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DETECTION OF BIOFILM PRODUCING COGULASE NEGATIVE STAPHYLOCOCCI AND THEIR ANTIBIOGRAM IN TERTIARY CARE HOSPITAL KOTA, SOUTH-EAST RAJASTHAN, INDIA

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ABSTRACT

Background: Over the last decades, Coagulase negative staphylococci (CoNS) have been accepted as major opportunistic pathogens of low virulence causing hospital-acquired infections (HAI). Predominantly cause infections in immune compromised patients or otherwise healthy individuals with breached skin and mucous barriers. **Objectives:** This study was conducted to isolate the CoNS in different clinical samples for detection of Biofilm formation by using three different screening methods and their AST pattern to know the pathogenicity and their drug resistant pattern in South-East Rajasthan. The ultimate goal of this study was to identify CoNS infection in early phase of disease and prevent future complications. **Methodology:** The present study was conducted in the central laboratory of Govt. Medical College, Kota from period of 1st January 2022 to 31th December 2022 to evaluate the Biofilm producing CoNS. 100 consecutive CoNS isolated from various clinical samples by the VITEK 2 system and conventional method were processed for the study and culture. AST of isolates was conducted using the Kirby-Bauer disk diffusion method against a panel of 17 antibiotics by using CLSI guidelines. The biofilm forming ability of all isolates was determined by three phenotypic methods; Congo red agar (CRA) method, Tube adherence method (TA) and Tissue culture plate (TCP) method. **Results:** Out of 100 CoNS isolates, Male: female ratio for CoNS was 1.70: 1, Maximum prevalence of CoNS was in 41-60 years age group (38%). Out of 100 CoNS Staph. epidermidis (58%), Staph. saprophyticus (18%), Staph. hemolyticus (15%), and Staph. lugdunensis (7%) and Staph. capitis (2%) were isolated respectively. CoNS were most commonly isolated blood samples (32%). Highest number of biofilm producers 65% were detected by TCP method, while by TA method were 56% and by CRA method were 21%. Out of 65 biofilm producers, Staph. epidermidis was most common biofilm producer i.e. 38 (58.46%), followed by Staph. saprophyticus 12 (18.46%), Staph. hemolyticus 10 (15.39%), Staph. Lugdunensis 4 (6.15%) and Staph. capitis (1.54%) respectively. The highest antibiotic resistance rates for strongly adherent isolates were observed against ampicillin (95.38%) and erythromycin (78.46%), but the isolates showed no resistance to vancomycin (0.0%), linezolid (0.0%) and tigecycline (0.0%). **Conclusion:** The clinical significance of CoNS is increasing day by day in device related infections, urinary tract infections, endocarditis. Very soon CoNS may emerge as one of the leading Nosocomial pathogen. This study calls attention to the Biofilm producing CoNS infection detected in early phase of disease and prevents morbidity, mortality and future complications.

KEYWORDS: CoNS (Coagulase Negative Staphylococci), HAI (Hospital-Acquired Infection), CRA (Congo Red Agar) method, TA (Tube Adherence) method, TCP (Tissue Culture Plate) method, AST (Antimicrobial Susceptibility Testing).

INTRODUCTION

CoNS have been major cause of nosocomial infections. CoNS are known to cause chronic infections, the major virulence factor determining the pathogenicity of CoNS has now well defined and found to be biofilm production. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate.[1] Coagulase negative staphylococci are gram-positive spherical cocci belonging to the genus Staphylococcus. They are catalase-positive, non-motile, non-spore-forming, noncapsulated. The absence of secreted enzyme coagulase is the key characteristic of CoNS, differentiating them from coagulase-positive staphylococci, such as Staphylococcus aureus, which is able to clot plasma and is generally accepted as a pathogenic bacterium related to acute infections.[2] Coagulase negative staphylococci of clinical importance are Staphylococcus epidermidis group, Staphylococcus saprophyticus group, Staphylococcus haemolyticus

