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A Study of Biofilm Producers and Its Correlation to Antimicrobial Resistance among Orthopaedic Implant Associated Infections in a Tertiary Care Centre

Shobha Medegar K.R.*, Pooja Mansabdar, Asha B. Patil***, Harshika Y.K.*, Ganesh Perumal**, Neeta P.N.******

Author Affiliation
*Tutor **Post Graduate Student ***Professor and HOD, Department of Microbiology, KIMS, Hubballi. ****Assistant Professor, Department of Community Medicine, VIMS, Ballari.

Reprint Request
Neeta P.N., Assistant Professor, Dept. of Community Medicine, Vijayanagar Institute of Medical Sciences, Hospet Rd, Cantonment, Ballari, Karnataka 583104.
E-mail: drnita10@gmail.com

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Abstract

Background: Biofilms are defined as microbially derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. These biofilm forming organisms are frequently associated with implant associated infections and they are intrinsically more resistant to antimicrobial agents than planktonic cells. *Objectives*: 1. This study aims to detect the biofilm formation in the orthopaedic implant associated infections 2. to correlate its antibiotic susceptibility pattern with emphasis on multi drug resistance pattern. *Methodology*: A prospective study was done on a total of 150 cases,of aspirated pus sample, of all orthopaedic implant associated infections over a period of one year and sent to the department of microbiology, KIMS, Hubballi, whereinthey were processed according to the standard laboratory protocol. The isolates were identified and subjected to biofilm detection by three methods (Tube method, Tissue culture plate method and Congo red Agar method) and subsequently antibiotic susceptibility testing was performed on Muller Hinton Agar (MHA) by Kirby Bauer's Disc Diffusion method according to Clinical and Laboratory Standard Institute(CLSI) guidelines 2016. *Results*: Among 150 samples which were processed for biofilm detection 48% were detected as positive. Majority were Gram Positive Cocci (GPC) accounting to 63.88%. Methicillin Resistant Staphylococcus Aureus (MRSA) accounted to 60%, multi-drug resistance (MDR) was noted in 69.56% in case of GPC and 100% MDR Gram negative bacteria (GNB). *Conclusion*: Biofilm detection methods and its antimicrobial susceptibility testing should be routinely employed especially in case of implant associated infections, so that we can formulate antibiotic regimen for the multi-drug resistant isolates, by appropriate screening of MRSA, Extended Spectrum Beta Lactamase (ESBL), AMP-C and MBL and thus prevent treatment failures.

Keywords: AMP-C Co-producers; Biofilm; ESBL; Implant; Multi-Drug Resistance and MRSA.

Introduction

Bone and joint degenerative and inflammatory problems affect millions of people worldwide [1]. The introduction of an implant in the body is always associated with the risk of microbial infection, particularly for the fixation of open-fractured bones and joint-revision surgeries [2]. Infection is a major

problem in orthopedics leading to implant failure due to formation of biofilm, making it challenging to treat [3]. Biofilms are a group of microbes along with their exopolysaccharide matrix which adhere on biotic and abiotic surfaces conferring antibiotic resistance especially in indwelling medical devices [4].

Biofilm formation is dependent on adhesion properties such as adsorption, extracellular polymeric substances, attachment to hydrophobic (Teflon)or hydrophilic (glass) substratum and presence of fimbriae, flagella, pilli or glycocalyx, oxygen concentration, nutrient composition of medium and antimicrobial drug concentration [5,6,7]. Microorganisms growing in a biofilm are intrinsically more resistant to antimicrobial agents, as high as 1000 times, when compared to planktonic cells, hence effective antimicrobial agents are needed to inactivate them [8].

Materials and Methods

This isa prospective study, carried out in the department of microbiology, KIMS, Hubballi, on all orthopaedic implant associated infections from September 2015 to September 2016. A total of 150 non repetitive clinical specimens of pus, collected from implanted area or swabs from discharging sinuses were taken for culture, out of which 101 were culture positive and subjected to biofilm detection. All the bacterial isolates were identified by standard biochemical tests. Antibiotic susceptibility test of bacterial isolates was performed by Kirby Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines. A reference strain of Staphylococcus epidermidis ATCC 35984 (positive biofilm producer) and Staphylococcus epidermidis ATCC 12228 (non biofilm producer) were used as positive and negative controls respectively. Biofilm detection was done by the following methods:

1. *Tube Adherence Method:* Described by Christensen et al [9] this is a qualitative method for biofilm detection.
2. *The Congo Red Agar (CRA) method:* According to the Freeman et al [10], it is a simple qualitative method to detect biofilm production.
3. *The Tissue Culture Plate (TCP) method:* This is a quantitative test considered as the gold standard method for biofilm detection. The interpretation of biofilm was done according to the criteria of Stepanovicet al [11].

