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Original Research Article

To determine antimicrobial susceptibility and biofilm production among coagulase negative staphylococci at a tertiary care hospital

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ABSTRACT

Introduction: CoNS are gaining importance due to increase in resistance rates to betalactam antibiotics and multi drug resistance. Although specific virulence factors are not as clearly established, it seems clear that factors such as bacterial polysaccharide components, and ability to form biofilm are involved in attachment and/or persistence of bacteria on foreign materials. Biofilms usually result in persistent infections that cannot be easily resolved with standard antibiotic treatments; therefore, the biofilm formation ability and the resistance to antimicrobial therapy can be intimately related.

Materials and Methods: A prospective cross-sectional study was done on purely isolated CoNS from various clinical samples from both out patients and inpatients. All the test strains were subjected to antimicrobial susceptibility testing. The ability to produce biofilm was detected by tube adherence method.

Results: Among 193 CoNS isolates 156 were from inpatients and 37 were from out patients. Methicillin resistant was seen in 80.31%. Of the total, 40.41% showed moderate biofilm formation by tube adherence method. 23.32% of isolates did not form biofilm. All the isolates from blood samples showed moderate (20/26) and strong (6/26) biofilm formation. Among non biofilm producers 66.67% were MS CoNS isolates and 33.33% were MRCoNS. 94.59% of biofilm producers were MRCoNS and 5.41% were MSCoNS. Production of biofilm was relatively more (1.16) among CoNS isolates of IPD than OPD.

Conclusions: As Coagulase negative Staphylococci are exhibiting multi drug resistance and are able to form biofilm, these organisms causing a major challenge for the physicians. Hence, such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification. The issue of antibiotic resistance among CoNS needs to be addressed through a more rational use of existing antibiotics as well as the development of new antimicrobial agents.

1. Introduction

Coagulase Negative staphylococci are the indigenous flora of the human skin and mucous membrane, but as per National Nosocomial Infections Surveillance System (NNIS) during the late 1980s and early 1990s that CoNS are among the five most commonly reported pathogens.[1] Due to increase in resistance rates to betalactam antibiotics and multi drug resistance CoNS are gaining importance. Although specific virulence factors are not as clearly established, it seems clear that factors such as bacterial polysaccharide components, and ability to form biofilm are involved in attachment and/or persistence of bacteria on foreign materials.[2,3] Biofilms are defined as structured communities of microorganisms embedded in a self-produced matrix of extracellular polymeric components (e.g., polysaccharides, proteins, lipids and nucleic acids).[2] Biofilm provide survival advantages to the organism by making the cells less accessible to the defence system of the host and also by impairing the action of antibiotics.[3]

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The ability to form biofilms appears to play an essential role in staphylococcal virulence.[4] Biofilms usually result in persistent infections that cannot be easily resolved with standard antibiotic treatments,[5] due to slow diffusion of conventional antibiotics through the extracellular polymeric substance[6] and often leads to removal of the foreign body for cure.[5] Therefore, the biofilm formation ability and the resistance to antimicrobial therapy can be intimately related.[2] There by these posing a major challenge for the physicians as these isolates can result in untreatable conditions[7] along with economic relevance as well. Such untreatable conditions can be minimised by detection of biofilm producers and appropriate antibiotic doses modification. Hence the present study was done to demonstrate the ability of CoNS to produce biofilm, along with their antimicrobial susceptibility pattern from various clinical samples.

2. Aims and Objectives

1. To know the frequency of CoNS isolates from various clinical samples
2. To know the antimicrobial susceptibility of CoNS
3. To determine the ability to form biofilm by CoNS
4. To determine the relative risk of CoNS from IPD to form biofilm.

3. Materials and Methods

A prospective cross sectional study was done at Department of Microbiology, Government Medical College and Government general Hospital, Kadapa from April 2019 to March 2020. All purely isolated CoNS from various clinical samples from both out patients and inpatients like urine, pus swabs, exudates, sputum, blood etc based on conventional methods. All the test strains were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method. Methicillin resistance was tested with Oxacillin and presence of mecA gene was tested cefoxitin Antimicrobial susceptibility was read by following CLSI guidelines.[8]

The ability to produce biofilm was detected by tube adherence method.

Tube adherence method: The obtained bacterial pure isolates (loopful of bacteria) were inoculated into Trypticase soy broth supplemented with 1% glucose (TSBglu) and incubated for 24 hours at 37°C. Tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed, and tubes were washed with deionized water and the experiment was done in triplicate manner. The controls for strong biofilm production and no biofilm production were S. epidermidis ATCC 35984 and S. epidermidis ATCC 12228 respectively.