group, Staphylococcus lugdunensis and Staphylococcus capitis.[2,3] They can produce serious human infections. They cause infections in debilitated or immunocompromised patients, also in patients with indwelling urinary catheters, cardiac valves and artificial joints.[4] Staph. epidermidis is the CoNS species most frequently isolated from human infections and should no longer be considered as a harmless commensal. Staph. epidermidis has been implicated as the etiological agent in infections of wound, urogenital tract, respiratory tract, meninges, conjunctiva and skin.[5,6] Staph. saprophyticus is second to coliforms as the most common cause of the acute urethral syndrome and UTI in women.[7,8] Multiple antibiotic resistances is a common finding among clinical CoNS isolates indicating its potential pathogenecity.[9] In Staphylococcus epidermidis polysaccharide intercellular adhesin (PIA), also known as a poly-N-acetylglucosamine is responsible for intercellular adhesion. It is a partially deacylated polymer of β-1, 6-N-acetylglucosamine, which with the other polymers such as teichoic acids and proteins can form a major part of the extracellular matrix. Recently, PIA homologous are identified in many pathogens with biofilm formation ability, which shows that the three-dimensional matrix formation plays an important role in bacterial virulence. Biofilm protects CoNS, against both antibiotics used to treat infections and host immune system responses.[10] Importantly, all CoNS species are isolated from different medical devices indicating their ability to cause device associated infections.[11] Several phenotypic and genotypic methods have been used to demonstrate biofilm forming properties of isolates from the surface of catheters and devices, as an indirect evidence of presence of biofilms. The Phenotypic methods namely, Congo red agar method, Tube adherence method and Tissue culture plate method are simple, rapid and fairly sensitive for detection of biofilm forming properties. Congo red agar method is a qualitative method; Tube adherence method and Tissue culture plate method are quantitative methods. The TCP assay described by Christensen is most widely used and is considered a standard test for detection of biofilm formation.[12,13,14,15]

AIM AND OBJECTIVES

This study was conducted to isolate the CoNS in different clinical samples for detection of Biofilm formation by using three different screening methods and their AST pattern to know the pathogenicity and their drug resistant pattern in South-East Rajasthan. The ultimate goal of this study was to identify CoNS infection in early phase of disease and prevent future complications.

MATERIALS AND METHODS

Study design: The present study was conducted in the central microbiology laboratory of the Govt. Medical College-Kota from period of 1st January 2022 to 31th December 2022 to evaluate the Biofilm producing CoNS. Total 100 consecutive Coagulase negative staphylococci (CoNS) isolated by the VITEK 2 system and conventional method from various clinical samples like central venous catheter(CVC), various medical devices, intravenous catheter tip, suction tip, drain tip, Double J (DJ) stent, tracheal tip, endotracheal tip, blood, wound/pus, urine, body fluids like pleural, ascitic, peritoneal fluid and CSF etc., urinary catheter, endotracheal aspirate and sputum of patients attending out-patient department or inpatient department of MBS Hospital and associated group of hospitals and were processed for the study.

Inclusion criteria

1. All isolates of coagulase negative staphylococci from patients hospitalized for more than 48 hours.
2. All coagulase negative staphylococci isolated from out-patients, with in-dwelling medical devices.

Exclusion criteria

1. All out-patients without indwelling medical devices.
2. Patients hospitalized for less than 48 hours.

Sample Processing: All specimens were processed according to standard operating procedure of laboratory and by VITEK 2 system`s manual guidelines. Samples first cultured on Blood & MacConkey agar. Examined after 24 hours of incubation for colony morphology, gram staining and microscopy. Then CoNS were isolate by doing catalase, coagulate tests and other reactions. Once the isolates were identified as CoNS, they were classified into species following simplified scheme proposed by Cunha et al. using several biochemical tests.[16] In this study clinical isolates of CoNS were screened by Congo Red Agar Method (CRA), Tube Adherence Method (TA) and Tissue Culture Plate (TCP) method for their ability to form biofilm. After that we performed Antimicrobial susceptibility Test by Kirby-Bauer disk diffusion method by CLSI guidelines.[17] S. epidermidis strain ATCC 35983 was used as positive control for biofilm production in all assays performed.

Detection of Biofilm formation

1. Qualitative Biofilm Testing

1(i)-Congo Red Agar (CRA) Method[13]

The Congo red agar method was used for qualitative biofilm testing. This method is a direct and non-quantitative approach that allows for the identification and differentiation of biofilm-forming microorganisms (black colonies) from non-biofilm-forming strains (pink/red colonies). The Congo red agar medium was prepared by mixing brain heart infusion agar, sucrose, and Congo red dye. The brain heart infusion (BHI) agar and the sucrose were prepared together, while the Congo red dye was separately prepared as a stock solution and autoclave at 121 ◦C for 15 min. The Congo red dye solution was then added to the BHI agar after both solutions had cooled to about 55 ◦C and allowed to set. The CRA plates were inoculated with one or more colonies of the CoNS isolates and incubated aerobically for 24 h at 37 ◦C. After incubation, the formation of

black colonies was considered positive for biofilm formation, while the formation of pink/red colonies was considered negative for biofilm formation. Brown colonies were considered moderate biofilm former.

Interpretation:- [figure 1 and figure 2]
Strong slime producers- Black colonies with a dry crystalline consistency

Moderate slime producers- Brown darkening of the colonies with absence of a dry crystalline colony.

Weak slime producers- Pink colonies or occasional darkening at the centers of colonies was observed.

