Original Article

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Speciation and Antibiotic Susceptibility Pattern of Coagulase Negative Staphylococci in a Tertiary Care Hospital of Manipur, India

Microbiology Section

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ABSTRACT

Introduction: Coagulase Negative Staphylococci (CoNS) are common opportunistic pathogens. They are increasingly being recognised as nosocomial pathogens and are associated with multiple antimicrobial resistance mechanisms particularly methicillin resistance. Therefore, rapid and reliable identification upto the species level is necessary to predict the potential pathogenicity or antibiotic susceptibility of each clinical isolate.

Aim: The aim of the present study was isolation and speciation of CoNS from various clinical samples, and to determine their antibiotic susceptibility pattern.

Materials and Methods: This study was a hospital-based cross-sectional study carried out in the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India, during the period from September 2017-August 2019. Total 135 CoNS isolates were identified using conventional microbiological procedures and speciation was done following the scheme of Kloos and Schleifer. Antibiotic susceptibility was determined by using the Kirby Bauer's disk diffusion method. Detection of methicillin resistance among CoNS was performed using cefoxitin disk (30 µg) diffusion method. Data analysis was done using descriptive statistics.

Results: CoNS isolates were identified from different clinical specimens, which included 88 (65.2%) from urine, 37 (27.5%) from blood, 3 (2.2%) from pus, 2 (1.5%) from catheter tip, 2 (1.5%) from wound swab, 1 (0.7%) each from aural swab, sputum and ascitic fluid. Predominant isolates were *Staphylococcus epidermidis* (40.7%) followed by *Staphylococcus haemolyticus* (19.3%), *Staphylococcus hominis* (11.9%), *Staphylococcus xylosus* (7.4%), *Staphylococcus saprophyticus* (6.0%), *Staphylococcus schleiferi* (5.2%), *Staphylococcus simulans* (4.4%), *Staphylococcus warneri* (3.0%), *Staphylococcus lugdunensis* (0.7%), *Staphylococcus capitis* (0.7%) and *Staphylococcus cohnii* (0.7%). Most isolates were resistant to penicillin (84.5%) and erythromycin (59.3%), and least to tigecycline (2.2%). No resistance to vancomycin and linezolid was seen. Methicillin sensitive CoNS (MSCoNS) was detected in 79 (58.5%) isolates and methicillin resistant CoNS (MRCoNS) in 56 (41.5%) isolates.

Conclusion: This study demonstrated the occurrence of various species of CoNS in our healthcare set up with varying antimicrobial susceptibility pattern. Therefore, there is a need for accurate identification to species level by simple, inexpensive methodology and their antibiogram.

Keywords: Antibiogram, Identification, Nosocomial, *Staphylococcus epidermidis*

INTRODUCTION

The CoNS are considered as the normal flora of human skin and mucous membranes. The definition of this group of bacteria is still based on diagnostic procedures that need to differentiate between *Staphylococcus aureus* and those staphylococci classified as being less or non pathogenic [1].

It is important to identify CoNS up to the species level, as the epidemiology, pathogenicity and drug resistance varies from species to species [2]. The CoNS constitute all species of staphylococci other than *Staphylococcus aureus*, also form clusters but small colonies on solid media and comprise of approximately 40 species, of which, several species have been recognised as potential pathogens to humans [3]. The most common human pathogens include *S. epidermidis, S. haemolyticus, S. hominis, and S. saprophyticus.* Other significant opportunistic but rarely isolated species are *S. warneri, S. lugdunensis, S. capitis, S. simulans, S. cohnii, S. saccharolyticus,* and *S. xylosu* [4].

In the past, CoNS were generally considered to be contaminants having little clinical significance. However, they are increasingly being recognised as nosocomial pathogens, probably due to their abilities to act as opportunistic pathogens or due to the ability to survive on synthetic medical devices and equipment like intravenous catheters, prosthetic heart valves, orthopaedic implants, and also on various surfaces in hospitals for weeks to months [5]. *S. epidermidis* is able to colonize foreign bodies such as vascular catheters or indwelling prosthesis. *S. saprophyticus* is an important pathogen of Urinary Tract Infection (UTI) in younger, sexually active women [6].

Another concern is the rising occurrence of methicillin-resistant MRCoNS in hospitalised patients [7]. Overall higher incidence of resistance to all antibiotics is observed with MRCoNS as compared to MSCoNS particularly to non-beta-lactam antimicrobials [8].

Though the occurrence of CoNS as important pathogens of nosocomial infections has been reported worldwide as well as from different parts of India [9-16], no such study has been undertaken extensively in Manipur, India. Hence, the proposed study is an attempt to identify and speciate CoNS and their antibiogram from the various clinical samples.

MATERIALS AND METHODS

This study was a hospital-based cross-sectional study carried out in the bacteriology section of Microbiology Department, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India, during the period from September 2017 to August 2019. Informed written consent was obtained from participating individuals. In case of minors, informed consent was taken from the parents/ legal guardians. Privacy and confidentiality was maintained in all the cases. Approval of ethical committee was obtained from the Institutional Ethical Committee (IEC) JNIMS vide no. Ac/06/IEC/ JNIMS/2017(PGT) dated: Imphal, the 26th August, 2017.