Tubes were then dried in an inverted position and observed for biofilm formation. Biofilm formation were considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Based on the intensity of the color formed, tubes were examined and the amount of biofilm was scored as 0-absent, 1- weak, 2-moderate or 3-strong. All the tests were done as per standard operative procedures.[9]

4. Results

Among 193 CoNS isolates 156 were from inpatients and 37 were from out patients. Most of the isolates were from pus swabs (79) followed by sputum samples (53)as shown in Table 1.

Table 1: Distribution of CoNS among OPD & IPD

Sample	OPD	IPD	Total
Urine	5	30	35(18.13%)
Sputum	17	36	53(27.46%)
Blood	-	26	26(13.47%)
Pus swabs	15	64	79(40.93%)
Total	37(19.17%)	156(80.83%)	193(100%)

All the test strains were resistant to penicillin. Methicillin resistant was seen in 80.31% and mec A gene was present in 65.81% of the isolates. Majority of the strains were sensitive to ceftriaxone (80.83%), cefepime (80.31%), vancomycin (84.97%), cefaperazone – sulbactum (97.93%), piperacillin-tazobactum (99.48%). Only one CoNS strain isolated from blood sample showed resistant to piperacillin – tazobactum as shown in Table 2.

Of the total 193 isolates of CoNS, 40.41% showed moderate biofilm formation by tube adherence method. 23.32% of isolates did not form biofilm. All the isolates from blood samples showed moderate (20/26) and strong (6/26) biofilm formation as shown in Table 3.

Among non biofilm producers 66.67% were MS CoNS isolates and 33.33% were MRCoNS. 94.59% of biofilm producers were MRCoNS and 5.41% were MSCoNS as shown in Table 4.

Production of biofilm was relatively more (1.16) among CoNS isolates of IPD than OPD as shown in Table 5.

5. Discussion

As CoNS are part of the microbial flora of the skin and mucous membranes, it is necessary to differentiate between clinically significant and contaminant bacteria in etiology of suspected infections.[10] The present study was done on 193 CoNS strains which were isolated purely from pus swabs (40.93%), sputum (27.46%), urine (18.13%) and blood (13.47%) samples. It was comparable with a study by Radhika et al[1] (pus- 41.35%). Though isolates

Table 2: Antimicrobial susceptibility of isolated CoNS

Antimicrobial	Pen		Ox		Cfx		Van		Ctr		CfS		PIT		Le		Cot		Cpm	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Urine	-	35	3	32	17	18	35	0	28	7	35	-	35	-	24	11	20	15	33	2
Sputum	-	53	9	44	12	41	38	15	44	9	53	-	53	-	39	14	47	6	52	1
Blood	-	26	1	25	6	20	21	5	20	6	24	2	25	1	18	8	6	20	10	16
Pus swabs	-	79	25	54	31	48	70	9	64	15	77	2	79	-	55	44	28	51	60	19
Percentage	0	100	19.69	80.31	34.19	65.81	84.97	15.03	80.83	19.17	97.93	2.07	99.48	0.52	70.47	29.53	52.33	47.66	80.31	19.69

Table 3: Categories of Biofilm formation by test strains

	No biofilm	Weak	Moderate	Strong	Total
Urine	12	10	8	5	35
Sputum	20	14	10	9	53
Blood	-	-	20	6	26
Pus swabs	13	14	40	12	79
Total	45(23.32%)	38(19.68%)	78(40.41%)	32(16.58%)	193

Table 4: Comparison between biofilm producers andnon biofilm producers with methicillin susceptibility

	Non biofilm producers	Biofilm producers	Total
Methicillin resistant(MR)	15(33.33%)	140(94.59%)	155
Methicillin sensitive (MS)	30(66.67%)	8(5.41%)	38
	45	148	193

Table 5: Comparison of CoNS from IPD & OPD with biofilm formation

CoNS isolates from	No. of Biofilm producers	No. of Biofilm non producers	Total
IPD	151(a)	5(b)	156(a+b)
OPD	31(c)	6(d)	37(c+d)
Total	182 (a+c)	11(b+d)	193(a+b+c+d)