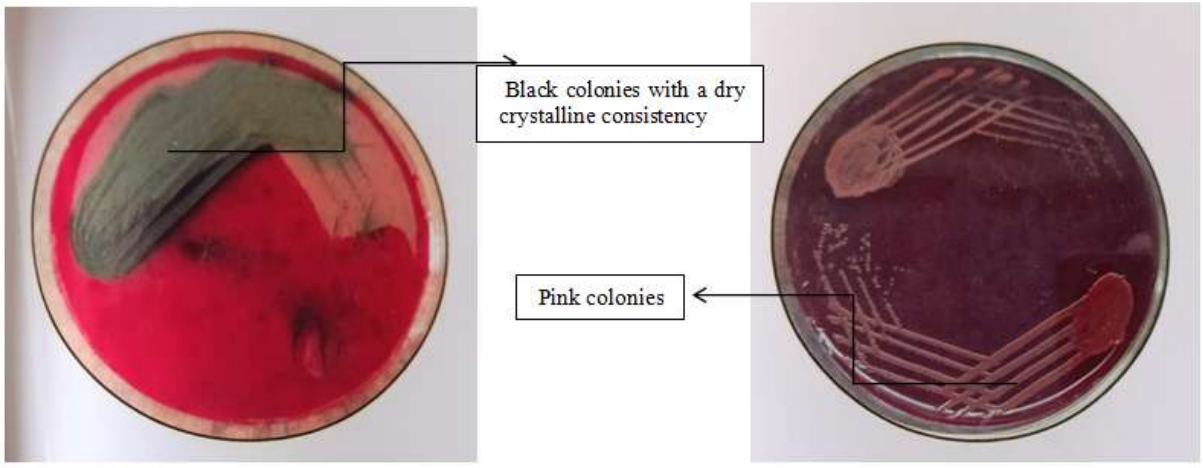

Figure 1: Biofilm Positive CoNS in CRA method.

Figure 2: Biofilm Negative CoNS in CRA method.

2. Quantitative Biofilm Testing

2(i)-Tube adherence (TA) Method[14]

This quantitative method for the detection of biofilm formation was performed as described by Christensen et al. A loop-full of microorganism was inoculated in trypticase soy broth (TSB) supplemented with 1% glucose. The tubes were incubated at 37ºC for 24 h. The tubes were decanted, washed with phosphate buffer saline (PBS) with pH 7.2 for 4 times and dried. Tubes were then stained with 0.1% crystal violet for 15 min. Excess stain was removed by washing with deionized water for 3 times. The tubes were then dried in inverted position and observed for biofilm formation. In this assay, biofilm formation was considered positive when a visible film was observed along the inner wall and bottom of tube. Depending on this, isolates were scored as 0 for absence, +for weak, + +for moderate and + + + for strong biofilm formation.

Interpretation:-[figure 3] Biofilm formation was 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. Tubes were examined and the amount of biofilm formation was scored as:- 0-absent, 1-weak, 2-moderate, 3-strong.

Figure 3: Tube Adherence (TA) Method.

2(ii)-Tissue Culture Plate (TCP) Method[12,15]

All the isolates were screened for their ability to produce biofilm by this quantitative method as described by Christensen et al with slight modification.[22] In this assay, a loop-full of organism was inoculated in TSB supplemented with 1% glucose and incubated at 37º C for 24 h. The overnight culture was diluted 1:100 with fresh media and 0.2 mL of this diluted culture was inoculated into individual wells of sterile polystyrene 96 well fat bottom tissue culture plates and incubated at

37 ºC for 24 h. After incubation, the content of tissue culture plate was removed by gentle tapping, and washed with PBS (pH 7.2) 4 times. Biofilms formed in the plate were fixed with 2% sodium acetate. It was then stained with 0.1% safranine for 20 min at room temperature. Excess stain was rinsed of by washing with deionized water for 4 times and plates were dried. Finally, 33% glacial acetic acid was added. The mean OD value obtained from control well was deducted from all the test OD values. Optical density (OD) of stained adherent bacteria was measured with micro ELISA auto reader at OD 630 nm. OD values from sterile medium, fixative and dye were averaged and subtracted from all test values. Bacterial adherence was classified based on OD values of the individual isolates. Mean OD value <0.120, 0.120–0.240 and >0.240 were classified as non/weak, moderate and strong biofilm adherence respectively.

