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A Study on Bio film Production in *Staphylococci* with their Antimicrobial Susceptibility Pattern in a Tertiary Care Centre

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A B S T R A C T

One of the reasons for organisms being capable of defending themselves from host immune systems is their capability to form biofilms. The interior of the bacterial biofilms presents greater resistance to opsonisation by antibodies and to phagocytosis, which explains the chronic character of these infections (Parsek *et al*., 2003). Furthermore, the information on the capacity of a clinical isolate to produce biofilm would help a clinician to evaluate the measure of its virulence and devise an appropriate treatment plan for the patient (Christensen *et al*., 1985). The objective of this study is to compare the biofilm production in *Staphylococcus aureus* and Coagulase negative Staphylococci (CONS) using Congo red agar and Tube method and also to compare antibiogram of Biofilm producers and non-biofilm producers. Among the total sample size of 497 clinical bacterial isolates, 60 isolates were Staphylococcus, out of which 46 isolates (76%) were *Staphylococcus aureus* and 14 (23%) were *Staphylococcus epidermidis* based on morphology and biochemical properties. From 46 *Staphylococcus aureus* isolated, 21 (47.82%) isolates were non-biofilm producers. 25 (54.34%) isolates produced biofilm as detected by Tube Method (TM),out of which, 11 (44%) biofilm producers were detected by both the Congo Red Agar (CRA) and Tube Method (TM).14(56%) biofilm producers were detected only by tube method. Among the 14 isolates of (23%) *Staphylococcus epidermidis*, 4 (28%) isolates were non-biofilm producers and 10 (71%) isolates were biofilm producers as detected by Tube Method (TM). Out of which, 5 (50%) biofilm producers were detected by both Congo Red Agar (CRA) and Tube Method (TM). 5(50%) biofilm producers were detected only by tube method In our study we found that,23 (92%) biofilm producers and 13 (68.42%) non biofilm producers of *Staphylococcus aureus* and 7 (70%) biofilm producers and 2(50%) non biofilm producers of *Staphylococcus epidermidis*, were resistant to Penicillin and biofilm producers of *Staphylococcus epidermidis*, showed higher resistance to Ciprofloxacin and Cotrimoxazole.

Introduction

Biofilm production is considered as a marker of clinically relevant infection. Previous observations have confirmed that microrganisms are not only resistant to antibiotics but also to a variety of disinfectants due to biofilm production

which emphasizes that their characterization is an important aspect of infection control (Chaudhary *et al*., 2009).

In a biological system, biofilm formation takes place in various steps: first there is an attachment to a surface; later, microbial surface adhesions recognize adhesive matrix molecules and start aggregating, attaching to each other and produce extra polymeric substances (EPS) that interact with host-derived components such as platelets to form a strong biofilm (Mackie & Mc Cartney, practical medical microbiology 14th edition). Under certain adverse circumstances such as deprivation of nutrition or a heavy shearing movement, breakage and dissemination of biofilm occurs due to the formation of certain defence proteins called auto-inducing peptides (AIP) with the release and dispersal of bacteria (Bose *et al*., 2009).

Biofilm appears to act as a barrier protecting bacteria from host defence mechanisms as well as from antibiotics, while providing a suitable environment for bacterial survival.

Materials and Methods

The study was done in a Tertiary care centre for a period of 6 months. Both in-patients and out patients were included in this study and total sample size was 497. All the Staphylococcal isolates from clinical samples such as, pus, wound swab, other body fluids, urine, respiratory and blood were included in this study. Organisms other than Staphylococci were excluded.

All the Staphylococcal isolates from various clinical samples were processed further for detection of Biofilm and Antibiotic sensitivity pattern.

Detection of Biofilm

The cultures were inoculated on trypticase soy agar (TSA) 16% (vol/vol) glycerol and kept at 20°C for 24 hours. The production of biofilm was detected by using special media and techniques like tube method (TM) and congo red agar (CRA).

Congo Red Agar (CRA) method

Procedure

Concentrated aqueous solution of the congo red stain was prepared and autoclaved at 121^0 C for 15 minutes. Then this solution, when it had cooled to 55^{0}C, was added to the prepared agar medium containing brain heart infusion (BHI) broth supplemented with sucrose. Then the plates were inoculated aseptically and incubated aerobically at 37^{0}C for 24 to 28 hours. The colonies with black dry crystalline morphology were graded as high biofilm producers, red colonies as moderate biofilm producers. Pink colonies were taken as biofilm negative.

Tube Method (TM)

Procedure

Trypticase soy broth (TSB) supplemented with glucose were prepared in 12 x 75 mm borosilicate test tubes. Then a loopful of microorganisms from overnight culture plates were inoculated onto the test tube containing the broth and incubated for 48 hours at 37°C.

