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Speciation, Detection of Virulence Factors and Antibiotic Susceptibility of Coagulase Negative Staphylococci

Deepthi S Dilip[a], Ardra R Menon[b,*]

[a] *Department of Microbiology, Government Medical College, Alappuzha, India*
[b] *Department of Microbiology, Government Medical College, Manjeri, India*

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ABSTRACT

Background and aim: Coagulase-Negative Staphylococci (CoNS), although predominant in the normal skin flora, can cause severe human diseases, as evident from their increasing incidence in recent years. Detailed characterization of CoNS isolates through speciation, and antibiotic susceptibility may be needed to differentiate pathogenic from contaminating isolates and plan effective therapy. Aim: To isolate and speciate CoNS from various clinical specimens, detect different virulence factors, and determine the antibiotic susceptibility profile.

Materials and methods: A hospital-based cross-sectional study was done using 110 clinical isolates of CoNS throughout one year in the Department of Microbiology, Government Medical College, Thrissur, Kerala, India. The isolates were identified, speciated using standard methods, virulence factors like biofilm formation and DNase were determined, as well as antibiotic susceptibility using the Kirby Bauer disc diffusion method. Statistical analysis was done by counts and percentages using MS Excel version 2010.

Results: Staphylococcus epidermidis was the most frequent isolate, 49.1%, Staphylococcus haemolyticus 27.3%, and Staphylococcus schleiferi 11.8%. Blood samples yielded maximum isolates. Biofilm production by the Congo Red Agar method was seen in 18.2% isolates, and 14.5% showed DNase production. Antibiotic susceptibility testing showed maximum resistance to Penicillin (95.5%) and Erythromycin (80.9%) with Methicillin resistance in 17.3% and 100 % sensitivity to Vancomycin. Biofilm-producing strains were more antibiotic-resistant.

Conclusion: The multiple antibiotic resistance and pathogenic potential of biofilm producers emphasize the importance of developing simple, reliable, and inexpensive methods to identify virulence factors and determine the antibiotic sensitivity of CoNS.

1. Introduction

Coagulase-negative Staphylococci (CoNS), previously thought to be non-pathogenic commensals of human skin, anterior nares, ear canals, respiratory and gastrointestinal mucus membranes, have emerged as a consequential source of healthcare-associated infections. Staphylococcus epidermidis, Staphylococcus saprophyticus, and Staphylococcus haemolyticus are the most prevalent species of CoNS implicated in human infections. Other species, such as S. hominis, S. warneri, S. simulans, S. lugdunensis, S. schleiferi, S. saccharolyticus, and S. cohnii, have only recently been discovered to play a role in human infections.[1] CoNS are responsible for 9% of nosocomial infections. They cause infections in immunocompromised hosts, especially patients with cancer, end-stage renal disease, renal transplantation, burns, and infections associated with indwelling catheters, shunts, and prosthetic devices.[2] They are acknowledged as the third commonest cause of bloodstream infections, leading to significant morbidity and mortality.[3] CoNS have become the commonest pathogen in Neonatal Intensive Care Units (NICU) and pose unique diagnostic and management challenges.[2] The ability to adhere to polymer surfaces and the subsequent biofilm production is the main virulence factor of CoNS. The biofilm acts as a protective barrier against the immune system and the antibiotics administered for treatment. Accurate identification of CoNS up to species level is necessary for an exact determination of the host-pathogen relationship. In addition to being a significant pathogen, multidrug-resistant CoNS may also act as a reservoir of resistance to other organisms.[4] Expression of methicillin resistance By CoNS, which involves all β-lactam antibiotics, can significantly limit therapeutic options. Methicillin resistance is due to the mecA gene, which encodes a penicillin-binding protein (PBP2a) with altered properties. Clindamycin, which can be used as an alternative therapy, has the disadvantage of developing inducible resistance leading to therapeutic failure. In all Staphylococcal species, including CoNS, PBP2a expression leads to complete β-lactam resistance, with the one exception being recently introduced MRSA cephalosporins like ceftobiprole and

* *Corresponding author. Ardra R Menon*
E-mail address: drardra@yahoo.co.in
Department of Microbiology, Government Medical College, Manjeri, India
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ceftaroline.[5] Increasing incidence and importance of CoNS infections require active surveillance, characterization of strains, and antibiotic sensitivity. CoNS form the major part of the skin flora of humans and can become pathogenic due to its virulence factors. Studies on CoNS characterization and prevalence will contribute to understanding their distribution and mechanisms of antimicrobial resistance. It will be very useful to develop new therapeutic strategies to prevent and treat CoNS infections. Therefore, the present study was undertaken in a tertiary care teaching hospital at Thrissur, Kerala, to identify the CoNS isolates and detect its virulence factors and antibiotic sensitivity pattern.

2. Materials and methods

A hospital-based cross-sectional study was done in the Department of Microbiology. The study was done with the permission of the Institutional Research Committee and the Institutional ethics review Board of the institution. Based on the previous laboratory records of the institute, a sample size of 110 was calculated.

Formula = (Zα)2 Pq/d2.

Prevalence(P) – 47% (Kavitha Y et al) 6.