Interpretation:-[figure 4]

Mean OD values	*Adherence*	*Biofilm formation*
<0.120	*Non*	*Non/weak*
0.120-0.240	*Moderately*	*Moderate*
>0.240	*Strong*	*High*

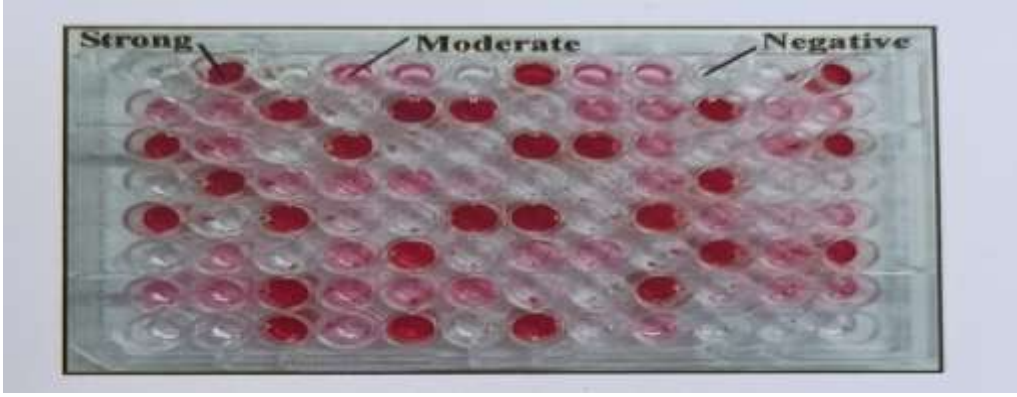

Figure 4: Tissue Culture Plate (TCA) Method.

Antimicrobial susceptibility testing (AST)

AST of the isolates was performed on Mueller Hinton Agar (MHA) by modifed Kirby-Bauer disk difusion method recommended by clinical laboratory standard institution (CLSI) guidelines.[17] Total seventeen (17) antimicrobial discs (Hi Media Laboratories) used in the study were: Ampicillin(10µg/disc), Erythromycin (15µg/disc), Amoxycillin-clavulinic acid(20/10µg/disc), Cefotaxime (30µg/disc), Linezolid(15µg/disc), Vancomycin (30µg/disc), Co-trimoxazole (1.25/23.75 µg/disc), Amikacin (30µg/disc), Levofloxacin (5µg/disc), Clindamycin (2µg/disc), Cefoxitin (30µg/disc), Ciprofoxacin (30µg/disc), Tetracycline (30µg/disc), Gentamicin (10µg/disc), Chloramphenicol (30µg/disc), Teicoplanin (30µg/disc) and Tigecycline (15µg/disc). The Staphylococcus aureus ATCC 25923 was used as reference strain for analyzing AST results.

Compilation of Data: All demographic and clinical data was collected and documented in pre-structured Performa.

Statistical Analysis of Data: Collected Data was entered into Microsoft Excel spreadsheet. Categorical variables were expressed as percentage basis and analyzed as a chi-square test, continuous data expressed as mean and Standard deviation and p value less than 0.05 was taken as statistically significant for all statistical analysis done.

RESULT

During the 1 year of study period from January 2022 to December 2022, a total of 100 CoNS were isolated from various clinical samples of patients from OPD and IPD of MBS Hospital and associated hospitals.The study revealed following results: CoNS were commonly isolated in male as shown in table 1. Out of 100 CoNS isolates 63% were prevalent in male and 37% in female. Male: female ratio for CoNS was 1.70: 1.

Table 1: Gender-wise Distribution of Study samples of CoNS.

Gender	Number of isolates (N=100)	%
Male	63	63.00%
Female	37	37.00%
Total	100	100%

In present study, Age-wise distribution CoNS were more common in was in 41-60 years age group, followed by 21-40 years age group as shown in table 2. Mean age of study group was 48.87 years. Maximum prevalence of CoNS was in 41-60 years age group (38%) and minimum prevalence in >80 years age group (1%).

Table 2: Age-wise Distribution of Study samples of CoNS.

Age	Number of isolates (N=100)	%
1-20 years	11	11.00%
21-40 years	31	31.00%
41-60 years	38	38.00%
61-80 years	19	19.00%
>80 years	1	1.00%
Total	100	100%

In present study, various species of CoNS were isolated as shown in table 3. Out of 100 CoNS Staph. epidermidis (58%), Staph. saprophyticus (18%), Staph. hemolyticus (15%), and Staph. lugdunensis (7%) and Staph. capitis (2%) were isolated respectively, as shown in figure 1.

Table 3: Species-wise Distribution of Study samples of CoNS.

Type of CoNS species isolated	Number of each CoNS species isolated (N=100)	%
Staph. epidermidis	58	58.00%
Staph. saprophyticus	18	18.00%
Staph. hemolyticus	15	15.00%
Staph. lugdunensis	7	7.00%
Staph. capitis	2	2.00%
Total	100	100.00%