After the overnight incubation, the contents were decanted and washed with PBS (Phosphate Buffer Saline) (pH 7.3) and left to dry at room temperature. Then the dried the tubes were stained with 1% solution of crystal violet. Each tube was then gently

rotated to ensure uniform staining and then the contents were gently decanted. The tubes were placed upside down to drain and then observed for biofilm formation. Ring formation at the walls and bottom of the tube was taken as high biofilm producers and those that appear only in the bottom were taken as moderate biofilm producers. Tubes which did not show the stain were taken as negative.

Antibiotic Sensitivity Testing

Antimicrobial susceptibility testing was performed on Muller Hinton agar by Kirby Bauer disc diffusion method. The antibiotic used for both *Staphylococcus aureus* and Coagulase negative staphylococci (CONS) were the same. However, novobiocin was used to differentiate *Staphylococcus epidermidis* from *Staphylococcus saprophyticus*. According to CLSI (Central Laboratory Standards Institute) guidelines, the following antibiotics disc were used: Penicillin (10 units) Cefoxitin (30 µg), Cephalexin (30 µg), Erythromycin (15 µg),Cotrimoxazole (1.25/23.75 µg),Gentamicin (10 µg),Clindamycin (21 µg),Ciprofloxacin (5 µg),Vancomycin (30 µg),Ofloxacin (5 µg),Linezolid (30 µg),Teicoplanin (30 µg),Rifampicin (5 µg),Amikacin (30 µg),Novobiocin.

Results and Discussion

Among the total sample size of 497 clinical isolates, 60 isolates were *Staphylococcus.* Out of 60 staphylococcal isolates, 46(76%) were *Staphylococcus aureus* and 14 (23%) were *Staphylococcus epidermidis.* Distribution of clinical samples from which *Staphylococcus aureus* and *Staphylococcus epidermidis* was isolated is shown in figure 1 and figure 2.

From 46 *Staphylococcus aureus* isolated from different clinical samples, 21(45.65%) isolates were non-biofilm producers. 25 (54.35%) isolates produced biofilm as detected by Tube Method (TM), out of which, 11 (44%) biofilm producers were detected by both the Congo Red Agar (CRA) and Tube Method (TM).14(56%) biofilm producers were detected only by tube method. Biofilm production by *Staphylococcus aureus* from different clinical isolates by Congo Red Agar (CRA) and Tube Method (TM) shown in Table 1.

Out of 14 *Staphylococcus epidermidis* isolates, 4 (28.57%) isolates were non-biofilm producers, and 10 (71.43%) were biofilm producers as detected by Tube Method (TM), out of which 5 (50%) biofilm producers were detected by both Congo Red Agar (CRA) and Tube Method (TM). 5(50%) biofilm producers were detected only by tube method as shown in the table 2.

Grading and Screening of Biofilm production

Among the 25 (54.34%) Biofilm producers of 46 *Staphylococcus aureus* isolates, 8 isolates (32%) were graded as high biofilm producers and 17 (68%) isolates were graded as moderate biofilm producers by the Tube method (TM).2 isolates (18.1%) were graded as high biofilm producers and 9 isolates (81.8%) as moderate biofilm producers by the Congo Red Agar method (CRA). Screening and grading of *Staphylococcus aureus* producing biofilm by Congo Red Agar (CRA) and Tube Method (TM) is shown in figure 3.

Among the 10 (71.43%) biofilm producers of 14 *Staphylococcus epidermidis* isolates, 4 (40%) isolates and 3 (60%) isolates were graded as high biofilm producers by Tube Method (TM) and Congo Red Agar (CRA) respectively. 6 (60%) isolates and 2 (40%) isolates were graded as moderate biofilm

producers by Tube Method (TM) and by Congo Red Agar (CRA) respectively. Screening and grading of *Staphylococcus epidermidis* producing biofilm by Congo Red Agar (CRA) and Tube Method (TM) is shown in figure 4.

Antimicrobial susceptibility testing

Regarding antibiogram of *Staphylococcus aureus*, all 46 (100%) isolates were susceptible to the Vancomycin and Linezolid. Detail description of the antibiotic sensitivity pattern is shown in the table 3.

45 (97.82%) *Staphylococcus aureus* strains were susceptible to Rifampicin. Only 10 (21.73%) strains were susceptible to penicillin. None of the isolates were Vancomycin resistant. 33(71.73%) were MSSA (Methicillin Sensitive *Staphylococcus aureus*) and 13 (28.26%) were MRSA (Methicillin Resistant *Staphylococcus aureus*) which was detected by Cefoxitin resistance.

All *Staphylococcus epidermidis* strains were sensitive to Vancomycin and Linezolid. 8(57.14%) were MSSE (Methicillin Sensitive *Staphylococcus epidermidis*) and 6(42.85%) were MRSE (Methicillin Resistant *Staphylococcus epidermidis*). Detailed description of the antibiotic sensitivity pattern is shown in the table 4.

All biofilm producers of *Staphylococcus aureus* were sensitive (100%) to Vancomycin, Linezolid and Rifampicin Antibiotic susceptibility pattern of biofilm producing *Staphylococcus*. aureus is shown in table 5.