Precision of study (d) – 20% of P = 20/100*47 = 9.4.

q = 100-P =100-47 =53

Calculated sample size = 110

All consecutive, non-repetitive CoNS isolates from various clinical specimens from both inpatients and outpatients received in the Department of Microbiology during the study period were included in the study after taking informed consent from the patients. CoNS mixed with other isolates were excluded from this study. A total of 110 consecutive culture isolates of CoNS from January 2018 to December 2018 were collected, and speciation, detection of virulence factors, and antimicrobial susceptibility were done simultaneously during the same period.

The isolates were obtained from clinical samples like pus, endotracheal tube tip, Umbilical venous catheter (UVC) and Central Venous Catheter (CVC) tips, urine, pleural fluid, amniotic fluid, blood. A smear was made from the sample and stained with Gram stain. Samples were inoculated onto Blood and MacConkey agar and incubated at 37°C for 24-48 hours aerobically. On the 2nd and 7th day of incubation, blood samples in the Brain heart infusion broth were sub-cultured onto Blood and MacConkey agar. Preliminary identification of CoNS was made by Colony morphology, Gram staining, Catalase test, Slide coagulase test, and Tube coagulase test.[7-9]

Characterization of coagulase negative staphylococci

The test isolates of CoNS were subjected to various tests viz., Urease test, Ornithine Decarboxylase Test, Sugar fermentation test with 1% Sucrose and Mannitol, Susceptibility to Polymyxin B (300U), and Susceptibility to Novobiocin.[7-9]

Urease test

It was done by Christensen's method. The surface of a urea agar slant is streaked with a portion of a well-isolated colony and Incubated at 37°C for 24 hours. The change in color of slant from light yellow to magenta was considered positive, and No color change (agar slant and butt remain light yellow) was negative.[9]

Ornithine decarboxylase test

It was done by Moeller's method. Inoculated 2-3 colonies of the culture into two tubes of Moller's decarboxylase test medium, one containing ornithine and the other without any amino acid as the control tube. Both the tubes were overlayed with sterile mineral oil to cover about 4-5 mm of the surface and incubated at 37 ºC for 18 to 24 hours. When the test tube changed to an alkaline (purple) colour compared to the control tube, it was taken as positive. When there was no color change or acid (yellow) color in the test and control tube, it was considered negative. The fermentation of dextrose in the medium causes the acid color change. It would not, however, mask the alkaline color change brought about by a positive decarboxylation reaction.[9]

Fermentation of sugars

An isolated colony of the test strain was inoculated into peptone water with pH indicator bromothymol blue and incubated for 4-6 hours. 3-4 drops of this peptone water were added to the corresponding sugar tubes with inverted Durhams tube inside and incubated aerobically at 37ºC overnight. On fermentation of the sugars, the colour changed from blue to yellow. The presence of air bubbles in the Durhams tube indicates gas production.[9]

Susceptibility to polymyxin B(300U)

A suspension of the organism equivalent to 0.5 McFarland turbidity standard was prepared in sterile peptone water and swabbed onto Muller Hinton Agar plate. A polymyxin B 300 units disc was applied to the inoculum, and the plate was incubated at 37 ºC overnight. Susceptible strains were indicated by a zone size of more than 10mm and resistant strains with zone <10mm.[7]

Susceptibility to novobiocin

A suspension of the organism equivalent to 0.5 Mcfarland turbidity standard was prepared in sterile peptone water and swabbed onto Muller Hinton Agar plate. A novobiocin disk (5µg) was applied to the inoculated area, and the plate was incubated for 18 to 24 hrs at 37ºC. Susceptible strains were indicated by the presence of a zone size more than 16mm in diameter and resistant strains with zone <12mm in size.[7]

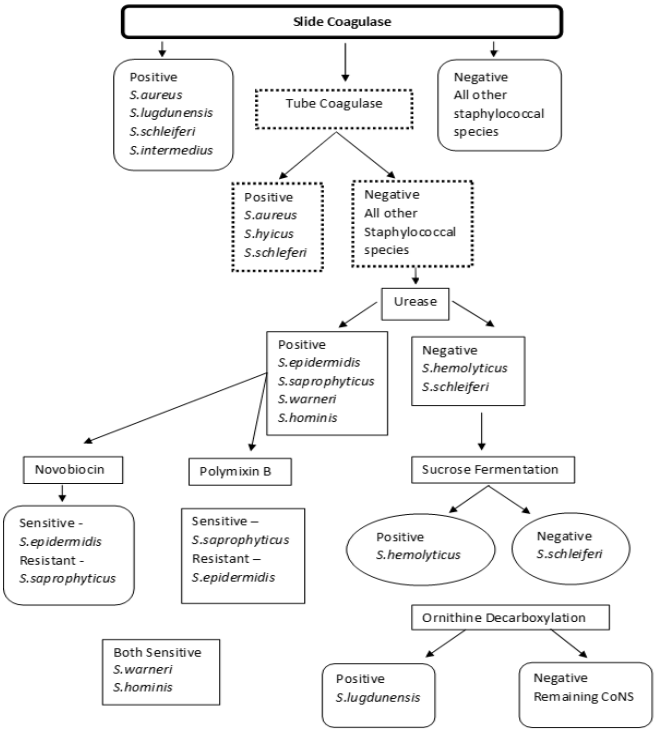

Fig. 1. Flow chart for speciation of CoNS.

