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Prevalence, observed in biofilm and beta-lactamase producing *Staphylococcus species* from nasal and throat isolates of hospital volunteers: A medical alert

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Abstract: **Background: The *Staphylococci* species are the predominant inhabitants of the nose and throat as normal flora, they are now becoming a threat because of its capacity to produce biofilm. Infections related to Biofilm arevery much difficult to be treated if it is not diagnosed early stages. The main aim of the present study is to to detect the prevalence of biofilm and beta-lactamase producing in Staphylococcus isolates collected from nasal and throat mucosal area in healthy volunteers.**

Methods: *Staphlococcal* isolates were collected from nasal and throat swabs of 100 hospital volunteers at Lata Mangeshkar medical College and Hospital, Nagpur. *Staphylococcus aureus* or Coagulase Negative *Staphylococcus* (CoNS) were classified on the basis of growth on Mannitol Salt Agar and the result of tube Coagulase test. Detection of Biofilm production is carried out by Christensen's tissue culture plate method and Congo red agar methods. Ability to produce Betalactamase amongst biofilm producers were further identified by iodometric tube method.

Results: Among all 100 *Staphylococcus* isolates, 41 were *Staphylococcus aureus* of which 61% were biofilm producers of which 46.3% were betalactamase positive. Among all the 59 CoNS isolates, 64.4% were biofilm producers of which following 30.5% were beta-lactamase positive. Predominating nasal *Staphylococcal* isolates were observed among the males at the age group of <20 years.
Conclusion: Biofilm-producing *Staphylococcus* inhabits the nasal and throat mucosa of healthy individuals. Beta-lactamase production was higher in *Staphylococcus aureus* positive for biofilm producers as compared to CoNS. Transmission of these biofilm producers with drug resistance factors from the healthy individuals to high risk patients with indwelling devices need to be considered.

Index Terms - **Keywords: Beta-lactamase; biofilm; coagulase negative *Staphylococcus*; *Staphylococcus aureus.***

Introduction:

Staphylococcus epidermidis **and** ***Staphylococcus aureus*** **are the predominant and persistent inhabitants in the anterior nares(Nostrils) and the throat as part of the normal flora.1-3 The nasal cavity serves as a site for multiplication of the** ***Staphylococcus species t*****o grow and remain as non-pathogens, until they disseminate through the blood stream or breached epithelial surface to other sites.4 There the growth and up regulation of adherence factors occur.5, 6 The virulence of the coagulase negative** ***Staphylococcus species*** **(CoNS) is related to its capacity to produce biofilms. Such biofilm-related infections are extremely difficult to treat and have to be diagnosed early.**

In biofilm producing ***Staphylococcus*** **Sp the major components of the extracellular polymeric substance (EPS) of consisting of poly-N-acetyl glucosamine (PNAG). Some of the strains lack PNAG and from these strains the extracellular teichoic acid was found to beas a new component of** ***Staphylococcal*** **biofilm.7**

Formation of biofilm in ***Staphylococcus*** **is suggested that it is a four step process, consisting of the following stages like adherence, accumulation, maturation and later dispersal.7 Biofilm is an important colonization factor as well as a virulence factor in bacterial adherence.4,8-12 Colonization occurs in the principle implants like central venous catheters, heart valves, ventricular assist devices, coronary stents, neurosurgical ventricular shunts, implantable neurological stimulators, fracture-fixation devices, arthro-prostheses, breast implants, cochlear implants, intra ocular lenses and dental implants.13-16**

The objective of this study was to determine the presence of biofilm formation and beta-lactamase production in ***Staphylococcus aureus*** **and CoNS inhabiting as the normal flora in the nostrils and throat of normal healthy individuals belonging to a Medical University.**

Methods:

The study was conducted among 100 healthy volunteers in Lata Mangeshker Medical College & Hospital . The nasal and throat swabs were aseptically collected and processed for Gram staining and isolation on Blood agar, Mannitol salt agar and Mac Conkey agar. The ***Staphylococcus*** **isolates were then categorized as** ***Staphylococcus aureus*** **and CoNS based on their different cultural and biochemical characteristics and standard tube method for coagulase test.17 The biofilm qualitative detection was done by two methods: Christensen's method and Congo Red Agar (CRA) method. In Christensen's method, a few colonies of the test organism were inoculated in 200µl trypticase soy broth with 1% glucose in triplicate into flat bottom polystyrene tissue culture plate wells and incubated for 24 and 48 hours at 37° C aerobically.**

The given sample contents were gently aspirated into the container aseptically and the wells were washed number of times with saline phosphate buffer with a pH of 7.2 to remove the free floating bacteria. The biofilm formed in the microwells were stained with 1% neutral red. The biofilm positive bacteria stained pink at the bottom and on the walls of the tissue culture plate wells [18]. In Congo Red Agar method, as described by Freeman et al. the Congo Red Agar was prepared with brain heart infusion broth, sucrose, agar, and Congo redis added as an indicator. Sterile Congo red stain was prepared as a concentrated aqueous solution. It was added to the sterilized brain heart infusion agar with sucrose at 55° C. The Conge Red agar plates were inoculated by the streak method with the test organisms and incubated at 37° C for 24 hours and after checking incubation was continued for another 24 hours aerobically.