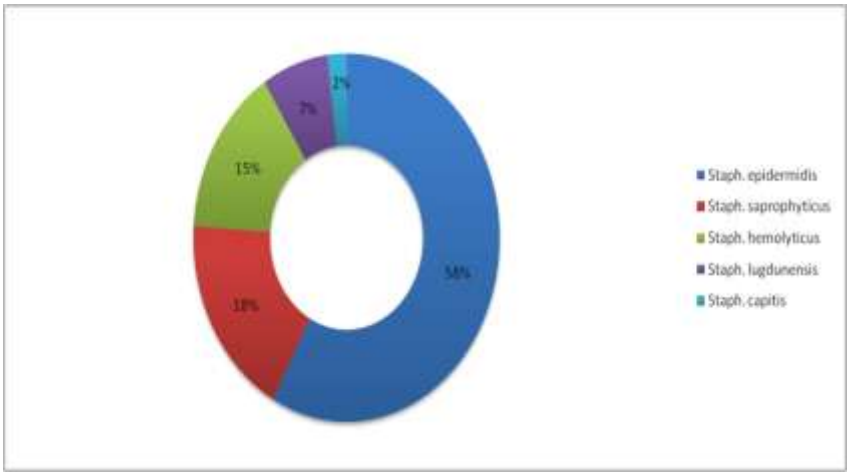

Figure 1: Species-wise distribution of CoNS.

In present study, CoNS were most commonly isolated from Blood samples, as shown in table 4. CoNS were most commonly isolated from pus from blood (32%), pus/wound (26%), catheters tip (12%), urine (11%), Central Intravenous catheters (7%), body fluids (6%), implants (5%), and sputum (1%) respectively as shown in figure 2. Staph. epidermidis was isolated form all clinical samples, it was more commonly isolated from blood, wound/pus. Staph. saprophyticus along with blood and wound/pus was also isolated from urine. All CoNS were isolated from catheter tip.

Table 4: Sample-wise Distribution of CoNS.

Type of various sample	Number of Isolates (N=100)	%
Blood	32	32.00%
Pus / Wound	26	26.00%
Catheters tip	12	12.00%
Urine	11	11.00%
Central Intravenous catheters (CVC)	7	7.00%
Various Body fluids	6	6.00%
Implants	5	5.00%
Sputum	1	1.00%
Total	100	100 %

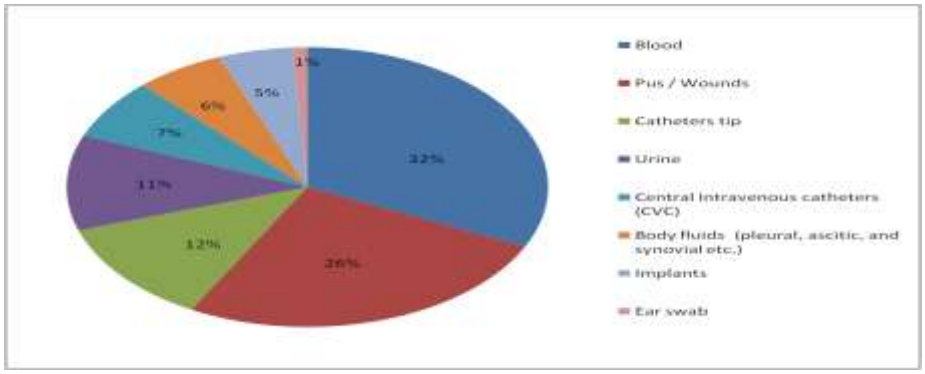

Figure 2: Sample-wise Distribution of CoNS.

Table 5 and figure 3 shows production of biofilm detected by three phenotypic methods. Congo Red Agar (CRA) method detected 9 (9%) of CoNS as strong biofilm producers. It detected moderate biofilm production in 12 (12%) isolates of CoNS and biofilm production was not detected in remaining 79 (79%) of CoNS. While the Tube adherence (TA) method detected strong biofilm production in 25 (25%) isolates of CoNS. It detected moderate biofilm production in 31 (31%) isolates of CoNS. By this method, 20 (20%) isolates were weak biofilm producers and remaining 24 (24%) isolates were non biofilm producers. Whereas Modified Tissue culture plate (TCP) method detected 30 (30%) of CoNS isolates as strong biofilm producers. It detected 35 (35%) isolates as moderate biofilm producers and remaining 35 (35%) as weak/non biofilm producers. For statistical analysis purpose in this study, strong and moderate biofilm producers have been placed in the biofilm producers and weak/non biofilm producers have been placed in the non biofilm producers. So it is evident that highest number of (Strong and moderate) biofilm producers 65 (65%) were detected by TCP method. Thus it was taken as gold standard to compare other two methods.

Table 5: Screening of CONS isolates for Biofilm formation by Tissue Culture Plate (TCP) Method, Tube Adherence (TA) method and Congo Red Agar (CRA) method.

