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ANALYSIS OF BIOFILM FORMATION IN STAPHYLOCOCCI BY MODIFIED TISSUE CULTURE PLATE METHOD.

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ABSTRACT

Context: Biofilm forming microorganisms are associated with chronic infections and are found to be highly resistant to antimicrobial agents. Staphylococci are one of the important pathogen associated with variety of human infections and are known to produce biofilm.

Aim: To detect biofilm formation in clinical isolates of *Staphylococcus spp.* To compare antimicrobial resistance in biofilm producing and non-producing isolates.

Settings and Design: Prospective observational study conducted in a tertiary care hospital.

Materials and methods: Total 166 staphylococci isolates obtained from pus samples were studied for biofilm production by modified tissue culture plate (MTCP) method. Antimicrobial susceptibility testing (AST) was done by Kirby-Bauer disc diffusion method.

Results: By modified tissue culture plate method, the biofilm production rate was found to be 66.9%. AST pattern revealed 59.6% isolates were Multi Drug Resistant (MDR).The rate of MDR isolates in biofilm positive isolates was 75.2% while only 39.7% non-biofilm producing isolates were found to be MDR.This difference in resistance pattern was found to be statistically significant.

Conclusion: Early identification of biofilm producing strains might help to modify the treatment and prevent relapse of such infection. Appropriate antibiotic selection for the treatment of such biofilm associated infections is extremely important.

KEYWORDS : Biofilm, Wound infection, Staphylococcus, Modified tissue culture plate method.

Staphylococci are associated with numerous infections like urinary tract infections, wound infections, endocarditis, osteomyelitis, pneumonia and other device related infections. A large number of virulence factors which are being produced by these organisms are implicated in their pathogenesis and tissue invasion.[1] Biofilm formation is considered as one of the important virulence factor of these Gram positive bacteria.

Biofilms are defined as microbially derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other, are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced.[2]

Literature suggests biofilms can be formed on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices (IUDs) and sutures.[3] They are a major contributor to diseases that are characterised by an underlying bacterial infection and chronic inflammation.[4] Biofilms are also found in wounds and are suspected to delay healing ultimately leading to non-healing chronic wounds.[5]

The present study was carried out to investigate the capacity of *Staphylococci* isolated from wound infections to produce biofilm alongwith their antibiotic sensitivity pattern. Also a comparative evaluation of antimicrobial resistance among biofilm producing and biofilm non-producing isolates was done.

Material and methods:

The present study was conducted from march – august 2016 on pus samples received in the deptt. of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India. The identification of the organisms was done by colony Gram staining, colony morphology, catalase test and coagulase test as per standard microbiological protocol.[6-8] AST were done by Kirby-Bauer disc diffusion method in accordance with CLSI guidelines 2016.[9] The antimicrobials tested included erythromycin (15µg), penicillin (10units), cefoxitin (30µg), cephalexin (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), linezolid (30µg), doxycycline (30µg), clindamycin (2µg), vancomycin (30µg), amoxicillin/clavulanic acid (20µg/10µg).

Biofilm production was detected by Modified Tissue Culture Plate (MTCP)[10] methods.

Isolates from fresh agar plates were inoculated in brain heart infusion (BHI) broth supplemented with 2% sucrose dispensed in 2 ml in test tubes and incubated for 18 hours at 37˚C in stationary condition. The broth with growth (visible turbidity) was diluted in the ratio of 1:100 with fresh medium. Then 200µl of this diluted culture broth was added to 96 well- flat bottom, non-adherent, non-treated polystyrene tissue culture plates. Un-inoculated broth served as control to check sterility and non specific binding of the medium while blank well served as control to check the quality of tissue culture plate. These tissue culture plates were incubated for 24 hours at 37˚C. After incubation, the contents of the wells were removed by gently tapping the plates. The wells were then washed four times with 0.2 ml of phosphate buffered saline (pH 7.2). Biofilm formed on the walls and bottom of wells were fixed with 2% sodium acetate for 30 minutes and stained with crystal violet (0.1% w/v) for 30 minutes. Excess stain was rinsed off by washing with distilled water and plates were kept for drying. After drying, 160µL of 33% glacial acetic acid was added into microwells for 15 min at room temperature, to solubilize the dried crystal violet stain which was adherent to any biofilm. Optical densities (OD) were then determined by an automated micro ELISA reader at wavelength of 570nm. These OD values were considered as an index of bacterial adhesion and biofilm formation. The biofilm formation was considered as weak/ none biofilm formation if OD value was less than 2.63, moderate if OD value was between 2.66-5.32 and strong when OD value was greater than 5.32.

