Detection of biofilm among Coagulase Negative Staphylococcus (CoNS) isolated from healthy population

[1]Leo Bernard Manuel, [2]Dr. Gopinath P
[1]BDS 2nd year, [2]Assistant Professor
Department of Microbiology
Saveetha Dental College, Saveetha University, Chennai.

***Introduction*: Coagulase-negative staphylococci have recently been recognized as important causes of hospital associated infections. An important step in the development of catheter or implant associated infections caused by Coagulase-negative staphylococci are the adhesion and attachment of these bacteria to different biotic and abiotic surfaces [1].**

Among the various mechanisms involved in bacterial adhesion, the production of an extracellular polysaccharide substance namely slime which plays a crucial role in the establishment of the infections. This substance enhances the surface adhesion like cement, permitting the agglomeration of bacterial cells into biofilms or multilayers. These biofilms render the cells less approachable to the defense mechanisms, thus impairing the action of antibiotics which inturn represents survival strategies of these microorganisms. Therefore, studies and diagnostic methods identifying virulent bacterial isolates with a propensity to form slime and consequent biofilm formation, are necessary to develop effective strategies for biofilm control and improvement of patient care [1, 2, 3].

Since biofilm-producing Coagulase Negative Staphylococci are significantly involved in the occurrence of catheter-associated infections, transforming microorganisms from the normal flora to important opportunistic pathogens in immunocompromised patients, this study intended to study the biofilm forming ability of this bacteria isolated from healthy individuals.

Materials & Methods:

Collections of CoNS:

20 samples were collected from different body sites such as anterior nares of nose and fore arm using saline moistened sterile cotton swabs and were seeded onto Blood agar and Mannitol Salt agar (MSA). Isolates were characterized by standard biochemical tests and confirmed. Isolates were preserved in semi-solid trypticase soy broth stock and stored at 4°C until further use.

Antibiotic sensitivity testing:

This has been done using routinely used different antibiotics such as Penicillin, Erythromycin, Clindamycin, Ciprofloxacin, Tetracyclin, Cotrimoxazole and Linezolid by Kirby-Bauer disc diffusion method. [4]

Detection of biofilm by TCP method

Overnight grown cultures of CoNS from agar plates were inoculated in 0.5 % of glucose and incubated at 37˚C overnight. Individual wells of sterile polystyrene 96 well flat bottom tissue culture plates were filled with 200µl of culture suspension from afore mentioned broth. Uninoculated broth served as negative control. The plates were incubated at 37˚C for overnight. After incubation, content of each well was gently discarded by tapping the plates downwards. The wells were washed three times with 200 µl of PBS (pH 7.2) in order to remove planktonic bacteria. Biofilms are formed by adherent sessile isolates in plates were fixed with 2 % sodium acetate and stained with 0.1% W/V crystal violet. Excess stain was removed by washing the wells with distilled water and plates were kept for drying at an inverted position. Optical density of stained adherent bacteria was determined with an ELISA reader (BioRad) at wavelength of 570nm. These OD values were taken as index of bacteria adhering the surface and formed biofilm. Experiments were carried in triplicate and their mean was taken for the analysis.The below mentioned interpretation charge was applied to categorize the ability of CoNS to form biofilm.[5]

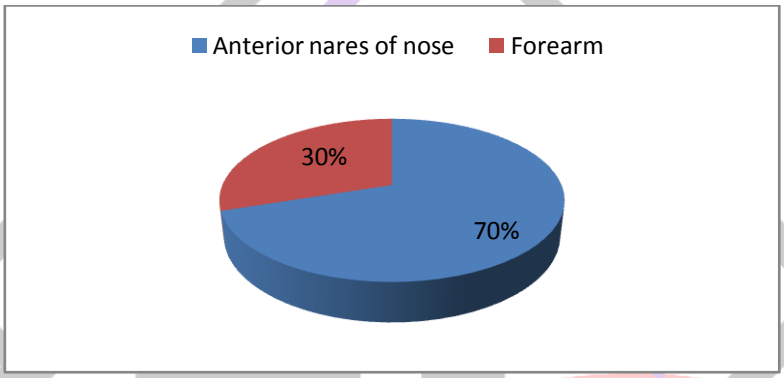
MEAN OD VALUES	ADHERENCE	BIOFILM FORMATION
<0.120	Non	Non / weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Table 1: Interpretation chart for biofilm formation by tissue culture plate method.