Detection of virulence factor

Biofilm formation

Congo red agar (CRA) method was used for identifying the Slime producing ability of the CoNS (Freeman et al., 1989).[10] The isolated CONS strains were inoculated onto a special solid medium composed of brain heart infusion broth (37g/l), sucrose (50 gms/l), agar no.1 (10 gms/l), and the Congo Red stain (0.8 gms/l). A concentrated aqueous solution of Congo red was autoclaved separately at 121°C for 15 minutes and added when the agar had cooled to 55°C. After inoculation, the plates were incubated aerobically for 24-48 hours at 37°C. Slime-positive isolates produced black colonies with dry crystalline consistency. Non-slime producers remained pink.[11]

DNase test

Inoculating the isolates on DNase agar (HIMEDIA) and incubating aerobically at 37°C for 13 to 24 hours was used for this test. When DNA is hydrolyzed, methyl green is released, and at a pH of 7.5, it combines with highly polymerized DNA, turning the medium colorless around the test organism.[9].

Antibiotic susceptibility testing

Determination of antibiotic susceptibility pattern was performed by the Kirby Bauer disc diffusion method on 5% Mueller Hinton agar for the following antibiotics- Penicillin (10U), Erythromycin (15µg), Clindamycin (2µg), Cefoxitin (30µg), Gentamicin (10µg), Cotrimoxazole (25µg), Nitrofurantoin (300µg), as per 2018 CLSI guidelines.[12] The Minimum inhibitory concentration for Vancomycin was determined by E -test method by HIMEDIA E- strips. Quality control was done using ATCC strain Staphylococcus aureus 25923.

Statistical analysis

After coding, data were entered and analyzed in Microsoft Excel version 10, and percentages of CoNS species, biofilm and DNase production, and antibiotic susceptibility was calculated.

3. Results

Of the 110 isolates of CoNS, 60 (54.5%) were obtained from female patients and 50 (45.5%) from male patients. The maximum number of patients, 31(28.2%), was 21-40 years old, followed by 41-60 years,29(26.4%). The majority of the CoNS isolates were from Surgery (22.7%), Medicine(20%), and Orthopedics wards (17.3%) (Table 1).

Table 1. Frequency Distribution of some demographic characteristics of patients from whom CoNS species were isolated.

Variable	Condition	Number (%)
Gender	Male	50(45.5%)
	Female	60(54.5%)
Range of year	0-20	26 (23.6%)
	21-40	31(28.2%)
	41-60	29(26.4%)
	Above 60	24(21.8%)
Distribution in various clinical wards	Surgery	25(22.7%)
	Medicine	22(20%)
	Orthopedics	19(17.3%)
	Others	44(40%)

The majority of the isolates were from blood 55 (50%) and pus swabs 32 (29.1%), and a few from pus aspirates 6 (5.4%) and urine samples 5(4.6 %). Only one strain (0.9%) was isolated from indwelling devices like central line, UVC, and shunt tip. S. epidermidis was the most frequent isolate 49.1% (54), followed by S. haemolyticus 27.3% (30), S. schleiferi 11.8% (13), S. saprophyticus 8.2% (9), S. hominis 2.7% (3), and S. lugdunensis 0.9% (1) (Table 2, Fig. 2).

Table 2. Species distribution of CONS in various clinical specimens.

Samples	Total No of CONS n*(%)	S. epidermidis	S. haemolyticus	S. schleiferi	S. saprophyticus	S. hominis	S. lugdunensis
Blood	55(50%)	28	18	5	1	2	1
Pus swabs	32(29.1%)	14	10	6	1	1	0

Pus aspirates	6(5.4%)	4	1	1	0	0	0
Urine	5(4.6%)	0	0	0	5	0	0
Vaginal swab	5(4.6%)	2	1	1	1	0	0
Tissue	2(1.8%)	1	0	0	1	0	0
Shunt tip	1(0.9%)	1	0	0	0	0	0
UVC tip	1(0.9%)	1	0	0	0	0	0
Central line tip	1(0.9%)	1	0	0	0	0	0
Ear swab	1(0.9%)	1	0	0	0	0	0
Bronchial wash	1(0.9%)	1	0	0	0	0	0
Total	110	54(49.1%)	30(27.3%)	13(11.8%)	9(8.2%)	3(2.7%)	1(0.9%)