Results:

A total of 166, *Staphylococcus aureus* (86) and coagulase negative staphylococci (CONS) (80) were isolated during the study period. The antibiotic sensitivity pattern showed *S. aureus* and CONS isolates to be 100% sensitive to vancomycin. Linezolid was also found highly effective, with 94.2% sensitivity in *S. aureus* and 96.2% sensitivity in CONS. The sensitivity pattern of other drugs in *S. aureus* isolates showed 72.1% sensitivity to clindamycin, 69.7% to doxycycline, 55.8% to erythromycin, 54.6% amoxicillin-clavulanate, 48.8% to cephalexin, 38.4% to trimethoprim-sulfamethoxazole, 37.2% to cefoxitin and 9.3% to penicillin. The CONS isolates showed equal susceptibility to doxycycline and clindamycin (70%), erythromycin (63.7%), amoxicillin-clavulanate (61.2%), trimethoprim-sulfamethoxazole (45%), cefoxitin (43.7%) and penicillin (12.5%). This has been shown in table 1.

Table 1: Antibiotic sensitivity pattern

Antibiotics	*S. aureus* (n=86)		CONS (n=80)	
	N	%	n	%
Erythromycin	48	55.8	51	63.7
Penicillin	08	9.3	10	12.5
Cefoxitin	32	37.2	35	43.7
Trimethoprime-sulfamethoxazole	33	38.4	36	45

Clindamycin	62	72.1	56	70
Cephalexin	42	48.8	36	45
Amoxicillin- clavulanate	47	54.6	49	61.2
Doxycycline	60	69.7	56	70
Linezolid	81	94.2	77	96.2
Vancomycin	86	100	80	100

The modified tissue culture plate method identified 60.5% isolates of *Staphylococcus aureus* and 73.7% CONS isolates as biofilm producers. The overall rate of biofilm production was found to be 66.9%. The grading of biofilm formation has been shown in table 2.

Table 2: Grading of biofilm formation.

Biofilm formation	MTCP	
	Number	Percentage
Strong	36	21.7
Moderate	75	45.2
Weak/None	55	33.1
Total	166	100

Out of 166 isolates, 99 (59.6%) isolates showed Multi Drug Resistance (MDR). The rate of MDR isolates in biofilm positive isolates was 75.2% while only 39.7% non-biofilm producing isolates were found to be MDR. This difference in resistance pattern was found to be statistically significant ($p< 0.05$). The resistance pattern of biofilm forming (BF) and non- biofilm forming (NBF) has been depicted in table 3.

Table 3: Antibiotic resistance pattern of biofilm forming (BF) and non-biofilm forming (NBF) *Staphylococcus* spp. (n=166)

Antibiotics	Resistance in BF isolates (n=93)		Resistance in NBF isolates (n=73)		'p' value
	n	%	n	%	
Erythromycin	47	50.5	20	27.4	<0.05
Penicillin	90	96.8	58	78.4	<0.05
Cefoxitin	74	79.6	25	34.2	<0.05
Co-trimoxazole	49	52.7	48	65.7	>0.05
Clindamycin	39	41.9	09	12.3	<0.05
Cephalexin	57	61.3	31	42.5	<0.05
Amoxicillin- clavulanate	54	58.1	16	21.9	<0.05
Doxycycline	36	38.7	14	19.2	>0.05
Linezolid	05	5.3	03	4.1	>0.05
Vancomycin	00	00	00	00	NA