All biofilm producing strains of *Staphylococcus epidermidis* were sensitive (100%) to Vancomycin and Linezolid. Detailed description of the above data with all the drugs is shown in the table 6.

Out of 25 biofilm producing strains and 21 non-biofilm producers of *Staphylococcus aureus*, Penicillin resistance was seen in 23 (92%) biofilm producers and 15 (71.43%) non-biofilm producers. Whereas 10 (40%) biofilm producers and 9 (42.86%) non-biofilm producers strains were resistant to Cotrimoxazole. Resistant pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus aureus* is shown in table 7.

It was observed that generally Biofilm producers of *Staphylococcus aureus* were showing more resistance to all the antibiotics than non-biofilm producers as shown in figure 5.

Out of 10 biofilm producing strains and 4 non-biofilm producers of *Staphylococcus epidermidis*, Penicillin resistance was seen in 7 (70%) biofilm producers and 2 (50%) non-biofilm producers. 60% of the biofilm producers of *Staphylococcus epidermidis*, were showing resistance to Ciprofloxacin and Cotrimoxazole. Resistant pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus epidermidis* is shown in table 8.

Biofilm producers of *Staphylococcus epidermidis* were showing more resistance to most of the antibiotics when compared to non-biofilm producers as shown in figure 6.

In this study, carried out in a Tertiary care centre for a period of 6 months, out of of 497 samples, 60 staphylococcal species were isolated from different clinical samples. According to our study we found that 35 (58.34%) staphylococcal isolates, (which includes *S.aureus* and *S.epidermidis*) show biofilm formation as detected by Tube Method (TM) and 25 (41.66%) isolates were non-biofilm producers. 16 (26.67%) isolates produce biofilm detected by Congo Red

Agar (CRA) and 35 (58.33%) isolates produce biofilm as detected by Tube method (TM). There is a difference in detection of biofilm between Congo Red Agar (CRA) and Tube Method (TM). This study similar with a study conducted by Yasmeen Taj *et al.*, where 4 (3.47%) isolates produces biofilm by Congo Red Agar (CRA), and 63 (57.78%) produces biofilm by Tube Method (TM) [6]. From 35 (58.33%) staphylococcal isolates, 5 (8.33%) produce black colonies and 11 (18.33%) were moderate biofilm producers by Congo Red Agar (CRA) method. There were 12 (20%) high biofilm producers and 23 (38.33%) were moderate biofilm producers detected by Tube Method (TM).

Table.1 Biofilm production by *Staphylococcus aureus* from different clinical isolates by Congo Red Agar (CRA) and Tube Method (TM)

Samples	Total number of samples	Non biofilm producers	Detection of biofilm production by			
			Tube Method (TM)	Congo Red Agar (CRA)	Both by CRA and TM	TM alone
Wound swab	24	10	14	5	5	9
Pus	15	7	8	5	5	3
Blood	3	3	NIL	NIL	NIL	NIL
Body fluid	2	NIL	2	NIL	NIL	2
Urine	1	1	NIL	NIL	NIL	NIL
Respiratory	1	NIL	1	1	1	NIL
Total	46	21	25	11	11	14

Table.2 *Staphylococcus epidermidis* isolates showing non-biofilm producers and biofilm producers by Congo Red Agar (CRA) and Tube Method (TM).

Samples	Total number of samples	Non biofilm producers	Detection of biofilm production by			
			Tube Method (TM)	Congo Red Agar (CRA)	Both by CRA and TM	TM alone
Wound swab	4	2	2	1	1	1
Pus	5	1	4	3	3	1
Urine	2	1	1	NIL	NIL	1
Body fluid	1	NIL	1	NIL	NIL	1
Blood	1	NIL	1	1	1	NIL
Ear swab	1	NIL	1	NIL	NIL	1
Total	14	4	10	5	5	5

Table.3 Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotics	Sensitive		Resistant	
	No. of isolates n=46	Percentage	No. of isolates	Percentage
Penicillin (P)	10	21.73%	36	78.26%
Cefoxitin (Cx)	33	71.73%	13	28.26%
Cephalexin (Cn)	35	76.08%	11	23.91%
Erythromycin (E)	34	73.91%	12	26.08
Cotrimoxazole (Cot)	28	60.08%	18	39.13%
Gentamicin (G)	31	67.39%	15	32.60%
Clindamycin (Cd)	37	80.43%	9	19.56%
Ciprofloxacin (Cip)	23	50%	23	50%
Vancomycin (V)	46	100%	NIL	NIL
Ofloxacin (Of)	27	58.69%	19	41.30%
Linezolid (Lz)	46	100%	NIL	NIL
Rifampicin (Rif)	45	97.82%	1	2.17%
Amikacin (Ak)	39	84.78%	7	15.21%