*n: Number

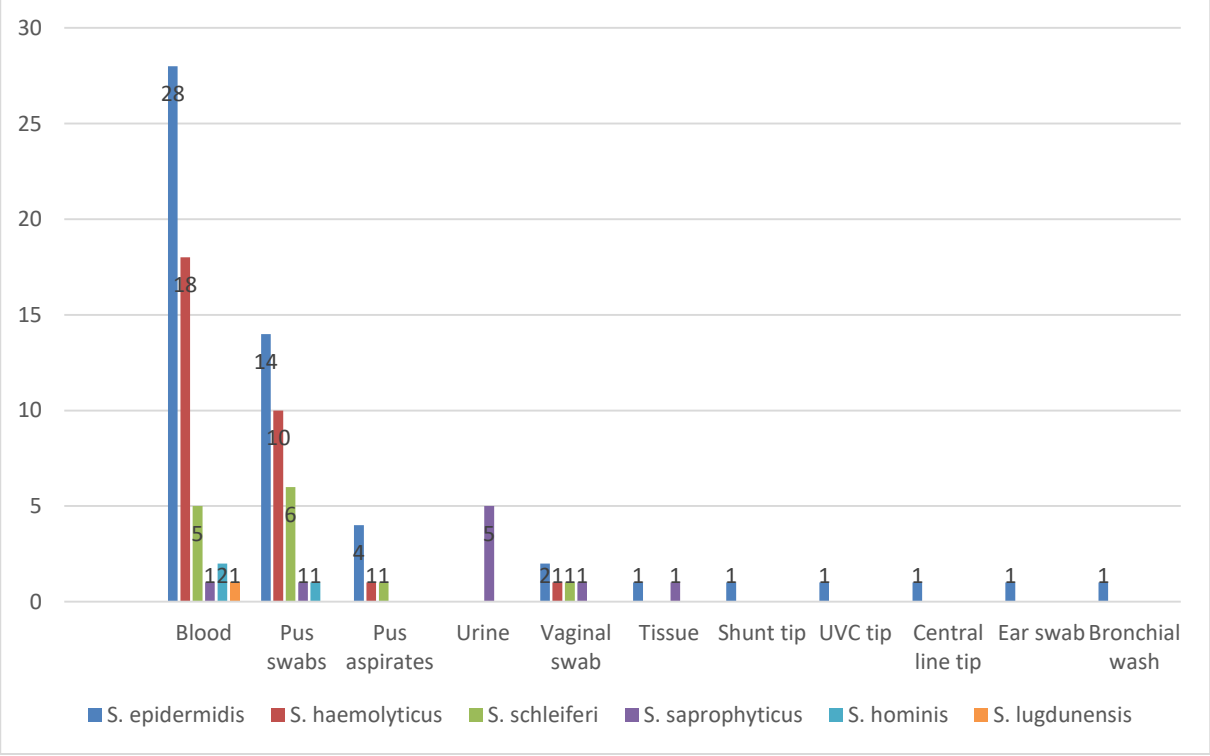

Fig. 2. Species distribution of CONS in various clinical specimens.

In our study, 18.2% of isolates showed biofilm production, and 14.5% showed DNase production. Maximum biofilm production was seen in blood isolates 27.3%, followed by pus swabs 12.5% and 16.7% pus aspirates. DNase production was observed from 28.1% of pus swab isolates, 10.9% of blood isolates, and a single bronchial wash sample. None of the isolates had both Biofilm and DNase production(Table 3). Biofilm was produced maximum by S. epidermidis (26%) followed by S. haemolyticus (16.7%) and S. schleiferi (8%). DNase was produced maximum by S. epidermidis (16.7%) followed by S. haemolyticus (16.7%) and S. schleiferi (15.38%) (Table 4 and Fig. 3).

Table 3. Distribution of virulence factors in various clinical samples.

Sample(n*)	Biofilm positive	Dnase positive
Blood (n= 55)	15(27.3%)	6(10.9%)
Pus swab (n=32)	4(12.5%)	9(28.1%)
Pus aspirate (n=6)	1(16.7%)	0
Bronchial wash (n=1)	0	1(100%)
Total (n=110)	**20**	**16**

***n= number**

Table 4. Distribution of virulence factors among the species isolated.

Samples(n*)	Biofilm positive	DNAse positive
S.epidermidis	14	9
S.hemolyticus	5	5
S.schleferi	1	2
Total	20(18.2%)	16(14.5%)

***n= number**

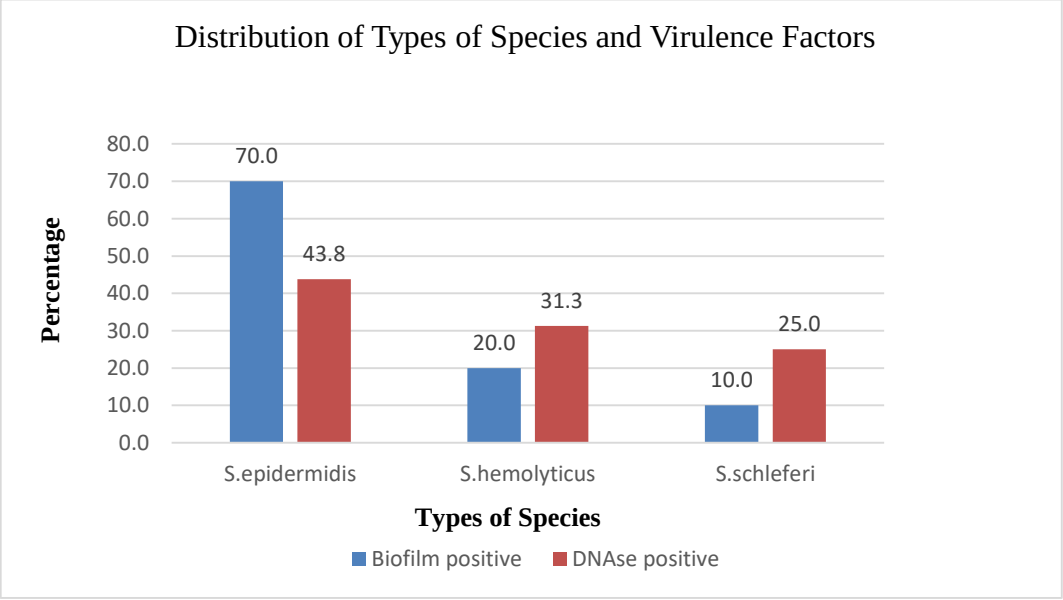

Fig. 3. Distribution of virulence factors among the species isolated.

