Original Article

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Antibiotic Susceptibility and Biofilm Production among Coagulase Negative Staphylococci Isolated from Clinical Samples at Tertiary Care Hospital

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ABSTRACT

Background: Coagulase Negative Staphylococci have been widely associated with medical device implant treatment and immune-compromised patients. Despite having increasing interest in Coagulase Negative Staphylococci, few studies from Nepal have reported the association of these organisms with urinary tract infections, conjunctivitis, high vaginal swabs, and cerebrospinal fluid. This study was carried out to determine antibiotic susceptibility pattern and biofilm production among Coagulase Negative Staphylococci isolated from clinical samples at tertiary care hospital.

Methods: This study was a hospital based cross-sectional study in which 3690 clinical samples were included. Isolation and identification of isolates was done following standard microbiological protocol. Coagulase Negative Staphylococci were identified phenotypically on the basis of gram staining, slide and tube coagulase test and by various sugar fermentation tests. Antibiotic susceptibility test was done following Kirby Bauer disk diffusion method (Clinical and Laboratory Standards Institute 2020). Biofilm production was determined by Tissue Culture Plate technique.

Results: A total of 113 isolates of Coagulase Negative Staphylococci were detected. Among them S. epidermidis (45.1%), S. saprophyticus (23.9%), S. haemolyticus (16.8%), S. hominis (5.3%), S. capitis (2.7%), S. cohini (1.8%), S. lugdunensis (1.8%) and S. sciuri (2.7%) were identified phenotypically. All isolates were found to be resistant against Ampicillin and 111 (98.2%) were sensitive against Linezolid.23.9% of CoNS were strong biofilm producers, 19.5% moderate and 56.6 % were non/weak biofilm producers.

Conclusions: It requires susceptibility test for prescribing antibiotics against Coagulase Negative Staphylococci in hospital and the misuse of antibiotics should be prevented.

Keywords: Clinical samples, CoNS, antibiotic susceptibility, biofilm

INTRODUCTION

Coagulase Negative Staphylococci (CoNS) is a large expanding group of bacteria with more than 50 species and 20 subspecies.[1] Despite of benign interaction with the host, CoNS are known to cause critical infections especially in immune compromised patients,[2,3] associated with a broad range of diseases of deep organs, such as heart, joints, bones, and even the central nervous system.[4]CoNS have ability to adhere and form biofilm on the surface of biomaterials which is assumed to be the most significant virulence factor.[5,6] About 80% to 90% of CoNS isolated from hospital are either methicillin resistant or resistant to multiple antimicrobial agents.[5] The increasing use of modern medical devices in treatment, is also increasing the chances of infections due to CoNS. The proper identification and diagnosis of CoNS help to minimize the threat among implant and immune compromised patients. Therefore, this study was

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carried out to isolate and identify CoNS phenotypically, determine antibiotic susceptibility pattern and the analysis of biofilm production of isolated CoNS at Nepal Armed Police Force Hospital (APF), Kathmandu, Nepal.

METHODS

This was a hospital based cross sectional study in which the collection, isolation, processing and phenotypic identification of CONS were carried out in the Department of Microbiology, Nepal Armed Police Force Hospital, Kathmandu from November 2021 to September 2022. A total of 3690 clinical samples (pus/wound swab, blood, urine, sputum, semen, body tissue, body fluids/tips), collected in sterile container were processed for routine culture. The samples received were subjected to gram staining and culture.

The clinical samples were inoculated into Blood agar (BA), MacConkey agar (MA) and cysteine lactose and electrolyte deficient (CLED) agar media (for urine sample). The incubation time for urine sample was 24 hours and for pus/wound swab, sputum and body fluids on MA and BA media, it was 48 hours aerobically at 37°C. Similarly, central venous catheter and catheter tips were first mixed with 2 ml of nutrient broth (NB) followed by vortexing and streaking on MA and BA media and incubated for 48 hours aerobically at 37°C. Whereas, the blood sample was poured in Brain Heart Infusion (BHI) broth in 1:10 ratio and sub cultured after 24 hours of enrichment at 37°C aerobically on MA and BA media for consecutive 7 days. The Gram positive bacterial isolates were sub-cultured in nutrient agar (NA) and Mannitol Salt Agar (MSA). CoNS were identified on the basis of gram staining, catalase test, O/F test, slide and tube coagulase test and phenotypically by various biochemical tests including various carbohydrate fermentation tests.[7-9]

Antibiotic susceptibility test: The antibiotic susceptibility of CONS was done as per Kirby Bauer disk diffusion method recommended by CLSI 2020 using Muller Hinton Agar (MHA).[10]