Table.4 Antibiotic susceptibility pattern of *Staphylococcus epidermidis*

Antibiotics	Sensitive		Resistant	
	No. of isolates n=14	Percentage	No. of isolates	Percentage
Penicillin (P)	5	35.71%	9	64.28%
Cefoxitin (Cx)	8	57.14%	6	42.85%
Cephalexin (Cn)	9	64,28%	5	35.71%
Erythromycin (E)	8	57.14%	6	42.85%
Cotrimoxazole (Cot)	9	64.28%	7	35.71%
Gentamicin (G)	13	92.85%	1	7.14%
Clindamycin (Cd)	11	78.57%	3	21.42%
Ciprofloxacin (Cip)	6	42.85%	8	57.14%
Vancomycin (V)	14	100%	NIL	NIL
Ofloxacin (Of)	6	42.85%	8	57.14%
Linezolid (Lz)	14	100%	NIL	NIL
Rifampicin (Rif)	12	85.71%	2	14.28%
Amikacin (Ak)	12	85.71%	2	14.28%
Novobiocin	14	100%	NIL	NIL

Table.5 Antibiotic susceptibility pattern of biofilm producing *Staphylococcus aureus*

Antibiotics	Sensitive		Resistant	
	No. of isolates	Percentage	No. of isolates	Percentage
Penicillin (P)	2	8%	23	92%
Cefoxitin (Cx)	20	80%	5	20%
Cephalexin (Cn)	20	80%	5	20%
Erythromycin (E)	20	80%	5	20%
Cotrimoxazole (Cot)	15	60%	10	40%
Gentamicin (G)	17	68%	8	32%
Clindamycin (Cd)	21	84%	4	16%
Ciprofloxacin (Cip)	19	76%	6	24%
Vancomycin (V)	25	100%	NIL	NIL
Ofloxacin (Of)	18	72%	7	28%
Linezolid (Lz)	25	100%	NIL	NIL
Rifampicin (Rif)	25	100%	NIL	NIL
Amikacin (Ak)	20	80%	5	20%

Table.6 Antibiotic susceptibility pattern of Biofilm producing *Staphylococcus epidermidis*

Antibiotics	Sensitive		Resistant	
	No. of isolates	Percentage	No. of isolates	Percentage
Penicillin (P)	3	30%	7	70%
Cefoxitin (Cx)	6	60%	4	40%
Cephalexin (Cn)	6	60%	4	40%
Erythromycin (E)	7	70%	3	30%
Cotrimoxazole (Cot)	4	40%	6	60%
Gentamicin (G)	8	80%	2	20%
Clindamycin (Cd)	8	80%	2	20%
Ciprofloxacin (Cip)	4	40%	6	60%
Vancomycin (V)	10	100%	NIL	NIL
Ofloxacin (Of)	6	60%	4	40%
Linezolid (Lz)	10	100%	NIL	NIL
Rifampicin (Rif)	9	90%	1	10%
Amikacin (Ak)	8	80%	2	20%
Novobiocin	10	100%	NIL	NIL

Table.7 Resistant pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus aureus*

Antibiotics	Biofilm Producers		Non-Biofilm Producers	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin (P)	2	23	6	15
Cefoxitin (Cx)	20	5	19	2
Cephalexin (Cn)	20	5	17	4
Erythromycin (E)	20	5	16	5
Cotrimoxazole (Cot)	15	10	12	9
Gentamicin (G)	17	8	17	4
Clindamycin (Cd)	21	4	18	3
Ciprofloxacin (Cip)	19	6	15	6
Vancomycin (V)	25	NIL	NIL	
Ofloxacin (Of)	18	7	15	6
Linezolid (Lz)	25	NIL	NIL	
R ifampicin (Rif)	25	NIL	NIL	
Amikacin (Ak)	20	5	15	6

Table.8 Resistant pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus epidermidis*

Antibiotics	Biofilm Producers		Non-Biofilm Producers	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin (P)	3	7	2	2
Cefoxitin (Cx)	6	4	3	1
Cephalexin (Cn)	6	4	2	2
Erythromycin (E)	7	3	3	1
Cotrimoxazole (Cot)	4	6	1	3
Gentamicin (G)	8	2	3	1
Clindamycin (Cd)	8	2	2	2
Ciprofloxacin (Cip)	4	6	2	2
Vancomycin (V)	10	NIL	4	NIL
Ofloxacin (Of)	6	4	3	1
Linezolid (Lz)	10	NIL	4	NIL
Rifampicin (Rif)	9	1	3	1
Amikacin (Ak)	8	2	3	1
Novobiocin	10	NIL	4	NIL

Fig.1 Distribution of clinical samples from which *Staphylococcus aureus* was isolated