Results:

Sample wise distribution of Coagulase negative Staphylococcus (CoNS):

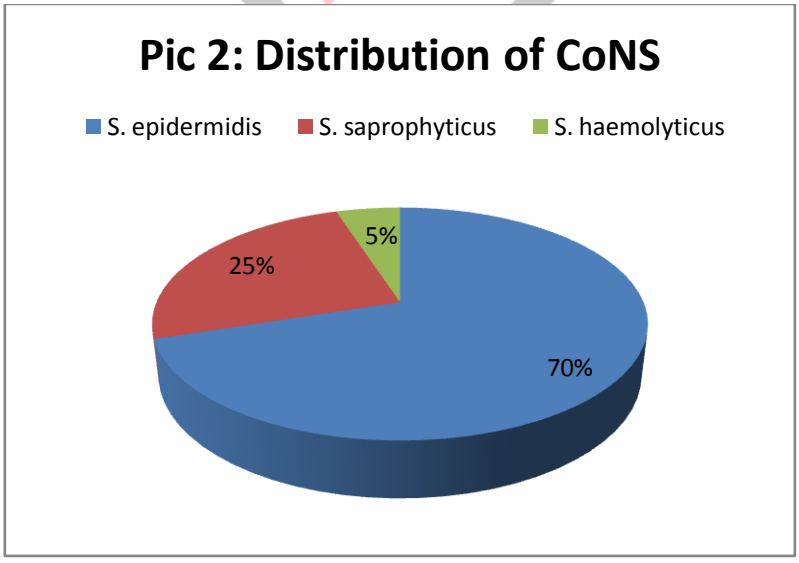
Of the 20 isolates of *CoNS*, 14/20 (70 %) isolates were from anterior nares of nose, 6/20 (30%) from fore arm region.



Pic 1: **Sample wise distribution of Coagulase negative Staphylococcus (CoNS)**

Distribution of CoNS:

Of the 20 CoNS isolates, 14/20 (70%) were found to be S. epidermidis, 5/20 (25%) and 1/20 (5%) were belong to S. saprophyticus and S. haemolyticus respectively.



Antibiotic sensitivity pattern result:

We have observed, total resistance (100%) to penicillin. For erythromycin and clindamycin, 10% of these isolates were shown to be resistant. Complete sensitivity has been demonstrated to linezolid. The detailed results of antibiotic sensitivity pattern to our isolates was shown in table 2.

ANTIBIOTICS	SENSITIVE(%)	INTERMEDIATE(%)	RESISTANT(%)
Penicillin	0	0	20 (100)
Erythromycin	16 (80)	2 (10)	2 (10)
Clindamycin	14 (70)	4 (20)	2 (10)
Ciprofloxacin	9 (45)	6 (30)	5 (25)
Tetracyclin	17 (85)	2 (10)	1 (5)
Cotrimoxazole	12 (60)	5 (25)	3 (15)
Linezolid	20 (100)	0	0

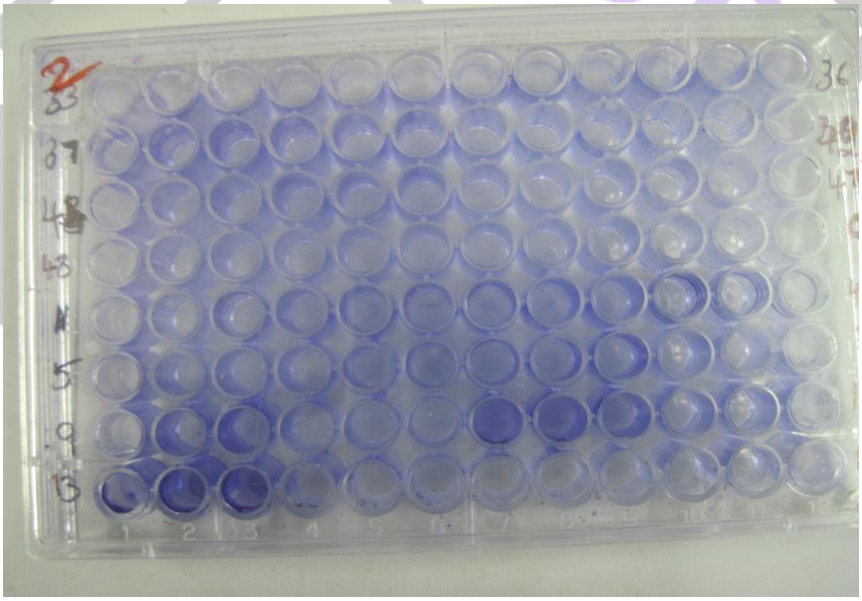
Table 2: Antibiotic sensitivity pattern to CoNS

Results of Biofilm by CoNS:

Of the 20 CoNS isolates, S. epidermidis showed comparatively more number of biofilm formers such as 3 strong and 4 moderate categories. The detailed result of biofilm ofd CoNS was shown in table 3.