Among the 110 isolates, all strains were sensitive to Vancomycin. Rate of resistance to Penicillin was 95.5% (105), Erythromycin 80.9% (89), Clindamycin 52.7% (58), Cotrimoxazole 36.36 % (20), Gentamicin 5.26 % (3). All five urinary isolates were sensitive to Nitrofurantoin. MR CoNS rate was (17.3) % (Table 5).

Table 5. Antibiotic susceptibility testing pattern of the isolated CoNS strains.

Antibiotics	Sensitive strains		Resistant strains	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Penicillin	5	4.5	105	95.5
Erythromycin	21	19.1	89	80.9
Clindamycin	52	47.3	58	52.7
Cotrimoxazole	35	63.6	20	36.3
Vancomycin	110	100	0	0
Cefoxitin	91	82.7	19	17.3
Gentamicin	54	94.73	3	5.26
Nitrofurantoin (For only 5 Urine samples)	5	100	0	0

Most of S. epidermidis were resistant to Penicillin (94.4%), followed by Erythromycin (81.5%), Clindamycin (51.9%), Cotrimoxazole (30.76%), Cefoxitin (18.5%). None of the isolates were resistant to Vancomycin, and all the urine isolates were sensitive to Nitrofurantoin (Table 6).

Table 6. Comparison of antibiotic resistance among the different strains.

Antibiotics	*S. epidermidis*(n=54)	*S. haemolyticus*(n=30)	*S. saprophyticus*(n=9)	*S. schleiferi(n=13)*	*S. hominis*(n=3)	*S. lugdunensis*(n=1)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Penicillin	51 (94.4%)	30(100%)	8(88.9%)	12(92.3%)	3(100%)	1(100%)
Erythromycin	44 (81.5%)	24(80%)	7(77.8%)	10 (76.9%)	3(100%)	1(100%)
Clindamycin	28 (51.9%)	16(53.3%)	4(44.4%)	8 (61.5%)	2(66.7%)	0
Cotrimoxazole	8(30.76%)	5(41.6%)	2(25%)	5 (62.5%)	0	0
Cefoxitin	10(18.5%)	6(20%)	0	3 (23.1%)	0	0
Gentamicin	1(3.6%)	2 (10.52%)	0	0	0	0
Vancomycin	0	0	0	0	0	0
Nitrofurantoin (For only 5 Urine samples)	0	0	0	0	0	0

† n= Number of resistant isolates

‡ %= Percentage of resistance

Among the 20 biofilm-positive isolates in the present study, Penicillin resistance was seen in 18.2% of blood isolates, 16.6 % of pus aspirates, and 9.3% of pus swab isolates. Among the Dnase positive isolates, the Penicillin resistance was seen in 10.9% of Blood isolates, 28.1% of pus swab isolates, and 100% of bronchial wash isolate. Cefoxitin resistance, i.e., Methicillin resistance, was seen only in one biofilm positive isolate(blood)and one Dnase positive isolate (pus swab). None of the biofilm positive and Dnase positive isolates showed resistance to Vancomycin, Nitrofurantoin, and Gentamicin (Table 7, Figs. 4 and 5).

Table 7. Distribution of virulence factors and antibiotic sensitivity patterns in clinical samples.

	Biofilm positive isolates(n=20)			Dnase positive isolate()n=16)		
Antibiotics	**Blood (n= 55)**	**Pus swab (n=32)**	**Pus aspirate (n=6)**	**Blood (n= 55)**	**Pus swab (n=32)**	**Bronchial wash (n=1)**
	%of resistance	**%of resistance**	**%of resistance**	**%of resistance**	**%of resistance**	**%of resistance**

Penicillin	10(18.2%)	3(9.3%)	1(16.6%)	6(10.9%)	9(28.1%)	1(100%)
Erythromycin	12(21.8%)	3(9.3%)	1(16.6%)	5(9.1%)	8(25%)	1(100%)
Clindamycin	5(9.1%)	2(6.3%)	0	5(9.1%)	6(18.8%)	1(100%)
Cotrimoxazole	1(1.8%)	2(6.3%)	0	0	1(3.1%)	0
Cefoxitin	1(1.8%)	0	0	0	1(3.1%)	0
Vancomycin	0	0	0	0	0	0
Nitrofurantoin	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0