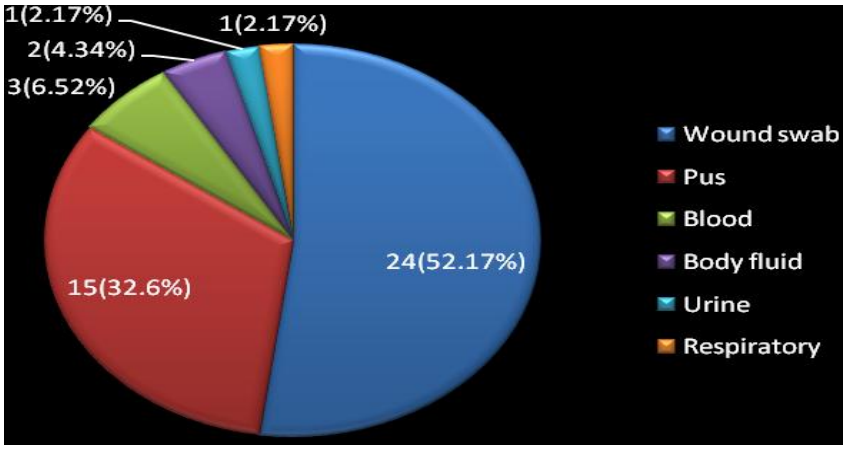


Fig.2 Distribution of clinical samples from which *Staphylococcus epidermidis* was isolated.

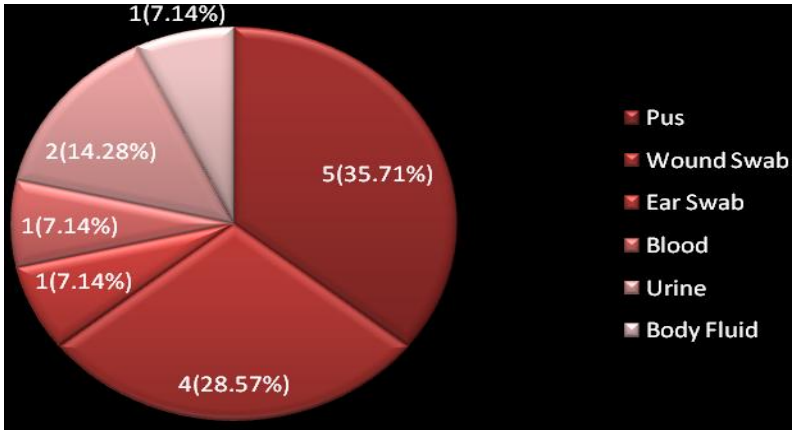


Fig.3 Screening and grading of *Staphylococcus aureus* producing biofilm by Congo Red Agar (CRA) and Tube Method (TM)

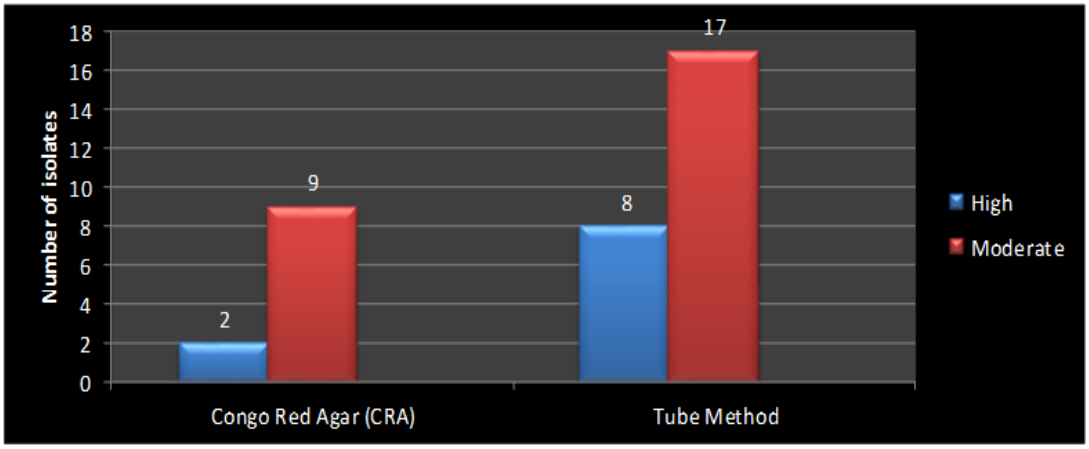


Fig.4 Screening and grading of *Staphylococcus epidermidis* producing biofilm by Congo Red Agar (CRA) and Tube Method (TM).