In the above cultures the organisms positive for biofilm production showed black colored dry colonies with crystalline consistency and biofilm negatives colonies produced pink colored smooth surface colonies.19-21 Further, these test isolates were screened for beta-lactamase production by the Iodometric tube method (ITM).The organisms screened positive for beta-lactamase were further proceed for antibiotic susceptibility testing by the standard Kirby Bauer disc diffusion method.22

Results:

The male to female ratio among the 100 healthy volunteers was 54:46. The gender participation in various age groups is shown in the Figure1. The age distribution was between 18 and 60 years. The majority of the participants (85%) were below 30 years of age.

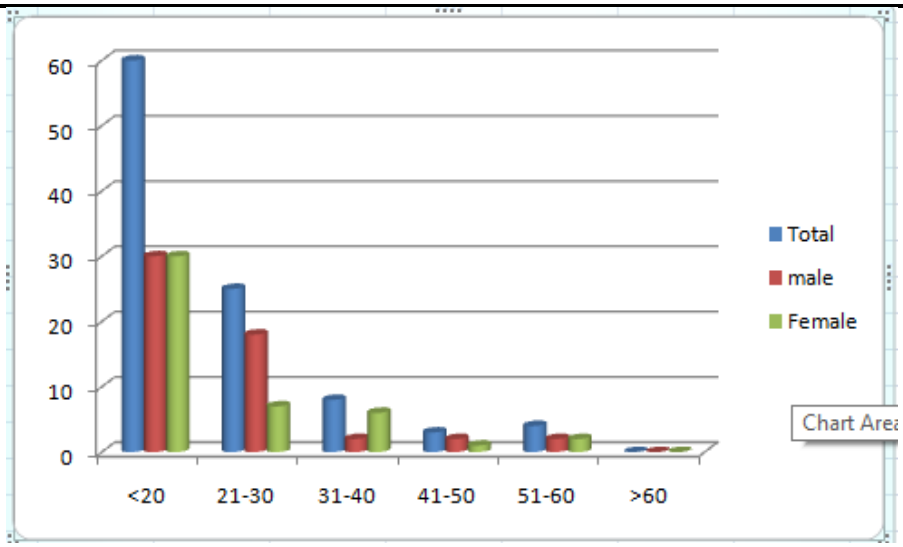

Figure 1. Gender participation ratios with age

As total 100 nasal swabs were collected 63 isolated *Staphylococcus* species, from which24 were Gram positive bacilli (Diphtheroids) and 13 swabs showed no growth. Among 100 throat swabs 37 isolated *Staphylococcus* species, 2 Gram negative bacilli (*Klebsiella pneumonia* & *Enterobacter* sp.), Total 41 Gram negative cocci in groups (non- pathogenic *Neisseria* Spp) and again 20 swabs showed no growth. *Staphylococcal* isolation was 37% from the throat and higher in the nasal site (63%) of which 21% were *Staphylococcus aureus*. Isolation rate was higher in the age group below 20 years of age (58%), followed by the 21-30 years (26%). In the remaining age groups isolation was minimal.

As shown in Table 1, In the present study, irrespective of age and gender, the CoNS isolates were significantly observed higher than *Staphylococcus aureus* in nasal and throat specimen (3:2). The number of *Staphylococcus* isolates was common among the younger age group (< 20 years).

Table 1.Distribution of *Staphylococcus* isolates depending upon age and gender of hospital volunteers

	S.aureus	CoNS	*S.aureus*	CoNS	Total
	No.	No.	No.	No.	No.
<20	13	20	10	15	58
21-30	08	06	02	10	26
31-40	02	01	02	04	08
41-50	01	01	01	00	04
51-60	0	01	01	01	03
>60	01	00	00	00	01
Total	25	29	16	30	100
%	46.2%	53.7%	34.7%	65.2%	

Table 2. Results of Biofilm and Beta-lactamase production in *Staphylococcus* isolates in present study.

Methods	Congo Red Agar Method (CRA)		Tissue culture Plate Method (TCP)		Tube Iodometric Method (ITM)	
	Biofilm(+)		Biofilm(+)		Betalactamase (+)	
	No.	%	No.	%	No.	%
Staphylococcus aureus	25	61	25	61	32	78
CoNS	34	57.6	38	64.4	22	37.2

The presence of the biofilm formation was tested by two methods CRA and TCP methods and Beta-lactamase by ITM. The detection biofilm by both the methods were alike in *Staphylococcus aureus* (60.9%),whereas more positivity (64.4%) were obtained by the TCP method compared to the CRA method (57.6%) in the CoNS. Betalactamase was observed positive for *Staphylococcus aureus* (78%) and positive for CoNS (37.2%)as shown in the Table 2.