Screening of biofilm production: Tissue Culture Plate (TCP) method was used to detect biofilm producers among CONS. Isolates were inoculated in 10 ml of trypticase soy broth with 1% glucose and incubated at 37°C for 24 hours followed by dilution in 1:100 ratio with fresh medium. Individual wells of sterile TCPs were filled with 200 µl of the diluted culture including negative controls (sterile media) and positive control (*S. aureus* ATCC 25923). Plates were incubated at 37°C for 24 hours. The contents of each well were then removed by gentle tapping and washed with 0.2 ml of phosphate buffered saline (pH 7.2) for four times. 200 µl of 2% sodium acetate was used as fixative, kept for 10 minutes and discarded. 200 µl of 0.1% crystal violet was filled in each well to stain the biofilm formed for 30 minutes. Excess stain was removed by using deionized water and plates were dried. Optical density of stained adherent biofilms was read by micro ELISA auto reader (model 680, Biorad, UK) at a wavelength of 570 nm. [11]The interpretation of biofilm production was done according to the criteria of Stepanovic et al. [12] The test was performed in triplicate for each test organism in a microtitre plate and tests were repeated for 3 times.

Average OD value Biofilm Production

OD ≤ ODc No biofilm production

ODc < OD ≤ 2*ODc Weak biofilm production

2*ODc < OD ≤ 4*ODc Moderate biofilm production

4*ODc < OD Strong biofilm production

Optical density cut-off value (ODc) = Average OD of negative control + 3* standard deviation (SD) of negative control.

Data analysis: Microsoft Excel for Windows 10 was used to manage the data gathered from the log entry and laboratory analysis.

Ethical approval: Ethical approval was received from Nepal Health Research Council (Ref. No. 727) and consent was obtained from patients.

RESULTS

A total of 3690 clinical samples including blood (382), urine (2137), pus/wound swab (498), sputum (487), body fluids/tips (146), semen (27) and body tissue (13) were subjected for culture. Out of 3690 clinical samples, 861 (23.3%) showed bacterial growth among which 489 (56.8%) were gram negative isolates, 26 (3%) *Streptococcus* spp and 346 (40.2%) were *Staphylococcus* spp. (Table 1)

Table 1. Sample wise distribution of Gram negative and Gram positive bacteria								
Culture	Pus/ Wound Swab	Urine	Blood	Body Fluids/ tips	Sputum	Semen	Body Tissue	Total
No growth	217(43.6)	1858(86.9)	319(83.5)	71(48.6)	348(71.5)	11(40.7)	5(38.5)	2829(76.6)
Gram negative isolates	165(33.1)	151(7.1)	28(7.3)	51(35)	87(18)	4(14.8)	3(23)	489(13.3)
Staphylococcus spp	116(23.3)	119(5.6)	34(8.9)	24(16.4)	36(7.4)	12(44.5)	5(38.5)	346(9.4)
Streptococcus spp	0(0)	9(0.4)	1(0.3)	0(0)	16(3.3)	0(0)	0(0)	26(0.7)
Total	**498(100)**	**2137(100)**	**382(100)**	**146(100)**	**487(100)**	**27(100)**	**13(100)**	**3690(100)**

Among 346 *Staphylococcus* spp, 233 (67.3%) isolates were found to be *S. aureus* and remaining 113 (32.7%) were CoNS. Among CoNS highest isolates were from urine 59 (52.2%) followed by pus/wound swab 33 (29.2%) and no isolation from sputum. (Figure 1)

