Original article:

Antimicrobial Resistance In Biofilm Producing Staphylococci Isolated From Clinical Specimens

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Abstract:

Introduction: Biofilm formation is one of the virulence markers in staphylococci which has been attributed to its pathogenicity and antimicrobial resistance. A study was undertaken to find out the antimicrobial resistance in biofilm producing staphylococci and compare the same against non-biofilm producing isolates.

Material & Methods: A total of 378 staphylococcal isolates recovered from various clinical specimens submitted to bacteriology department during a period of one year were included. All isolates were speciated by standard methods and antimicrobial susceptibility testing was performed by Kirby Bauer's disk diffusion method. Biofilm production was determined using Freeman's Congo red Agar method and Christensen's tube method. Isolates positive by both methods were considered as biofilm producers.

Results: Out of total 378 isolates, 148 were *S.aureus* and 230 were Coagulase negative staphylococci (CoNS). Biofilm formation was observed in 79 of the *S.aureus* isolates and 105 of the CoNS. Penicillin resistance was observed in 87% and 83% amongst biofilm producing as compared to 19% and 25% amongst non-biofilm producing *S. aureus* and CoNS respectively. Similarly methicillin resistance was 60% and 61% in biofilm producing as against 7% and 4% of non-biofilm producing *S. aureus* and CoNS respectively.

Conclusion: Biofilm production in staphylococcal isolate indicates probable antimicrobial resistance as has been observed in our study. Incorporating simple test like growth on Congo red agar for detection of biofilm, in routine staphylococcal identification, may provide useful information to clinicians and may aid in selecting the antimicrobials for therapy.

Keywords: Biofilm, Staphylococci, Antimicrobial resistance

Introduction:

Staphylococci are the most common cause of localized suppurative lesions and continue to be one of the most important pathogens isolated in the clinical microbiology laboratory. [1] Biofilm production is one of the virulence trait in staphylococci which has been attributed to its pathogenicity and antimicrobial resistance. Biofilm makes organisms more resistant to both, administered antibiotics and host defense mechanisms and acts as a diffusion barrier for most antimicrobial agents. The bacterial population within biofilm remains in dormant stages thereby protecting them against antibiotics which act upon actively multiplying bacteria.[2,3,4]

The differentiation of staphylococci with respect to biofilm phenotype might provide a clue about probable resistance pattern and thus help clinicians in deciding upon the antimicrobial selection, considering the high preponderance of Methicillin Resistant *Staphylococcus aureus* (MRSA) & Methicillin Resistant Coagulase Negative Staphylococci

(MRCoNS) in hospital acquired infections. In view of this, a study was undertaken to characterize staphylococcal isolates phenotypically to species level, determine biofilm production amongst them and to study antimicrobial susceptibility pattern and its correlation with biofilm production.

Material and methods:

Staphylococci isolated from various clinical specimens submitted to the laboratory for bacterial culture during a period of one year were included in the study. A total of 378 staphylococci were studied. All coagulase positive staphylococci grown from these specimens were included in the study. Coagulase negative staphylococci grown as a sole organism along with suggestive microscopy were also included. The strains were speciated by standard bacteriological methods. All isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method using commercially available discs from HiMedia, Mumbai and BD diagnostics Ltd., Oxford, UK. Biofilm production was determined using Congo red Agar method described by Freeman[5] et al 1989 and tube method described by Christensen[6] et al 1982.

Results and observations

Total number of isolates of staphylococci studied = 378

(*S. aureus* – 148, CoNS – 230)

Table 1. Biofilm production amongst staphylococci (n= 378).