CoNS isolates	Strong biofilm	Moderate biofilm	Non Biofilm
S. epidermidis (14)	3	4	7
S. saprophyticus (5)	1	0	4
S. haemolyticus (1)	0	1	0
Total (20)	4 (20%)	5 (25%)	11 (55%)

Table 3: Results of **Biofilm by CoNS using TCP method**



Pic 3: Representative picture showing biofilm by CoNS by TCP method

Discussion:

The main problem associated with infections produced by biofilm formers is multitude of resistance to different antibiotics.Study conducted by Rao et al., 2008 employed both tube and tissue culture plate (TCP) methods, showed 62% as strong producers of biofilm, 25.4% weakly adherent by tube method were additionally picked up by TCP method confirming its sensitiveness.[6]However, as adherence alone may not complete the cycle of process of biofilm production and there might be several other mechanisms which could involve in adherence.[7][8]

Study performed by Rodreguez-Ban documented 63% of biofilm positivity in their isolates, which was incomparable with our results, as our study showed 35% and 25% of moderate and strong biofilm producers respectively. Biofilm forming

isolates were less frequently resistant to carbapenem and ciprofloxacin in his study.[9] Whereas, our study showed positive association between production of biofilm and multiple antibiotic resistance, especially in some antibiotics.[10]

Conclusion:

CoNS gaining much importance in clinical settings as it frequently being reported from clinical specimens with multitude of drug resistance. Biofilm is considered to be a one of the main factor for the persistence and establishment of pathogens in biotic and abiotic surfaces, it is proven that we have also seen biofilm formers CoNS from healthy individuals. Hence, judicial usage of antibiotics and proper sanitation of clinical environment are the need of hour to control such notorious pathogen.

References:

1. ARCIOLA CR., CAMPOCCIA D., GAMBERINI S., CERVELLATI M., DONATI E., MONTANARO L. Detection of slime production by means of an optimized Congo red agar plate test based on a colourimetric scale in *Staphylococcus epidermidis* clinical isolates genotyped for *ica* locus. *Biomaterials*, 2002, 23, 4233-9.

2. AMMENDOLIA MG., DI ROSA R., MONTANARO L., ARCIOLA CR., BALDASSARRI L. Slime production and expression of the slime-associated antigen by staphylococcal clinical isolates. *J.Clin. Microbiol*., 1999, 37, 3235-8.

3. RCIOLA CR., CAMPOCCIA D., GAMBERINI S., DONATI ME., BALDASSARI L., MONTANARO L. Occurrence of *ica*genes for slime synthesis in a collection of *Staphylococcus epidermidis* strains from orthopedic prosthesis infections. *Acta Orthop. Scand.*, 2003, 74, 617-21.

4. Clinical and laboratory standard institute performance standards for antimicrobial disc susceptibility test; approved standard. Edn 10. CLSI document M 100-S18, Wayne; 2011.

5. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A, Detection of biofilm formation among the clinical isolates of Staphylococcus: an evaluation of three different screening methods. J Med Microbiol,24,2006,25-29.

6. Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S. Prashanth K, Correlation between biofilm production and multiple drug resistance imipenem resistant clinical isolates of Acinetobacter baumannii. Indian J Med Microbiol,26,2008,333-337.
7. Costerton JW, Stewart TS, Greenverg EP, Bacterial biofilms: A common cause of persistent infection. Science,284,1999,1318-22.
8. Lewis K, Riddle of biofilm resistance. Antimicrob Agents Chemother,45,2001,999-1007.
9. Rodrguez-Ban OJ, Marthi S, Soto S, Fernandes Cuenca F, Cisneros JM, Pachon J, Biofilm information in Acinetobacter baumannii: Assoicated features and clinical implications. Clinical Microbiol Infect,14,2008,276-8.

10. Karthic A, **Gopinath P.** Detection of biofilm among clinical isolates of Pseudomonas aeruginosa by tissue culture plate (TCP) method. Journal of Chemical and Pharmaceutical Sciences 2016; 9(4):3236-3238.