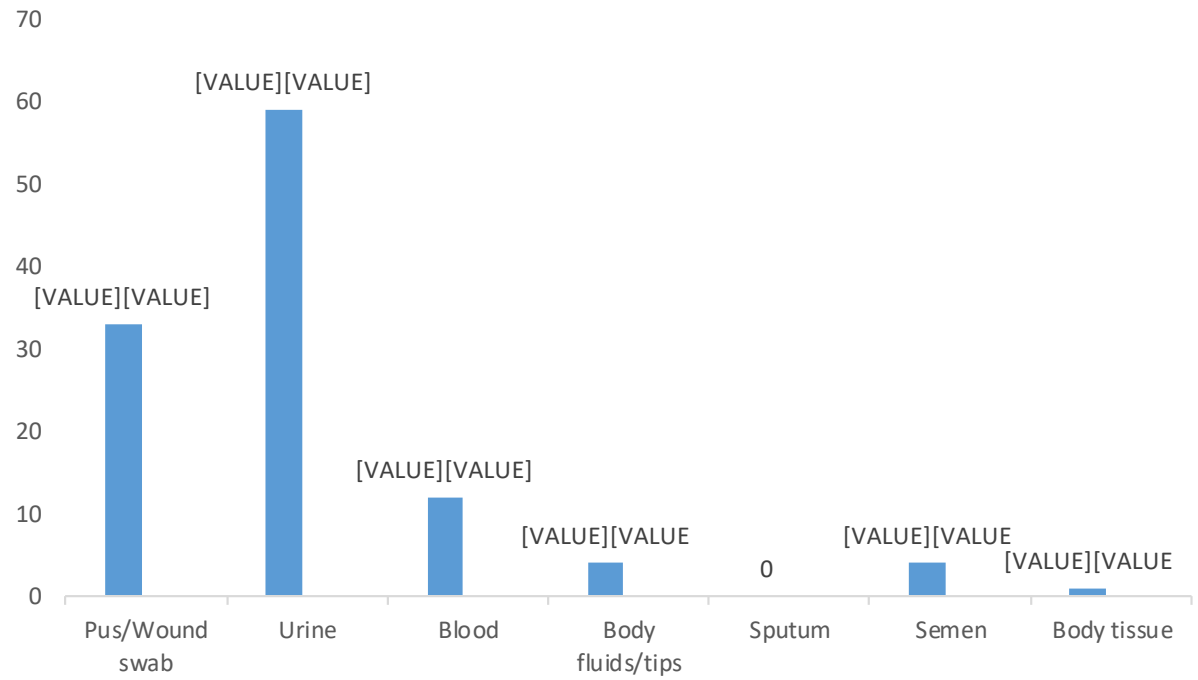

Figure 1. Distribution of CoNS among different clinical samples.

Based on various biochemical and sugar fermentation tests, 8 species of CoNS were determined. Highest isolates were *S. epidermidis* 51 (45.1%), followed by *S. saprophyticus* 27 (23.9%), *S. haemolyticus*19 (16.8%), *S. hominis*6 (5.3%), *S. capitis*3 (2.7%), *S. cohini* and *S. lugdunensis*2 (1.8%) each, whereas *S. sciuri* isolates were 3 (2.7%).

S. epidermidis, S. saprophyticus and *S.capitis* were identified mostly from urine sample 32 (62.7%) , 25 (92.6%) and 2 (66.7%) respectively, *S. haemolytics* and *S. hominis* from pus/wound swab 12 (63.2%) and 5 (83.3%), *S. cohini* 1 (50%) each from blood and body fluids/tips, *S. lugdunensis* 2 (100%) from body fluids/tips and *S. sciuri* from 2 (66.6%) blood. (Table 2)

Table 2.Distribution of CoNS species among clinical samples.							
Species	**Pus/Wound Swab**	**Urine**	**Blood**	**Body fluids/tips**	**Semen**	**Body tissue**	**Total**
S. *epidermidis*	14(27.5%)	32(62.7%)	3(5.9%)	1(2%)	1(2%)	0	51 (45.1%)
S. *saprophyticus*	0	25(92.6%)	0	0	2(7.4%)	0	27(23.9%)
S. *haemolyticus*	12(63.2%)	0	5(26.3%)	0	1(5.3%)	1(5.3%)	19(16.8%)
S. *capitis*	1(33.4%)	2(66.6%)	0	0	0	0	3(2.7%)
S. *cohini*	0	0	1(50%)	1(50%)	0	0	2(1.8%)
S. *lugdunensis*	0	0	0	2(100%)	0	0	2(1.8%)
S. *hominis*	5(83.3%)	0	1(16.7%)	0	0	0	6(5.3%)
S. *sciuri*	1(33.4%)	0	2(66.6%)	0	0	0	3(2.7%)
Total	33	59	12	4	4	1	113

Similarly, among 113 isolates of CoNS, maximum number of isolates 111 (98.2%) were sensitive against Linezolid, whereas 113 (100%) were resistant to Ampicillin. Individual resistance pattern of CoNS species is elaborated in below. (Table 3)