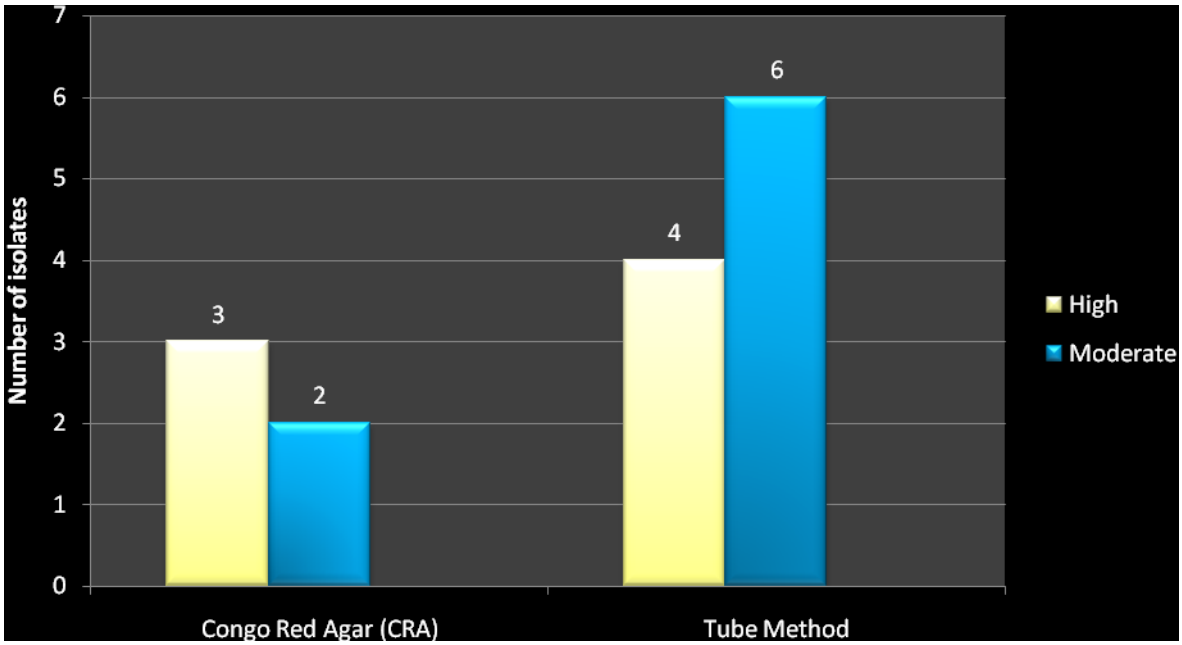


Fig.5 Graphical representation of Resistance pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus aureus*

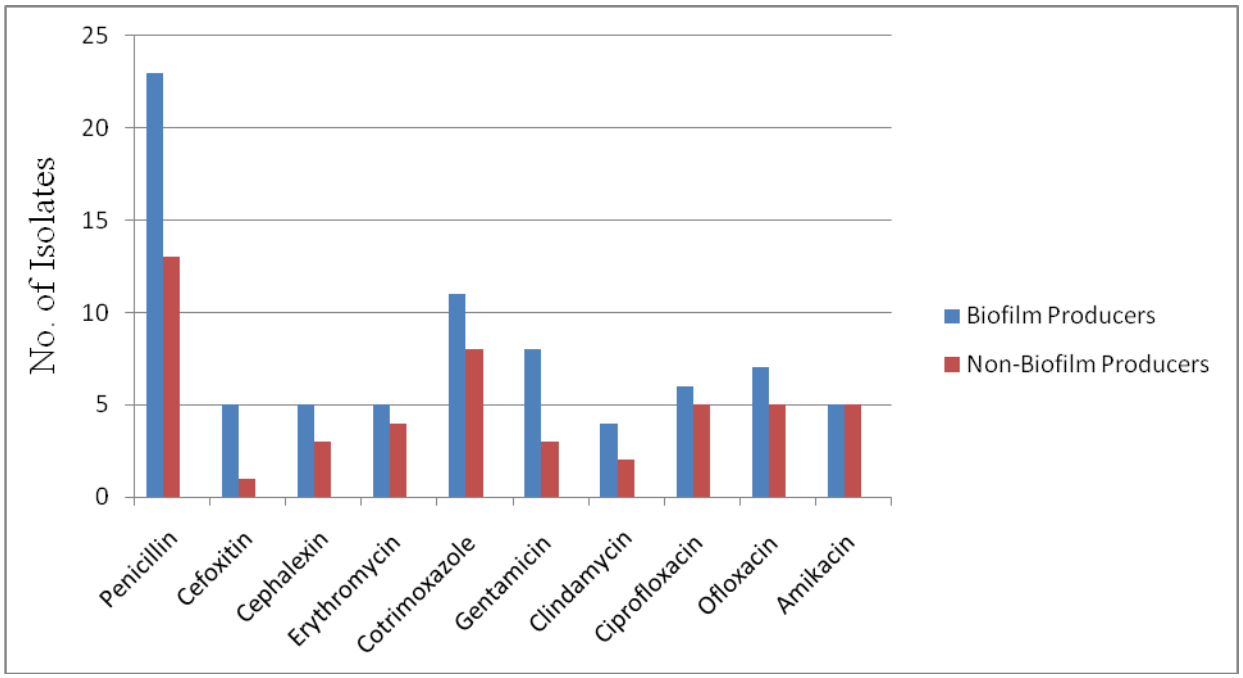


Fig.6 Graphical representation of Resistance pattern among biofilm producers in comparison with non-biofilm producers in *Staphylococcus epidermidis*