Biofilm production	***S. aureus*** **n= 148**	**CoNS** **n= 230**	**Total** **n= 378**
Congo red positive, tube test positive	79 (53.37%)	105 (45.65%)	184 (48.68%)
Congo red negative, tube test negative	54 (36.48%)	105 (45.65%)	159 (42.06%)
Congo red negative, tube test positive	10 (6.75%)	13 (5.65%)	23 (6.08%)
Congo red positive, tube test negative	5 (3.4%)	7 (3.05%)	12 (3.18%)

Table No 1 shows that 79 out of 148 *S. aureus* (53.37%) and 105 out of 230 CoNS (45.65%) were biofilm producers by both tube as well as congo red agar method, thus overall biofilm producing strains were 184 (48.68%).Non-biofilm producing strains amongst *S. aureus* were 54 (36.48%) and 105 (45.65%) amongst CoNS. Fifteen isolates of *S. aureus* and 20 isolates of 13 CoNS gave discordant results between congo red and tube test. These isolates with discordant results were not included in any of the group (biofilm producer or non biofilm producer) for data analysis.

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Table 2 . Biofilm production amongst coagulase negative staphylococci (congored and tube method positive) (n=230)

Species	Total isolates	Biofilm producers	Percentage
S. epidermidis	81	36	44.45%
S. hemolyticus	34	15	44.18%
S. saprophyticus	19	5	26.31%
S.auricularis	15	5	33.34%
S. caprae	13	10	76.92%
S. hominis	11	9	81.82%
S. xylosus	11	4	36.36%
S. warneri	8	5	62.5%
S. intermedius	8	3	37.5%
S. simulans	6	2	33.34%
S. schleiferi	5	4	80%
S. capitis	4	2	50%
S. hyicus	3	2	66.67%
S. cohnii	3	1	33.34%
S. sciuri	2	1	50%
S. gallinarum	1	1	100%
S. chromogenes	2	0	0 %
S. carnosus	2	0	0 %
S. lugdunensis	1	0	0 %
S. lentus	1	0	0%
Total	**230**	**105**	**45.65%**

Out of 230 CoNS, 105 were biofilm producers. Amongst these, majority were *S. epidermidis* & *S. hemolyticus.*

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Table 3. Comparison of antimicrobial resistance amongst biofilm producing and non biofilm producing staphylococci.

Antibiotic	*S. aureus*		CoNS	
	Biofilm producing (n=79)	**Non Biofilm producing (n=54)**	**Biofilm producing (n=105)**	**Non Biofilm producing (n=105)**
Penicillin	69 (87.34%)	10 (18.5%)	87 (82.85%)	26 (24.76%)
Cefoxitin*	47 (59.5%)	4 (7.4%)	64 (60.95%)	4 (3.8%)
Cotrimoxazole	63 (79.75%)	27 (50%)	85 (80.95%)	53 (50.47%)
Erythromycin	63 (79.75%)	19 (35.18%)	85 (80.95)	29 (27.61%)
Clindamycin	24 (30.38%)	5 (9.25%)	43 (40.95%)	6 (5.71%)
Vancomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Linezolid	9 (11.4%)	0 (0%)	6 (5.71%)	0 (0%)
Urinary isolates (n=66)	**n=10**	**n=10**	**n=19**	**n=21**
Nitrofurantoin	0 (0%)	1 (10%)	4 (21.0%)	0 (0%)
Norfloxacin	10 (100%)	6 (60%)	18 (94.74%)	15 (71.42%)

*Surrogate marker for oxacillin

Penicillin resistance was observed in 87% and 83% amongst biofilm producing as compared to 19% and 25% amongst non-biofilm producing *S. aureus* and CoNS respectively. Similarly methicillin resistance was 60% and 61% in biofilm producing as against 7% and 4% of non-biofilm producing *S. aureus* and CoNS respectively. In present study, 59% of all staphylococcal isolates showed resistance to macrolides & lincosamides group of antibiotics. (*S. aureus* – 64%, CoNS – 44%), by one or the other mechanism. i.e. inducible or constitutive. In the present study, none of the staphylococcal strains, including biofilm producing ones, were resistant to vancomycin. None of the non-biofilm producing staphylococci showed resistance to Linezolid. However, it was observed in 11% of biofilm producing *S.aureus* & 6% of CoNS.