Results

Among the total 150 samples which were processed, 101 samples were culture positive. Out of these, biofilm producing organisms were 72 isolates (which were positive by any one of the method)

Table 1 shows that highest number of isolates were of Staphylococcus aureus 54.45%(55 isolates), followed by Klebsiella species 14.8%(15 isolates), CONS 11.8%(12 isolates), Pseudomonas species 4.9% (5 isolates), NFGNB 4.9% (5 isolates), Citrobacter freundi 3.9%(4 isolates), Escherichia coli 2.9%(3 isolates) and Providencia species 1.9% (2 isolates). For the Staphylococcal isolates, screening for Methicillin Resistant Staphylococcus aureus (MRSA) was done by using 30µg of cefoxitin disk on Muller Hinton Agar (MHA). The isolate with zone of inhibition <=21 mm was considered to be methicillin resistant.

Table 1: The distribution of isolates and biofilm production

Organisms Isolated	Biofilm Producers	Non Bio Film Producers
Escherichia coli	02(66.66%)	01
CONS (Coagulase negative staphylococcus)	06(50%)	06
Klebsiella species	12(80%)	03
Pseudomonas species	05(100%)	00
NFGNB (Non fermenting gram negative bacteria)	03(60%)	02
Citrobacter species	03(75%)	01
Providencia species	01(50%)	01

The high percentage of MRSA (60%) in biofilm producers was a contributing factor for high rate of drug resistance among them. Table 2 shows that majority of the isolates were highly resistant to Beta lactam antibiotics and macrolides. The isolates were sensitive to aminoglycosides, flouroquinolones, linezolid and 100% sensitive to teicoplanin and Vancomycin. The resistance of ampicillin and amoxyclav was statistically significant among biofilm producers and non biofilm producers. The statistical significance was calculated by using Chi Square test with p value <0.05 considered as significant.

Table 2: Drug resistance pattern among grampositive cocci isolates(n=67)

Antibiotics (mcg)	Biofilm producer	Non biofilm producer
Ampicillin(10)	89%(41)	57%(12)
Amoxicillinclavulanic acid(30)	78.2%(36)	42.86%(9)
Erythromycin(15)	65.2%(30)	57.14%(12)
Clindamycin(2)	41.3%(19)	38.09%(8)
Cefoxitin(30)	58.67%(27)	47%(10)
Linezolid(30)	13%(6)	0%
Vancomycin(30)	0%	0%
Teicoplanin(30)	0%	0%
Gentamycin(10)	19.5%(9)	4.76%(1)
Amikacin(30)	10.86%(5)	0%
Ciprofloxacin(30)	32.6%(15)	19.04%(4)
Levofloxacin(5)	4.34%(2)	0%
Cefepime(30)	30.4%(14)	23.8%(7)

Table 3: Multi drug resistance pattern in biofilm producing gram positive cocci(GPC) isolates:

Antibiotics	Number of isolates
A, AX, E, Cl,	10
A, AX,E,Cl,Cip,Cfm	8
A,AX, E, Cl,Gen,Ami,	4
A,AX,Cip,CX	8
A,AX,E,Cl,LN,CX	2

Table 3 shows that among the 46 biofilm producing GPC, 32 isolates were multidrug resistant accounting to 69.56%. Ampicillin (A), Amoxycillin clavulanic acid (AX), Erythromycin (E), Clindamycin (Cl), Ciprofloxacin (Cip), Cefepime (Cfm), Gentamycin (Gen), Cefoxitin (CX), Amikacin (Ami), Linezolid (LN)

Table 4 shows that there is high resistance among biofilm producers for Beta-lactam group of antibiotics. Resistance amongst biofilm and non biofilm producers for ampicillin, amoxy clavulanic acid, co-trimoxazole, ceftazidime and cefoxitinwas statistically significant and less resistance to Quinolones, Piperacillin tazobactam and aminoglycoside group of antibiotics was seen. All the isolates were sensitive to imipenem. The statistical significance was done by Fischer's test with p value <0.05 were considered significant.

Table 4: Theantibiotic resistance pattern among biofilm and non biofilm isolates of gram negative bacteria(n=34)

Antibiotics (mcg)	Biofilm producers(26)	Non-biofilm producers(8)	Chi square value	p- value
Ampicillin (10)	24(92%)	03(37.3%)	11.18	0.004
Amoxicillin clavulanic acid(30)	22(84.6%)	02(25%)	10.44	0.002
Ciprofloxacin(30)	11(42.3%)	02(25%)	1.329	p>0.05
Levofloxacin (5)	02(25%)	00(0%)	0.765	p>0.05
Co-trimoxazole(25)	22(84.7%)	02(25%)	10.3	0.001
Ceftriaxone(30)	21(80.7%)	05(62.5%)	0.126	p>0.05
Gentamycin(10)	18(69.2%)	02(25%)	4.936	0.03
Amikacin(30)	08(30.7%)	02(25%)	0.3	p>0.05
Ceftazidime(30)	21(80%)	03(37.8%)	5.5	0.01
Cefoxitin(30)	22(84.6%)	03(37.8%)	6.97	0.008
Pipercillin/tazobactam (100/10)	07(26.92%)	00(00%)	3.12	p>0.05
Imepenem(10)	00	00	00	0