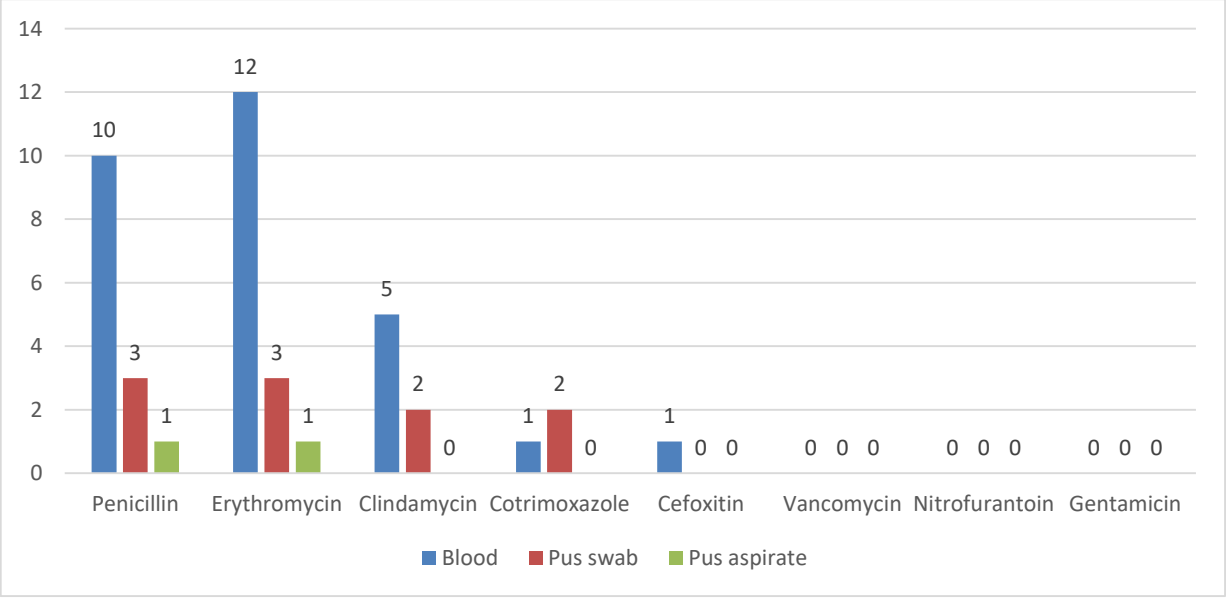

Fig. 4. Antibiotic resistance pattern of biofilm positive isolates in various samples.

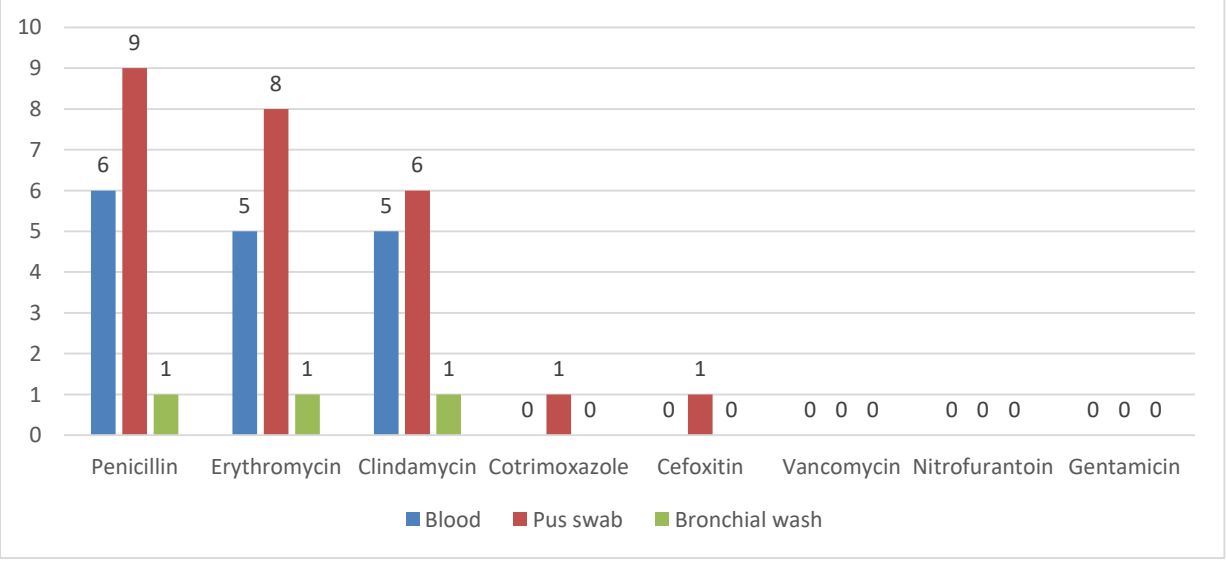

Fig. 5. Antibiotic resistance pattern of Dnase positive isolates in various samples.

In our study, among the 14 biofilms producing isolates of S. epidermidis, 12(85.71%) showed resistance to Penicillin followed by 12(85.71%) to Erythromycin, 5(35.71%) to Clindamycin, 2 (14.28%) to Cotrimoxazole,1(7.14%) to Cefoxitin (Table 8).

Table 8. Antibiotic resistance pattern of biofilm positive isolates.

Antibiotics	S. epidermidis(n=14) %of resistance	S. haemolyticus(n=5) %of resistance	S. schleiferi(n=1) % of resistance
Penicillin	12(85.71%)	1(20%)	1(100%)
Erythromycin	12(85.71%)	3(60%)	1(100%)
Clindamycin	5(35.71%)	1(20%)	1(100%)
Cotrimoxazole	2(14.28%)	0	1(100%)
Cefoxitin	1(7.14%)	0	0
Vancomycin	0	0	0
Nitrofurantoin	0	0	0
Gentamicin	0	0	0

In our study, among the nine biofilms producing isolates of S. epidermidis, all were resistant to Penicillin, Erythromycin, and Clindamycin. Maximum resistance was seen in S. schleiferi. (Table 9).