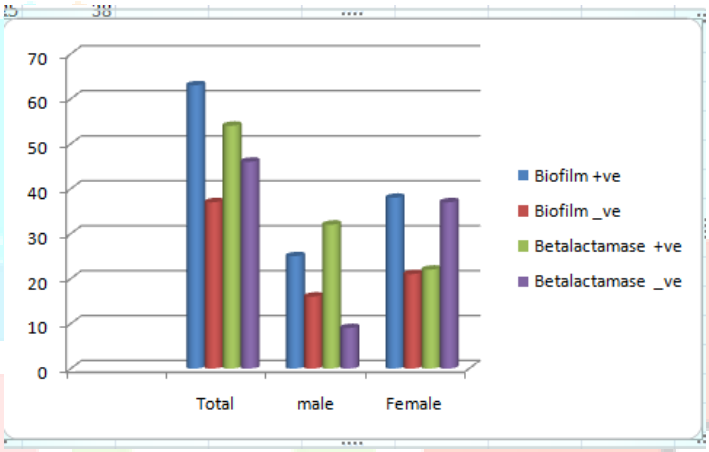

Figure 2. Biofilm production by Tissue Culture Plate method and Beta-lactamase production by Tube Iodometric method in *Staphylococcus* isolates.

Figure 2 shows the number of positive and the negative results for biofilm production by both the tissue culture plate method and beta-lactamase production by Tube iodometric method among the *Staphylococcus aureus* and CoNS. From the total 100 *Staphylococcus* isolates 37 were screened negative for biofilm production and 46 were negative for betalactamase production. The beta-lactamase positive isolates were resistant to Penicillin and Ampicillin and susceptible to Vancomycin, Oxacillin and Rifampicin.

Discussion: Biofilm formation in bacteria is now a major problem in the medical field since it is responsible for many recalcitrant infections and also difficult to be eradicated. It contributes to virulence factors like the ability to avoid host immune response, restricted penetration of antimicrobial agents into the biofilm and exhibition of resistance to antibiotics due to various mechanisms including beta-lactamase production.

***Staphylococcal* isolation was high in the nasal site among the Japanese population, as reported by Tadayukiin.23 Our results were comparable, with increased *Staphylococcus* isolation (62%) from the nasal site. Karina et al. conducted a similar study among the medical students in Brazil and observed a percentage of nasal *Staphylococcus aureus* isolation of 40.8%.24 In our study the nasal *Staphylococcus aureus* isolation was 21%. Samie et al. conducted a similar study on biofilm and beta-lactamase detection using similar methods and detected 42 % were biofilm producers among which 16% were beta- lactamase positive.25**

In our study, 63% were biofilm producers among which 37%were betalactamase positive. In your present study the detected 63% of biofilm producing *Staphylococcus* isolates, which was similar to a study done in Pakistan (54.8%) by Joanna et al.26 The resemblance in the present results may be

due to similar culture, living conditions and geographical location. Here both the conventional methods for biofilm production and detection (CRA and TCP) provides similar results in *Staphylococcus aureus* isolated, whereas the positive in CoNS was slightly higher by the TCP method, agreed with the earlier studies.27-28 However, Ruzicka et al. had gone through the genetic studies detecting ica operon responsible for the biofilm production and compared with the similar conventional methods adopted in this study.28

The present results had shown higher correlation with Christensen's method than with the CRA method.27 Our findings differ from the earlier reports where the authors had suggested the CRA method to be superior to TCP.22,30,31. Colonization of biofilm forming CoNS is the current threat to effective antibiotic therapy given because of the increasing difficulty in detection and management of infections, leading to fatal outcomes. All the beta-lactamase producers were resistant for Penicillin and Ampicillin but showed 100% sensitivity to Vancomycin corresponding with the earlier reports.24 There is a possibility of transmission of these virulence factors from the healthy individuals to those at high risk such as patients on long term catheterization, or having indwelling devices in a medical set up, which may be difficult to treat with the commonly available antimicrobial agents.

Conclusion: Biofilm detection is more reliable by the Tissue culture plate method than with the Congo Red Agar method. Also, the virulence factors, biofilm production and beta-lactamase production, seem to be present in the *Staphylococcus* isolates which normally inhabit the upper respiratory tract. Since the biofilm and beta-lactamase virulence factors seem to be present in the normal flora of healthy individuals there is a need to screen for them among the healthy individuals who are posted at high risk units where medical device implantation, catheterization etc. are commonly carried out.

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