Table 5: Multi drug resistance in biofilm producing Gram negative bacteria isolates(n=26)

Antibiotics	Number of isolates
A, AX, Cip,Ctr, Caz	10
A,AX, Cip, Gen, Ami,Caz, Ctr	06
A,AX,Ctr, Caz, Cx, Cot	05
A,AX, PTZ, Caz, Cx, Ctr	05

Table 5 shows that among the 26 biofilm producers all were multidrug resistance accounting to 100% (A:Ampicillin, AX:Amoxyclavulinic acid, Cip: Ciprofloxacin, Ctr: Ceftriaxone, Gen: Gentamycin, Ami:Amikacin, Caz: Ceftazidime, Cx: Cefoxitin, PTZ: Piperacillin/tazobactam, Cot:Cotrimoxazole). Among the total GNB organisms, (ESBL) producing isolates were 20%, AMP-C producers were 06%, ESBL and AMP-C co-producers were 44%. All the Pseudomonas isolates were biofilm positive conferring 100% biofilm producing isolates andall were non Metallo Beta Lactamase (MBL) producers.

Discussion

Implant related infection is a major concern, to the patients and in the orthopaedic community. The use of prosthetic implants in orthopaedics provides an ideal environment for biofilm formation as they are highly susceptible to infection. This is due to preoperative/post-operative infection, local host immune response or device rejection leading to device failure [12]. This necessitates further studies to determine the causative organisms and their susceptibility pattern to treat the patient. The diagnosis and treatment of these infections are complicated by the formation of a bacterial biofilm and an increase in the number of multidrug resistant bacteria. This stresses the value of an early diagnosis, leading to appropriate therapy of these patients.

In the present studya total of 150 samples were processed from all implant associated infections by collecting pus/swabs from wound discharge. Out of them 101 were cultures positive. Majority of the isolates were gram positive cocci accounting for 67 in number (66.33%) in comparison to gram negative organisms which were 34 in number (33.66%). Among the Gram positive cocci (GPC) Staphylococcus aureus isolates were 55 and Coagulase negative Staphylococcus species (CoNS) were 12 in number. Among Gram negative bacteria (GNB), majority were Klebsiella species 15 in number, followed by Pseudomonas species. A total of 72 biofilm producers were isolated which almost matches to a study conducted by Carla Renata Arciola et al [13] which shows 66% biofilm producers in orthopaedic implantsout of 80 isolates. Among the Staphylococcus aureus isolates 60% biofilm producers were Methicillin Resistant Staphylococcus aureus (MRSA) and 37.5% were MRSA in non biofilm producers. The present study results were in accordance to Khosravi et al [14] and Anisha F et al [15] which also reported Staphylococcus aureus as the most frequent isolatein orthopaedic implant associated infections.

The antimicrobial susceptibility testing revealed high rate of antimicrobial resistance in Staphylococcus aureus isolates to most of the routinely used antibiotics. All the Gram Positive Cocci, showed sensitivity to Vancomycin and Teicoplanin as seen in Afreenish Hassan et al [16] and Nixon M et al where Vancomycin was the most effective antibiotic [17]. The percentage of Methicillin Resistant Coagulase negative Staphylococcus was 50% in biofilm producing isolates and in gram negative biofilm producing organisms high prevalence of non MBL and high ESBL and Amp C co-producer isolates accounting to 44%, contributing to implant failures. Probably the prolonged hospitalization in these patients contributes to hospital acquired infection leading to such high drug resistance [18]. Also the lowered immune status, extremes of age, patient with steroid therapy and other conditions like diabetes mellitus and open wound fractures could have contributed to high rate of infections. Studies also suggest use of titanium implants rather than stainless steel implants to minimize the risk of infections. But affordability is also a major concern in a government setup unless the government subsidizes it.

We found the following antibiotics-vancomycin, teicoplanin, amikacin, levofloxacin to be more effective for biofilm producing Gram Positive Cocci and amikacin, levofloxacin,Imipenem and piperacillin/tazobactam effective for biofilm producing Gram Negative Bacilli.

Conclusion

The result of this test shows that there is high prevalence of biofilm producing organisms in orthopaedic implants, showing multi drug resistance, hence routine screening tests for MRSA, ESBL and AMP-C producing isolates should be emphasized to prevent treatment failure.

Recommendation

However we would recommend that similar studies need to be done with larger sample size to identify biofilm producing isolates and their antibiotic susceptibility pattern in prosthetic implants.

Conflicts of Interest: None

Source of Support: Nil

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