Table 9. Antibiotic resistance pattern of Dnase positive isolates.

Antibiotics	S. epidermidis(n=9) %of resistance	S. haemolyticus(n=5) %of resistance	S. schleiferi(n=2) %of resistance
Penicillin	9 (100%)	5(100%)	2(100%)
Erythromycin	9 (100%)	3(60%)	2(100%)
Clindamycin	9 (100%)	2(40%)	1(50%)
Cotrimoxazole	0	0	1(50%)
Cefoxitin	0	0	1(50%)
Vancomycin	0	0	0
Nitrofurantoin	0	0	0
Gentamicin	0	0	0

4. Discussion

Coagulase Negative Staphylococci have emerged as pathogens in an increasing number of severe hospital-acquired infections over the past few years. Biofilm formation and frequent antibiotic resistance make treatment difficult. Distinguishing between clinically significant and contaminant strains is a key challenge for clinical microbiologists. Therefore, this study was initiated to provide a detailed characterization of CoNS isolates, including speciation, virulence factors, and antibiotic sensitivity, in order to differentiate between the pathogen and the contaminant and plan appropriate treatment.

In the present study, the CoNS infection was more common in females (54.5%), in contrast to most of the studies.[3,13] The increased isolation in females can be attributed to more females being included in the study than males. This finding was also seen in a by Kashid et al.[14] Out of the 110 CoNS, 31 (28.2%) cases were 21-40 years of age, followed by 29(26.4%) in 41-60 years. Other studies have also observed that CoNS infections were more common in 2nd and 3rd decades of life.[3, 15] It may be due to a higher incidence of Urinary tract infections and post-operative wounds in these age groups.

In the present study, CoNS isolates were predominantly found in patients from the surgery department 25/110 (22.72%) followed by medicine 22/110 (20%) and orthopedic departments 19/110 (17.2%)(Table 1). The higher isolation rates in these patients are probably due to the more invasive procedures done in these departments, use of central venous catheters, prolonged parenteral nutrition, use of IV lipid emulsions, immunosuppression, critical conditions, and prolonged stay in hospitals.[16]

Among the 110 isolates of CoNS, 55 (50%) were isolated from blood, 32(29.1%) from pus swabs, 6 (5.5%) from pus aspirates, and 5(4.5%) from urine samples. Various other studies also have corroborated the increased isolation from blood.[5, 6, 17] The primary source of endogenous CoNS infections is the colonization of various skin and mucous membranes. They are, however, primarily transferred by medical and nursing procedures.[18] Studies have related the detection of two or more positive blood cultures to clinical bacteremia; however, it has been found that about 34% of nosocomial bacteremia have only one positive blood culture.[19] Coagulase-negative staphylococci are the most frequent cause of bacteremia associated with residential devices.[18] We had three isolates from indwelling devices in the present study, and the corresponding blood culture was also positive. Most of these infections are acquired in hospitals, and recent studies show that they are often due to strains that move through hospital patients.[19] In this study, pus isolates were mainly from post-operative wound infections, implying that CoNS can be a significant pathogen in infected wounds.

Identifying the CoNS species, biotypes, antimicrobial susceptibility, and clonality is one of the best methods to determine true bacteremia. There is a lower risk of contamination if the isolated CoNS species are similar or highly clonally related, indicating true bacteremia.[19] Although S. epidermidis has a high level of genomic diversity, other CoNS species like S. haemolyticus, S. lugdunensis, and S. schleiferi, have a lower level of diversity, as evidenced by pulsed-field gel electrophoresis (PFGE).[18]

In the present study, six different species/groups of CoNS were identified. S. epidermidis 54(49.1%) was the most frequent isolate, followed by S. haemolyticus 30 (27.3%), S. schleiferi 13 (11.8%), S. saprophyticus 9 (8.2%), S. hominis 3(2.7%), and S. lugdunensis 1 (0.9%). This is similar to the findings of various studies where S. epidermidis and S. haemolyticus were the most common isolates.[5, 17, 20] Even though S. epidermidis is a common member of the normal flora, its growing position as a nosocomial pathogen responsible for post-operative sepsis, medical implant device-related infections in immunocompromised patients, and other infections necessitates research into its virulence factors and antibiotic resistance.[17] The current study's recognition of S. epidermidis as a major CoNS supports this.

Among the 55 blood isolates in the present study, S. epidermidis was the predominant isolate, followed by S. haemolyticus. S. epidermidis is emerging as an important cause of morbidity and mortality in immunocompromised patients. It is the most common cause of septicemia in leukemia and lymphoma patients. CoNS account for most foreign body-related bloodstream infections (FBRIs) in both temporarily and permanently implanted devices. Most FBR-BSIs are catheter-related bloodstream infections (CRBSIs).[18] S. epidermidis was the species recovered from the three indwelling devices in the present study. S. saprophyticus was the only isolate from urine in the present study. S. saprophyticus subsp. saprophyticus prefers the rectum and genitourinary tract of young women and prefers hemagglutination and fibronectin attachment. It is also the second most reported cause of uncomplicated lower Urinary tract infections(UTIs) among sexually active young females.[19]