Phenotypic Methods		CoNS					
		Total No. (N=100)	%	Total Biofilm producers	%	Total Non-Biofilm producers	%
TCP Method	Strong	30	30%	65	65%	35	35%
	Moderate	35	35%				
	Weak/non	35	35%				
TA Method	Strong (3)	25	25%	56	56%	44	44%
	Moderate (2)	31	31%				
	Weak (1)	20	20%				
	Non (0)	24	24%				
CRA Method	Strong	9	9%	21	21%	79	79%
	Moderate	12	12%				
	Weak	79	79%				

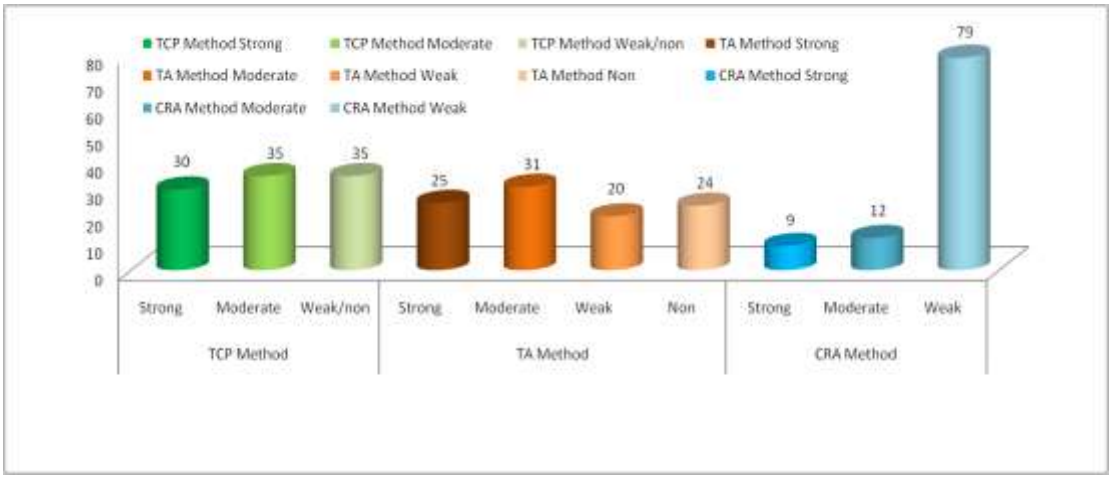

Figure 3: Detection of Biofilm formation in CoNS by three (3) different Phenotypic methods.

Species wise biofilm production in CoNS, as shown in table 6. Out of 65 biofilm producers, Staph. epidermidis was most common biofilm producer i.e. 38 (58.46%), followed by Staph. saprophyticus 12 (18.46%), Staph. hemolyticus 10 (15.39%), Staph. lugdunensis 4 (6.15%) and Staph. capitis (1.54%) respectively as shown in figure 4.

Table 6: Distribution of Biofilm producers and Non-Biofilm producers in Isolated species of CoNS.

Type of CoNS species isolated	Number of Isolates (N=100)	% (N=100)	Biofilm Producers CoNS (N=65)	% (N=65)	Non-Biofilm Producers CoNS (N=35)	% (N=35)
Staph. epidermidis	58	58.00%	38	58.46%	20	57.14%
Staph. saprophyticus	18	18.00%	12	18.46%	6	17.14%
Staph. hemolyticus	15	15.00%	10	15.39%	5	14.29%
Staph. lugdunensis	7	7.00%	4	6.15%	3	8.57%
Staph. capitis	2	2.00%	1	1.54%	1	2.86%
Total	100	100.00%	65	100.00%	35	100.00%

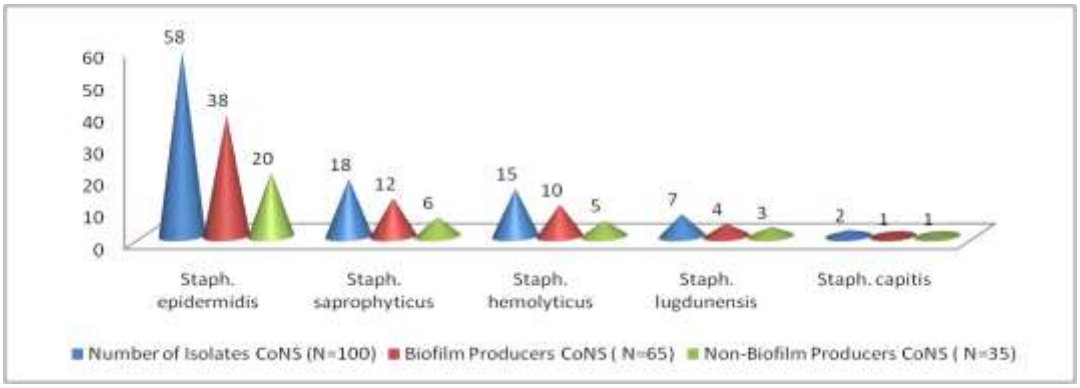

Figure 4: Distribution of Biofilm producers and Non-Biofilm producers in Isolated species of CoNS.