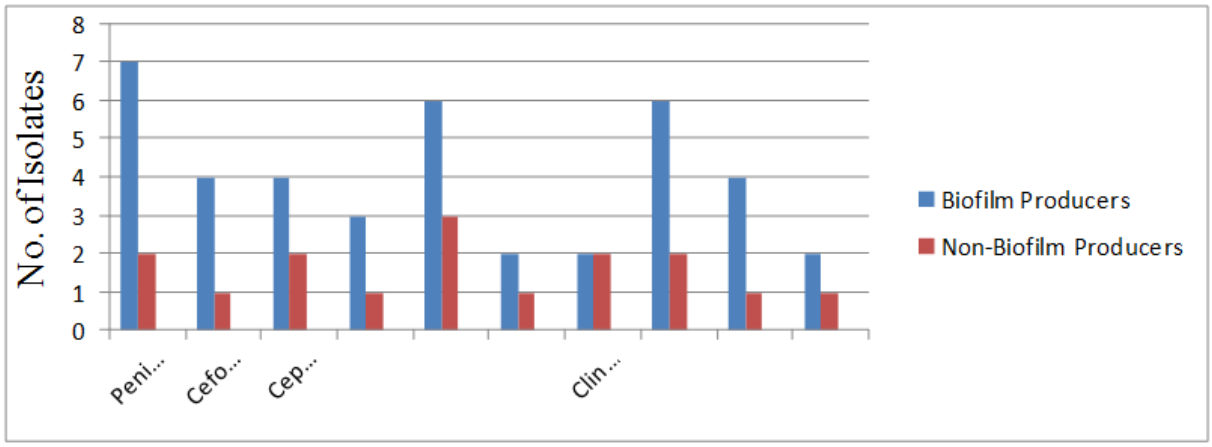
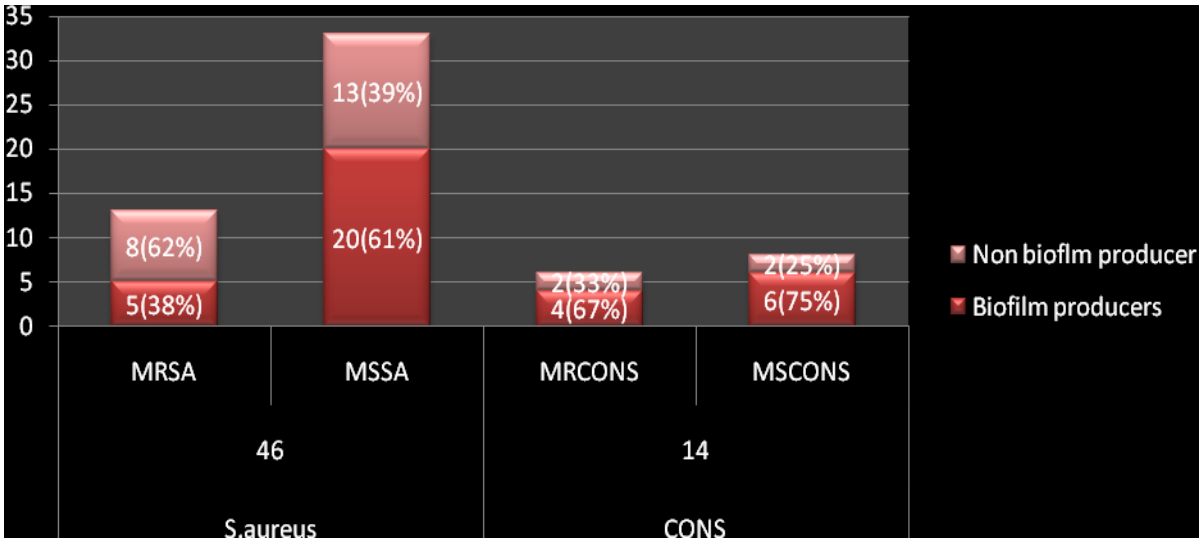


Fig.7 Distribution of Bio-film Producers and Non Biofilm Producers among *Staphylococcal* isolates :



Our study was similar to the study conducted by Mathur *et al.*, 2 (1.31%) produce black colonies and 6 (3.94%) were moderate biofilm producers by Congo Red Agar (CRA) method. 18 (11.84%) were high biofilm producers and 45 (29..33%) were moderate biofilm producers detected by Tube Method (TM) (Mathur *et al.*, 2006).

Among 60 staphylococcal isolates, 46 (76.66%) were *Staphylococcus aureus* and 14 (23.33%) were *Staphylococcus epidermidis*. Out of which 25 (54.34%) *Staphylococcus aureus* produce biofilm as detected by Tube Method (TM) and 11 (23.91%) by Congo Red Agar (CRA) method. Whereas 10 (71.42%) isolates of *Staphylococcus epidermidis* produce biofilm as detected by Tube Method (TM) and 5 (35.71%) isolates by Congo Red Agar (CRA) method. Our study is similar to the observation conducted by Bose L *et al*, wherein out of 179 *Staphylococcus* spp., 111 were *S.epidermidis* and 68 were *S.aureus*,44.69% of *S.epidermidis* and 32.96% *S.aureus* were slime producers, 76

(42.46 %) by TM and 11 (6.15%) by CRA method (Bose *et al*., 2009).