The biofilm development mode, which is involved in both chronic and acute infections and facilitates adherence and colonization on artificial materials, is a major contributor to CoNS pathogenesis in clinical settings. Furthermore, the proximity of cells inside a biofilm can promote plasmid exchange, allowing antimicrobial resistance to spread faster.[17] Among the 110 isolates, only 20 (18.2%) isolates showed biofilm production by Congo red agar method. It is very low in contrast to other studies where the slime production in CoNS ranged from 39.5%-74.6%5.[21-24] However, in the majority of the studies, higher biofilm production was detected by the Tissue culture plate (TCP)method, which was taken as the gold standard.[5, 21-24] The Congo red agar method was easier and faster to perform than other phenotypic methods, but the CRA method detected biofilm production comparably poorly. In various studies, it was discovered that adding Vancomycin at a sub-MIC(Minimum inhibitory concentration) (0.5 g/mL) to modified CRA and performing the CRA method on strains freshly isolated from clinical specimens increased the expression of genes related to biofilm formation.[24, 25] The differences in biofilm detection may be due to the various sources from which strains were isolated. Maximum biofilm production was seen in blood isolates (15/55 -27.3%), followed by pus swabs (4/32- 12.5%) and (1/6 - 16.7%) pus aspirates. Biofilm was produced maximum by S. epidermidis (26%) followed by S. haemolyticus (16.7%) and S. schleiferi (8%). Infections induced by medical devices, such as contaminated implants, urinary catheterization, and prosthetic valves are closely linked to biofilm development. S. epidermidis isolates were found to develop biofilms in all three cases with indwelling devices. This correlates with a study by Prasad S et al., who found that 26 (65.2 %) of 55 S.epidermidis isolates from various device-related infections were biofilm producers.[26] On the other hand, slime formation can be influenced by a variety of factors, including medium composition, the presence of starch, iron, and CO2, as well as oxidation. Biofilm was found in 14 adult patients, three children (1-10 years old), and three babies in our sample. These patients have various medical histories, including sepsis, pneumonia, surgical site infection, acute glomerulonephritis, and gangrene.

DNase (Deoxyribonuclease enzyme) converts tissue components into nutrients, allowing bacteria to expand and invade more easily. They also play a minor role in pathogenesis. Although DNase is not a fully accurate predictor of CoNS pathogenicity, it has been confirmed that CoNS has DNase activity.[27] In the present study, the rate of DNase positivity was 14.5%. Production of DNase was shown ranging from 1.8%-43.5% in various studies.[21, 28] It is a well-documented fact that DNase production is more for S. aureus when compared to S. epidermidis.

Antibiotic resistance in CoNS strains has been documented in growing numbers to widely used antibiotic groups such as Beta-lactam groups, macrolides, aminoglycosides, and, as a last resort, antibiotics such as glycopeptides.[19] The long-term efficacy of glycopeptides against methicillin-resistant coagulase-negative Staphylococci (MR-CoNS) has become a hot topic since the first reported cases of teicoplanin resistance in MR-CoNS in the United States and the United Kingdom.[29] Glycopeptide-resistant CoNS has been identified in patients receiving long-term vancomycin treatment since then, reducing therapeutic options. In this study, resistance to Penicillin was high 95.5%, followed by Erythromycin 80.9%, Clindamycin 52.7%, Cotrimoxazole 18.2%, Cefoxitin 17.3%, Gentamicin 2.7%. The increased resistance to Penicillin and Erythromycin has been reported by various studies.[5, 14, 19, 30] It may be attributed to Penicillin's selective pressure in analogues, widely prescribed in outpatient cases. All isolates were sensitive to Vancomycin. In various studies conducted in India, all isolates were found to be sensitive to Vancomycin.[5, 22, 30] According to the CDC's guidelines, Vancomycin is only recommended when CoNS is isolated from several blood cultures to prevent the spread of vancomycin resistance.

MR-CoNS isolates were 17.3% in the present study. Various studies have shown that the Methicillin resistance rate in CONS ranges from 16.28%-65.33%.[3, 5, 14, 30] The high prevalence of methicillin resistance among CoNS may be due to a genetic exchange of resistance between CoNS and S. aureus, posing a therapeutic challenge. Methicillin resistance rises with time spent in the hospital and exposure to antibiotics, especially semisynthetic Penicillins. In a study by Kashid et al. in health workers, it was found that 35,7% of MR-CONS was from anterior nares of doctors, making them an important link in the chain of transmission of infection.[14] The biofilm serves as a barrier to bacterial cell eradication and confers high resistance to antimicrobial agents. In our research, antibiotic resistance was found to be higher in biofilm-producing CoNS isolates than in non-biofilm-producing CoNS isolates. Various studies have come up with similar results.[5, 22, 23] In the war against virulent multidrug-resistant CoNS, a combination of successful identification and good infection management practice is crucial.

5. Conclusion

The study revealed S. epidermidis as the predominant CoNS isolate from various clinical samples with maximum biofilm and Dnase production in our institution. There was also Increased resistance to Penicillin and Erythromycin. Given that the etiological significance of CoNS has been overlooked in the past, the current study confirms that species-level recognition and antibiograms and the integration of methods to detect biofilm development in routine laboratory setup may help improve antibiotic choice and prevent microbiologists from issuing incorrect reports.

Conflict of Interest

The authors declared that there is no conflict of interest.

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