Table 3. Antibiotic resistance pattern of individual species of CoNS [N(%)] .								
Antibiotics	S. *epidermidis* N= 51	S. *saprophyticus* N= 27	S. *haemolyticus* N= 19	S. *hominis* N= 6	S. *capitis* N= 3	S.*cohini* N= 2	S.*lugdunensis* N= 2	S. *sciuri* N= 3
Gentamicin	2(3.9)	6(22.2)	4(21.1)	4(66.7)	0	2(100)	1(50)	3(100)
Azithromycin	45(88.2)	24(88.9)	14(73.7)	6(100)	3(100)	2(100)	2(100)	3(100)
Ciprofloxacin	6(11.8)	5(18.5)	7(36.8)	5(83.3)	2(66.7)	2(100)	2(100)	3(100)
Levofloxacin	31(60.8)	8(29.6)	10(52.6)	3(50)	2(66.7)	1(50)	2(100)	3(100)
Norfloxacin	27(52.9)	9(33.3)	5(26.3)	5(83.3)	3(100)	1(50)	2(100)	3(100)
Clindamycin	21(41.2)	12(44.4)	12(63.2)	4(66.7)	3(100)	1(50)	2(100)	2(66.7)
Cotrimoxazole	24(47.1)	17(63)	9(47.4)	1(16.7)	2(66.7)	2(100)	1(50)	3(100)
Chloramphenicol	15(29.4)	13(48.1)	13(68.4)	2(33.3)	2(66.7)	2(100)	2(100)	2(66.7)
Ampicillin	51 (100)	27(100)	19(100)	6(100)	3(100)	2(100)	2(100)	3(100)
Linezolid	0	0	0	1(16.7)	0	0	0	0
Ceftriaxone	14(27.5)	7(25.9)	5(26.3)	3(50)	1(33.3)	0	1(50)	2(66.7)

27 (23.9%) were strong biofilm producers, 22 (19.5%) moderate and 64 (56.6%) were non/weak biofilm producers. Among 8 species, highest number of S. *haemolyticus* 10 (8.8%) were strong biofilm producers and all 3 (2.7%) isolates of S. *sciuri* were also strong biofilm producers. (Table 4)

Table 4. Biofilm production by various species of CoNS.									
Biofilm Formation	S. *epdermidis*	S. *saprophyticus*	S. *haemolyticus*	S. *capitis*	S. *cohini*	S. *lugdunensis*	S. *hominis*	S. *sciuri*	Total
Strong	4(3.5)	7(6.2)	10(8.8)	1(0.9)	0	1(0.9)	1(0.9)	3(2.7)	27(23.9)
Moderate	16(14.2)	2(1.8)	3(2.7)	0	0	1(0.9)	0	0	22(19.5)
Non/Weak	31(27.4)	18(15.9)	6(5.3)	2(1.8)	2(1.8)	0	5(4.4)	0	64(56.6

DISCUSSION

Patients under treatment with indwelling devices or immune compromised are more prone to get CoNS infections. Therefore, medical devices used in the treatment, have often been associated with nosocomial infections, [13, 14, 15]increasing the clinical significance of CoNS. In this study, 3690 clinical samples were included, among which 861 (23.3%) samples were culture positive. Among total culture positive samples, we isolated 346 (40.2%) *Staphylococci* spps. The majority species were *S. aureus* 233 (67.3%) and remaining 113 (32.7%) were CoNS. In the studies conducted by Pandey et al. [16] and Abdel et al. [17], *S. aureus* isolates were more than CoNS.

Among CoNS, 8 species of CONS have been identified phenotypically. The maximum isolates were *S. epidermidis* (51, 45.1%), followed by *S. saprophyticus* (27, 23.9%), *S. haemolyticus*(19, 16.8%), *S. hominis*(6, 5.3%), *S. capitis*(3, 2.7%), *S. cohini* and *S. lugdunensis*(2, 1.8%) each, whereas *S. sciuri* isolates were (3, 2.7%). This finding correlates with other studies in which the maximum isolates are *S. epidermidis.*[19] Similarly the highest frequency of *S. saphrophyticus* from urine sample, is consistent to with previous findings[18,19] and can be related to the capacity of *S. saprophyticus* to colonize the urinary tract of woman.[20]

Antibiotic resistance is the major challenge in the treatment of patient. This study revealed 100% CoNS were resistant to Ampicillin followed by Azithromycin 99 (87.6%). However, Linezolid was highly effective against 111 (98.2%) CoNS isolates followed by Gentamicin 91 (80.5%). The higher resistance to Ampicillin and sensitivity towards Linezolid was also previously reported in Nepal.[18]

Biofilm production is one of the strong factor for pathogenicity in *Staphylococcus* spp. In our study, 27 (23.9%) were strong and 22 (19.5%) moderate biofilm producers. The findings were lower in comparison to other studies conducted in Nepal in 2021 and 2017.[18,19] The difference could be due to the variation in environment, nutrition, stress and so on.[5]

CONCLUSIONS

The isolation and identification of CoNS from various clinical samples showed the variety of infections that can be caused by CoNS. The CoNS isolates showed resistance to commonly used antibiotics urging the need to implement proper strategies to discourage over or self- prescribed medication. Strong biofilm production by *S. haemolyticus* isolates also suggests that the possible treatment failure in systemic infection could be due to production of biofilm.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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