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Discussion:

Staphylococci continue to be a pathogen of major importance, evolving new pathogenic capabilities. The coagulase negative staphylococci, often considered as commensals or contaminants, have been increasingly recognized to cause serious infections. However the clinical significance of various species remains to be defined.[7] The virulence of CoNS is probably multifactorial. Strains associated with disease produce a wider range of extracellular toxins and enzymes than commensal strains. Biofilm is considered as an important risk factor in CONS infections. Many studies have shown that the slime producing capacity of an infecting strain correlates well with the clinical outcome of infection.[8] Biofilm is believed to facilitate the establishment of CoNS as a pathogen in various infections and is associated with multi drug resistance.[9] Detection of biofilm production in a CoNS has a positive predictive value of above 80% in predicting its pathogenic role, especially in bacteremia.[10] Congo red method and Christensen's tube method are simple, cost-effective, rapid, sensitive and reproducible methods of detecting biofilm production.[5,10] Incorporation of these tests in the standard set of tests for CoNS in the laboratory would be helpful to decide; if probing ahead to identify species and perform antimicrobial susceptibility testing is worth? Moreover, a significantly higher percentage of antimicrobial resistances in a biofilm producing staphylococci warrants a special attention and emphasizes such a need.

Table number 3 shows comparison of antimicrobial resistance pattern amongst biofilm producing and non biofilm producing staphylococci. It is evident from this table that antimicrobial resistance is significantly greater amongst all biofilm producing strains than non biofilm producing strains for all antimicrobials.

Biofilm is believed to make microorganisms more resistant to both, administered antibiotics and host defense mechanisms.[4] The mechanism of biofilm resistance is multifactorial and includes impaired penetration, reduced growth rate and a distinct phenotype exhibited by biofilm producing bacteria including expression of resistant genes. The biofilm environment promotes genetic exchange of antimicrobial resistant genes, increasing bacterial virulence and contributing to the development of multi resistant phenotypes.

Betalactams are the mainstay in the therapy of staphylococcal infections. However, increased resistance to these antimicrobials in biofilm producing staphylococci may lead to treatment failures. The percentages of penicillin & methicillin resistance in staphylococci is clearly higher in the biofilm producing isolates than in non biofilm producing isolates. The Macrolides, Lincosamides and Streptogramin B (MLS_B) family of antibiotics serves as an alternative to treat staphylococcal infections in penicillin allergic patients in MRSA especially skin and soft tissue infection.[11]However, widespread use of MLS_B antibiotics has lead to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics by way of both inducible as well as constitutive resistance mechanisms. Moreover, biofilm producing staphylococci are more likely to be resistant to this group of antibiotics than their non biofilm producing counterparts as has been observed in our study.

The glycopeptide vancomycin has been regarded and remains as the drug of choice for the treatment of infections due to methicillin resistant staphylococci.[12]In the present study, none of the staphylococcal strains, including biofilm producing ones, were resistant to vancomycin. This suggests that vancomycin can still be considered a drug of choice for MRSA and MR-CoNS infections. It might also be an alternative in infections due to biofilm producing strains of staphylococci. However, Vancomycin resistance in staphylococcal species has been reported in some studies including India.[12,13,14,15,16] *Enterococcus faecium* is intrinsically resistant to vancomycin. This organism is said to have offered drug resistance gene to staphylococci. Considering this transferability of vancomycin resistance the clinical fraternity must be alarmed against indiscriminate or injudicious use of this

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precious antibiotic.[13,16] Considering the ease of administration and low cost of linezolid compared to vancomycin, it may serve as an alternative to vancomycin in MRSA and MR-CoNS infections.

Thus it is conclusive that biofilm producing organisms are more drug resistant as compared to the non biofilm producing isolates. The resistance of microbes residing in the biofilm towards various types of antimicrobial agents poses a serious threat not only to the infected patient and hospitals but also to the pharmaceutical industries.[17]These facts advocate inclusion of test for biofilm detection in routine laboratory protocols for staphylococci. The results will certainly aid the clinicians in deciding upon antimicrobial therapy in staphylococcal infections.

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