Antibiotic resistance pattern of isolated CoNS, biofilm producer CoNS and non-producer CoNS isolates is shown in table 7 and figure 5. In present study, No resistant isolates were found in present study for vancomycin, linezolid and tigecycline. There was high resistant pattern among biofilm producers in comparison with non- biofilm producers. The resistance pattern of biofilm producing CONS against antibacterial agents showed that the majority of the biofilm producing isolates were resistant to Ampicillin (95.38%) followed by Erythromycin (78.46%), Amoxycillin-clavulinic acid (75.38%), Cefoxitin (73.85%), Clindamycin (69.23%), Cefotaxime (56.92%), Levofloxacin (53.85%), Co-trimoxazole (43.08%), Ciprofloxacin (41.54%), Tetracycline(29.23%), Amikacin (27.69%), Gentamycin (16.92%), Chloramphenicol (10.77%) and Teicoplanin (10.77%) respectively when compared to isolated CoNS and non biofilm producing strains. Thus, this study is clearly shows that biofilm producing CoNS isolates shows higher antibiotic resistance than isolated CoNS and non biofilm producers.

Table 7: Antibiotic resistance pattern in Isolated CoNS, Biofilm producers CoNS and Non-Biofilm producers CoNS.

Antibiotics Name	No. of Resistant isolates CoNS (N=100)	% (N=100)	Biofilm producers CoNS (N=65)	% (N=65)	Non-Biofilm producers CoNS (N=35)	% (N=35)
Ampicillin	87	87.00%	62	95.38%	25	71.43%
Erythromycin	69	69.00%	51	78.46%	18	51.43%
Amoxycillin-clavulinic acid	67	67.00%	49	75.38%	18	51.43%
Cefoxitin	65	65.00%	48	73.85%	17	48.57%
Clindamycin	63	63.00%	45	69.23%	18	51.43%
Cefotaxime	55	55.00%	37	56.92%	18	51.43%
Levofloxacin	51	51.00%	35	53.85%	16	45.71%
Co-trimoxazole	38	38.00%	28	43.08%	10	28.57%
Ciprofloxacin	37	37.00%	27	41.54%	10	28.57%
Tetracycline	29	29.00%	19	29.23%	10	28.57%

Amikacin	28	28.00%	18	27.69%	10	28.57%
Gentamycin	16	16.00%	11	16.92%	5	14.29%
Chloramphenicol	11	11.00%	7	10.77%	4	11.43%
Teicoplanin	9	9.00%	7	10.77%	2	5.71%
Vancomycin	00	0.00%	00	00%	00	00%
Linezolid	00	0.00%	00	00%	00	00%
Tigecycline	00	0.00%	00	00%	00	00%

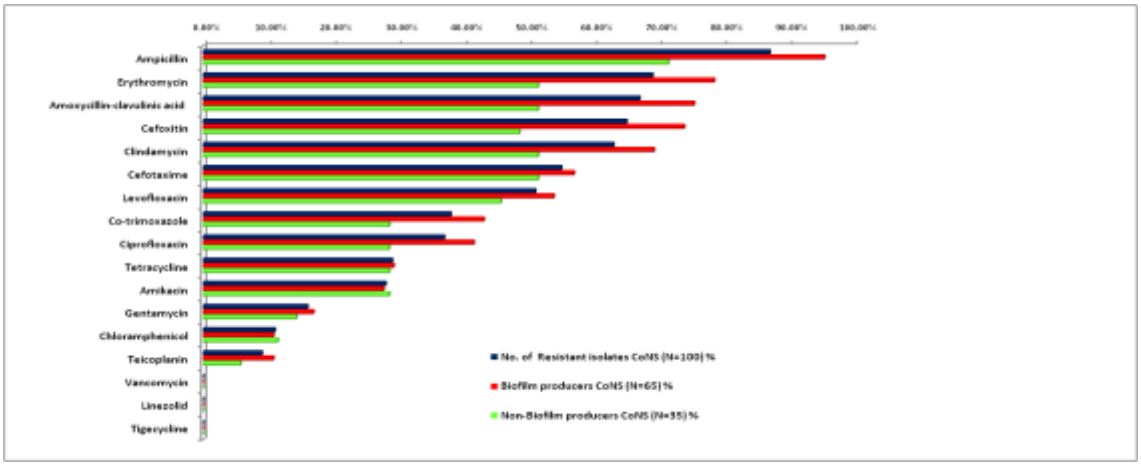

Figure 5: Antibiotic resistance pattern in Isolated CoNS, Biofilm producers CoNS and Non-Biofilm producers CoNS.