In our study we found that 23 (92%) biofilm producers and 13 (68.42%) non-biofilm producers are resistant strains of *Staphylococcus aureus* and 7 (70%) biofilm producers and 2 (50%) non-biofilm producers are resistant strains of *Staphylococcus epidermidis* towards Penicillin drugs. Also in case of *Staphylococcus epidermidis*, (60%) of the biofilm producers are resistant to drugs like Ciprofloxacin and Cotrimoxazole. Our study correlates with that of Bose L *et al*, where 100% resistant to penicillin shown by both biofilm producers and non-biofilm producers and Cotrimoxazole 60% resistantance shown by biofilm producers and 40% resistance shown by non-biofilm producers strains of *Staphylococcus aureus* and *Staphylococcus epidermidis.*

The information on the capacity of a clinical isolate to produce biofilm would help a clinician to evaluate the measure of its virulence and devise an appropriate treatment plan for the patient. Moreover, most of the biofilm strains of Staphylococcal isolates, especially CONS showed resistance to antimicrobial drugs, more than the non-biofilm producing strains. Almost all biofilms producing strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* showed resistance to Penicillin and Cotrimoxazole drugs. However, Vancomycin and Linezolid were some of the promising and effective drugs for both biofilm and non-biofilm producers including MRSA.

References

Anathanarayan, R. and Paniker, C.K. 2009. Text book of microbiology 8th Ed. Chennai, India; Orient Longman publications:.

Baveja, C.P. 2005. Text Book of microbiology, 2nd Ed. New Delhi; Arya publications.

Bose, S., Khodke, M., *et al*. 2009. Detection Of Biofilm Producing Staphylococci. *J. Clin. Diag. Res.,* (3): 1915-1920.

Chaudhary, A., M. Nagaraja and A.G. Kumar. 2009. Potential of biofilm formation by staphylococci on polymer surface and its correlation with methicillin susceptibility. *Ind. J. Med. Microbiol.,* 27: 377-378.

Christensen, G.D., W.A. Simpson, J.A. Younger, L.M. Baddour, F.F. Barrett, D.M. Melten and E.H. Beachey, 1985. Adherence of coagulase negative staphylococci to plastic tissue cultures: A quantitative model for the adherence of staphylococci to medical devices. *J. Clin. Microbiol.,* 22: 996-1006.

Forbes, Betty, A., Daniel, F., Sam, Ellis, S., Weisfield. 2002. Bailey and Scott's diagnostic microbiology, vol.7, 8 and 9.

Franklin, D. Lowy a study on Staphylococcal Infections.

Friedrich, Gotz, Tammy Bannerman and Karl-Heinz Schleifer. (Prokaryotes (2006) 4:5–75 DOI: 10.1007/0-387-30744-3_1).

Koneman, E.W., Allen, D., Janda, W.M., Schreekenberger, P.C., Winn, W.C. 1997. In Colour atlas and textbook of diagnostic microbiology 6th Ed. Philadelphia, USA: Lippincott, Williams and Wilkins publications.

Mackie & Mc Cartney. Practical medical microbiology 14th edition.

Mathur, T., S. Singhal, S. Khan, D.J. Upadhyay, T. Fatma and A. Rattan, 2006. Detection of biofilm formation among clinical isolates of staphylococci: An evaluation of three

different screening methods. *Ind. J. Med. Microbiol.*, 24: 25-29.

Nayak, N., Nag, T.C., Satpathy, G., Ray, S.B. 2007. Ultrastructural analysis of slime positive and slime negative *Staphylococcus epidermidis* isolates in infectious keratitis. *Indian J. Med. Res.*, 125: 767-771.

O'Gara, J.P. and H. Humphreys, 2001. *Staphylococcus epidermidis* biofilms: Importance and implications. *J. Med. Microbiol.,* 50: 582-587.

Parsek, M. and P.K. Singh, 2003. Bacterial biofilms: An emerging link to disease pathogenesis. Annu. Rev. Microbiol., 57: 677-701.

Patel, R. 2005. Biofilms and antimicrobial resistance. *Clin. Orthop. Relat. Res.*, 437: 41-47.

Patel, R. 2005. Biofilms and antimicrobial resistance. *Clin. Orthop. Relat. Res.*, 437: 41-47.

Rasha, A., Nasra, Hala, M., Abu Shady, Hussein, S. Hussein. Biofilm formation and presence of ica AD gene in clinical isolates of staphylococci.

The Staphylococci in human disease by Kent B. Crossley, M.D. and Gordon L. Archer. University of Minnesota Medical School, 1997.

Yasmeen Taj, Farhan Essa, Faisal Aziz, Shahana U. Kazmi, Dow Medical College Karachi, Karachi, Pakistan 2Immunology and Infectious Diseases Research Laboratory (IIDRL), Department of Microbiology, University of Karachi, Karachi, Pakistan.

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