Relative risk: a/a+b÷c/c+d = 151/156 ÷31/37 = 1.16

of CoNS were more from pus samples (49/96) in a study by Tilakavarthy et al[11] the percentage (51.04%) was high when compare to our study. It was also observed in our study that more isolates were from inpatients than out patients, which represents hospital stay and medical interventions can precipitate colonization of CoNS. CoNS gaining much importance in clinical settings as it frequently being reported from clinical specimens with multitude of drug resistance. Therapeutic options for the treatment of CoNS are limited because the vast majority of clinically recovered isolates are methicillin resistant.[12] In our present study all the isolates were penicillin resisitant (100%). The same was observed in a study by Hasanvand et al[5] but it was 96.1% in Sowmya et al[3]99.3% of CoNS, isolated from hospital environment showed resistant to penicillin in a study by rathanin et al.[13] Methicillin resistant was 80.31% in present study and it was comparatively less in studies by Sowmya et al[7] (70%), Radhika et al[1](60.71%) and Shrestha et al[14] (58%). vancomycin susceptibility in present study was less (84.97%) when compare to Radhika et al[1] (100%), Hasanvand et al[5] (100%), Shrestha et al[14] (100%), and Sowmya et al[7] (93%). Very less percentage of resistant was observed with piperacillin – tazobactum (0.52%) and ceftriaxone – sulbactum (2.07%) in present study, explained that resistant to beta lactam drugs could be overcome by administration drugs along with beta lactamase inhibitors. This could be helpful to the clinician in treating patients by choosing empirical antimicrobials correctly. Along with exhibiting multi drug resistance CoNS are known to have the ability to form polysaccharide intercellular adhesin and chemically diverse biofilm,[3] which is formed by a four-step process involving attachment, accumulation, maturation, and detachment,[9] that's why today CONS represents one of the major pathogens among immune compromised and hospitalized individuals, with a considerable impact on morbidity and mortality[1] a also posing a major challenge for the physicians along with economic relevance as well.[7] Such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification.

As test tube method is most suitable and reproducible method for detecting such strains[7]it was adopted in our present study. 76.68% of CoNS isolates showed ability to form biofilm in our present study. It was more in studies by Sowmya et al[3] (87.5%), Rathanin et al[13] (81.9%) and less in studies by Fernando et al2 (57%), Bose et al[15] (45%), Fathima et al[16] (63.74%), Saumya et al3 (50%), Szcuska

et al[4] (64%), Shrestha et al 14 (65.38%), Bernard et al[17] (45%) and observed very less in studies by Radhika et al1(23.63%), Tilakavarthy et al[11] (30.2%), Shareori et al[18] (36.3%). It was also observed in our present study that more isolates were in category of moderate biofilm producers (40.41%), where as it was observed differently in a study by Fernando et al[2] that weak biofilm producers were more (34%) than moderate (10%) and strong (13%). The present study was also observed that all isolates from blood samples were moderate to strong biofilm producers. Hence pure isolation of CoNS from blood samples should be considered as pathogen rather than considering either contaminant or commensal. Many studies including our present study observed that antimicrobial resistance was high among biofilm producers than non biofilm producers. In our present study it was observed that biofilm production was high in methicillin resistance (94.59%) than methicillin susceptible CoNS (5.41%) but it was 60.71% and 39.29% respectively in a study by Radhika et al.[1] The increased antibiotics resistance of biofilm producing strains might be due to their slow rate of metabolism and infrequent division resulting in decreased sensitivity to antibiotics targeted at cellular functions such as protein and DNA synthesis.[3] As there is a chance of persistent infections with organisms exhibiting biofilm production and multi drug resistance, these can land in untreatable conditions[7] and also creates a serious problem on public health.[6] The predominance of CoNS isolates in exhibiting (multi)resistance to antibiotics and antiseptics, as well as their capacity for biofilm production, is strongly indicative of selection processes facilitated by modern medicine, i.e., mainly from (over)use of antibiotics and insertion of foreign body devices.[12] In the present study it was observed that the relative risk of biofilm production was high (1.16) among CoNS isolated from inpatients(151/182) than out patients (31/182).

Coagulase Negative Staphylococci are now being considered as emerging multidrug resistant pathogens, hence, studies on their distribution, antibiotic sensitivity, and biofilm production are very important. The CoNS isolates of current study exhibited multiple antibiotic resistance similar to the other global reports. Studies on the prevalence of biofilm production and drug resistance can help to understand their role and interaction with each other and are necessary to identify new targets to develop therapeutic approaches. Further studies are needed to define the roles of the different components of undetermined biofilms and their regulation. Resistance to vancomycin can have serious impact because of the possibility to spread this to other bacterial strains. Thus, proper strategies should be adopted for the control and prevention of infections and this requires close monitoring and periodic inspections of these potential multidrug resistant pathogens.

6. Conclusions

As Coagulase negative Staphylocooci are exhibiting multi drug resistance and are able to form biofilm, these organisms causing a major challenge for the physicians along with economic relevance as well. Hence, such problems can be prevented by detection of biofilm producers and appropriate antibiotic doses modification. Therefore, test tube method can be adopted as most suitable and reproducible method for detecting such strains. The issue of antibiotic resistance among CoNS needs to be addressed through a more rational use of existing antibiotics as well as the development of new antimicrobial agents. Adherence to proper disinfection measures in hospital environment is also need to control such organisms.

7. Conflicts of Interest

Nil.

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