DISCUSSION

The clinical significance of CoNS is increasing day by day in device related infections, urinary tract infections, endocarditis. Very soon CoNS may emerge as one of the leading nosocomial pathogen. In present study 100 CoNS isolates were phenotypically characterised and their ability to form biofilms was determined by phenotypic methods i.e. CRA method, TA method and TCP method, taking TCA method as gold standard. In present study Male: Female ratio for CoNS isolates was 1.70: 1. This finding correlated with study by S S Vijayasari et al.[18] who reported Male: Female ratio for CONS was 1.70: 1 whereas Puja Gupta et al.[19] reported Male: Female ratio for CoNS was 1.1: 1. In present study CoNS were most commonly isolated from 41-60 years age group (38.00%), followed by 21-40 years age group (31%), In study of Puja Gupta et al.[19] CoNS were most commonly isolated from 41-60 years age group (39.00%), which is comparable to present study and CoNS were most common in age group of 21-40 years age group (31.81%) in study of S S Vijayasari et al.[18] Among 100 CoNS isolates Staph. epidermidis (58%) was the most predominant species in present study. This observation was supported by Priyanka Mane et al,[20] S S Vijayasari et al,[18] S A Sardar et al l,[21] and Samant Sarvari et a,[22] who reported Staph. epidermidis as most commonly isolated species in their studies respectively. In present study Staph. Saprophyticus (18%) was second most commonly isolated species, this finding was favoured by S A Sardar et al.[21] and Samant Sarvari et al,[22] Staph. Saprophyticus as second most commonly isolated species respectively in their studies whereas Staph. hemolyticus (22.00%) was second most commonly isolated species, was reported by Priyanka Mane et al.[20] and S S Vijayasari et al.[18] in their studied respectively. In present study CoNS were most commonly isolated from from blood (32%) followed by pus/wound (26%) which is comparable with the study of Samant Sharwari et al.[22] and Usha M G et al.[23] TCP method is suggested as best method based on present study findings. It is also reported as gold standard by Mathur et al.[9] Similar finding have been reported by Hassan et al,[24] Riyaz et al.[25] and Puja Gupta et al.[19] Hence TCP method was considered as standard method for further interpretation of results. In present study, Staph. epidermidis (58.46%) were most common biofilm producers species, which was comparable with the studies of S A Sardar et al.[21] (55.00%), S S Vijayasari et al.[18] (66.66%) and Priyanka Mane et al.[20] (52.94%). All above studies including Samant Sarvariet al.[22] reported Staph. epidermidis as most frequently isolated and biofilm producing species of CoNS. In present study, there was high resistant pattern among biofilm producers in comparison with non-biofilm producers. The Susceptibility pattern of biofilm producing CoNS against antibacterial agents comparable to other studies Shreshta et al,[26] Pankaj Joshi et al,[27] Roberto et al,[28] and Chincholkar et al,[29] when compared to non biofilm producing strains. No resistant isolates were found in present study for vancomycin, linezolid and tigecycline this was comparable to other studies. So vancomycin, linezolid and tigecycline were most effective against biofilm producing strains. This result was favoured by most of the studies.

LIMITATION OF STUDY

Limitations of this study include this study is a only 100 samples study so we are unable to assess the exact incidence and prevalence of CoNS in different risk groups although we describe biofilm producing CoNS isolates shows higher antibiotic resistance than non biofilm producers. We exclude some patients from study due to incomplete data and who were hospitalized only for 48 hours. Currently due to lack of resources, We have not been able to do Genotyping methods. In the future, We would like to this methods also at our institute.

SUMMARY AND CONCLUSION

Over the last decades, Coagulase negative staphylococci (CoNS) have been accepted as major opportunistic pathogens of low virulence causing Hospital-acquired infections (HAI). Predominantly cause infections in immune compromised patients or otherwise healthy individuals with breached skin and mucous barriers. CoNS are known to cause chronic infections, the major virulence factor determining the pathogenicity of CoNS has now well defined and found to be biofilm production. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate. The biofilm producing CoNS isolates showed higher antibiotic resistance than non biofilm producers. Biofilm production had a strong association with medical device related to orthopaedic implants, urinary catheterization and intravenous catheters.[30] TCP method can be recommended for the identification of biofilm producing organism due to cost effectiveness, short turnaround time and capability of being used in routine diagnostics. Resistance among CoNS are increasing especially among the patients with the indwelling medical devices due to biofilm production. Among various phenotypic methods TCP method showed high sensitivity and specificity. Tissue culture plate method was proved to be simple, economical method can be recommended for early and prompt diagnosis of biofilm production.The virulence of CoNS is directly related to its capability to establish multi-layered, highly structured biofilms on artificial surfaces. There is association between biofilm production with persistent infection and antibiotic failure. So, This study calls attention to the Biofilm producing CoNS infection detected in early phase of disease and prevent morbidity, mortality and future complications.

Declarations

Conflict of interest: The authors have no conflict of interest.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human or animal subjects performed by authors.

Bio-Safety: All standard precautions, bio-safety measures & Biomedical Waste Management in our study according to Biological Waste Management's Rules 2016 and it`s new amendment were observed.

Data availability: All datasets generated of analyzed during this study are included in the manuscript.

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