural conjugative plasmids induce bacterial biofilm development. Nature 2001 26;412(6845):442-5.
18. Ammendolia, M. G., Rosa, R. D., Montanaro, L. M., Arciola, C. R. and Baldassarri, L. Slime production and expression of the slime associated antigens by Staphylococcal clinical isolates. Journal of Clinical Microbiology 1999;37(10): 3235-3238.
19. Bose, S., Khodke, M., Basak, S. and Mallick, S. K. Detection of biofilm producing Staphylococci: Need of the hour. Journal of Clinical and Diagnostic Research 2009;3:1915-20.
20. Donlan, R. M. and Costerton, W. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clinical Microbiological Reviews 2002; 15(2):167-193.
21. Stickler DJ. Bacterial biofilms in patients with indwelling urinary catheters. Nat Clin Pract Urol. 2008;5(11):598-608.
22. Treter1 J, Macedo1 A. J. Catheters: a suitable surface for biofilm formation. Science against microbial pathogens: communicating current research and technological advances. FORMATEX 2011: 835-842.

Conflict of interest: None

Funding: None

Cite this Article as: Suraj S, Madhumati P. Antibiotic Resistance Pattern of A Biofilm Forming Bacteria, Isolated From Implanted Catheters. Natl J Integr Res Med 2017; 8(6):55-59