Discussion

Staphylococci are commonly implicated in wound infections which are often difficult to treat due to high level of resistance to multiple antibiotics. In the present study, the *Staphylococcus* spp. showed high resistance to penicillin (89.1%), cefoxitin (59.6%) and trimethoprime – sulfamethoxazole (58.4%) while all of them showed 100% sensitivity to vancomycin. Huma et al studied 94 isolates of *Staphylococcus* spp. which showed 97.87% resistance to penicillin, 81.91% for trimethoprim –sulphmethaxazole, 68.08% for erythromycin, 57.44% for cefoxitin, 54.25% for clindamycin, 50.0% for doxycyclin and 6.38% for linezolid.[11] Jaychandran et al studied 100 isolates of *S. aureus* and noted 61% resistance to amoxicillin, 36% resistance to erythromycin, 33% resistance to cephalexin and 19% to cefoxitin. Vancomycin and linezolid were found effective against all the isolates.[12] Astha et al studied 78 isolates of staphylococci obtained from pus samples and found majority of isolates were resistant to doxycycline (67.9%), oxacillin (61.5%), clindamycin (51.3%), amoxicillin – clavulanate (47.4%) and erythromycin (46.1%). All the isolates were found sensitive to linezolid and vancomycin.[13]

Authors of present study found 66.9% of the isolates were biofilm producers. Bose et al studied 179 clinical isolates of *Staphylococcus* spp. and found biofilm production rate to be 54.2%.[14] Astha et al found 52.6% isolates to be biofilm producers.[13] In a study by Apurva et al, 38 clinical isolates of *S. aureus* were evaluated for biofilm production and found 52.6% of the isolates to be slime producers.[15] The rate of biofilm production by staphylococci in our study was comparable to the results of these studies.

Identification of infection caused by biofilm producing S. aureus might help to modify the antibiotic therapy and prevent infection.

REFERENCES

1. Foster. The Staphylococcus aureus superbug. J Clin. Invest. 2004;114:1693-6.
2. Donlan RM, Costerton W. Biofilms: Survival mechanisms of clinically relevant Microorganisms. Clin Microbiol Rev. 2002;15(2):167-93.
3. Wolcott R, Costerton JW, Raoult D, Cutler SJ. The polymicrobial nature of biofilm infection. European society of Clin Microbiol Infect. 2013;19(2):107-112.
4. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. J. Clin. Invest. 2003;112(10):1466–1477.
5. Thomas JG, Ruiz JC, Cutting KF, Leaper D, Snyder RJ, Wolcott R. Advancing your practice: understanding wound infection and the role of biofilms. Association for the advancement of wound care. Malvern, PA. 2008.
6. Colle JG, Marr W. Specimen collection, culture containers and media. In: Colle JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone. 1996: 95-111.
7. Colle JG, Miles RB, Watt B. Tests for identification of bacteria. In: Colle JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone. 1996:131-149.
8. Duguid JP. Staining Methods. In: Collee JG, Fraser AG, Marmon BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone. 1996:793-812.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility test: Twenty forth information supplement. CLSI document M100-S25. Wayne, PA: CLSI;2015.
10. Saxena S, Banerjee G, Garg R, Singh M. Comparative study of biofilm formation in Pseudomonas aeruginosa isolates from patients of lower respiratory tract infection. J Clin Diagn Res. 2014;8(5):9-11.
11. Huma Z, Javed I, Mushtaq S. Biofilm formation and detection in Multi-drug resistant Staphylococcus. Int J Pathol. 2015;13(2):50-54.
12. Jayachandran AL. Biofilm formation and Antibiotic susceptibility pattern among Staphylococcus aureus in a tertiary care hospital in Kanchipuram: An Evaluation of screening methods for biofilm formation. Int J Bioassays 2016;5(4):4991-4995.
13. Astha, Rathi R, Rishi S. Detection of biofilm producing Staphylococci and their Antimicrobial Susceptibility Pattern from pus samples in A tertiary health care centre. Ind J App Res. 2016;6(3):89-92.
14. Bose S, Khodke M, Basak S, Mallick SK. Detection of biofilm producing Staphylococci: Need of the hour. J Cin Diagnostic Res. 2009;3(6):1915-1920.
15. Apurva J, Barate D, Musaddiq M. Biofilm Forming Abilities and Antibiotic Susceptibility Pattern of Clinical Isolates of Staphylococcus aureus. Ind J App Res. 2013;3(5):41-44.