Inclusion criteria: Patients of all age group and both sex with a history of UTI, prolonged urinary catheterisation, neonatal sepsis, intravenous access for delivery of medications and transfusions or nutrition, presence of intravascular catheters or implants and wound infections, attending outpatient and inpatient departments of Medicine, Surgery, Obstetrics and Gynaecology, Paediatrics, Orthopaedics and intensive care unit were included in the study.

Exclusion criteria: Clinical samples yielding polymicrobial growth, patients with history of prior antimicrobials administration and who refused to participate were excluded.

Study Procedure

Specimen collection: Clinical samples such as urine, blood, pus, wound swab, aural swab, catheter tip, ascitic fluid or sputum were collected from various inpatient and outpatient departments.

Identification, speciation and antibiogram of the isolates: A total of 135 CoNS isolates were identified on the basis of conventional microbiological procedures [17]. Speciation of CoNS was done following the scheme of Kloos and Schleifer which was based on slide and tube coagulase tests, ornithine decarboxylase, Voges-Proskauer (VP) test, urease test, novobiocin (5 µg) disk test, and sugar fermentations of mannose, mannitol, trehalose, lactose, and xylose [18].

Antibiotic susceptibility was determined by using the Kirby Bauer's disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) recommendations [19] using the Mueller Hinton agar (Hi-Media, Mumbai, India) and commercially available 6 mm antimicrobial disks of penicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), nitrofurantoin (300 µg), cotrimoxazole (1.25/23.7 µg), ciprofloxacin (5 µg), amikacin (30 µg), linezolid (30 µg) and tigecycline (15 µg).

Antimicrobial susceptibility testing of vancomycin was performed using vancomycin Minimum Inhibitory Concentrations (MIC) E-test strip E-test -Vancomycin (E-VA) having concentration of 0.016 to 256 µg/mL (Bio Mérieux India Pvt., Ltd., New Delhi, India) following manufacturer guidelines.

Detection of methicillin resistance among CoNS was performed using cefoxitin disk (30 µg) diffusion method. Diameter of the circular zone of inhibition ≥25 mm was interpreted as sensitive and ≤24 mm as resistant for CoNS, except for *S. lugdunensis* for which zone diameter ≤21 mm was considered as resistant [19].

Quality control: Every batch of media prepared was checked for sterility for 24 hours. Potency of disk used will be checked with *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923.

STATISTICAL ANALYSIS

Descriptive statistics like percentage and proportion were used to present the data. Analysis was done using Epi Info 7. Level of significant in methicillin sensitive and methicillin resistant CoNS isolates was determined using Chi-square test. A $p<0.05$ was considered significant.

RESULTS

During the study period of two years, 135 CoNS isolates were identified from different clinical specimens, which included 88 (65.1%) from urine, 37 (27.4%) from blood as shown in [Table/Fig-1].

Samples	No. of isolates	%
Urine	88	65.2
Blood	37	27.5
Pus	3	2.2
Catheter tip	2	1.5
Aural swab	1	0.7
Wound swab	2	1.5
Sputum	1	0.7
Ascitic fluid	1	0.7
Total	135	100

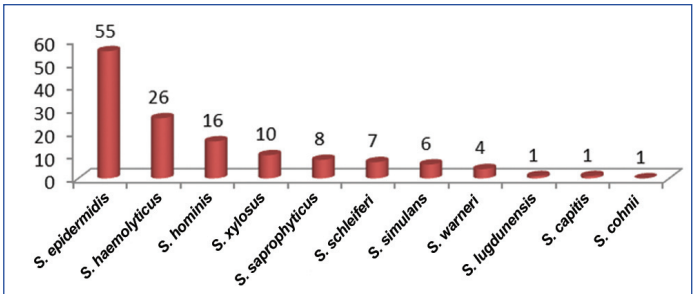
[Table/Fig-1]: Distribution of samples.

A total of 39 (28.88%) isolates were identified in age group of 20-29 years and least 3 (2.2%) isolates in 80 years and above. Majority of the isolates were recovered from female (74.8%) as compared to male (25.2%) [Table/Fig-2].

Age groups (years)	No. of samples collected (%)	Gender	
		Male (%)	Female (%)
<10	33 (24.4)	17 (12.59)	16 (11.85)
10-19	13 (9.6)	6 (4.4)	7 (5.2)
20-29	39 (28.98)	2 (1.48)	37 (27.4)
30-39	20 (14.8)	1 (0.74)	19 (14.1)
40-49	10 (7.4)	3 (2.2)	7 (5.1)
50-59	9 (6.7)	3 (2.2)	6 (4.4)
60-69	8 (6%)	2 (1.48)	6 (4.4)
70-79	0	0	0
80-above	3 (2.2)	0	3 (2.2)
Total	135 (100)	34 (25.2)	101 (74.8)

[Table/Fig-2]: Distribution of isolates among different age groups and gender.

The predominant isolates were *S. epidermidis* (40.7%) followed by *S. haemolyticus* (19.3%) and *S. hominis* (11.9%) as shown in [Table/Fig-3,4].



[Table/Fig-3]: Bar graph showing CoNS isolates.
Values given as frequency (n)

CoNS species	Urine (%)	Blood (%)	Pus (%)	Catheter tip (%)	Aural swab (%)	Sputum (%)	Ascitic fluid (%)	Wound swab (%)	Total (%)
S. epidermidis	33 (60)	17 (31)	1 (1.8)	2 (3.6)	1 (1.8)	1 (1.8)	0	0	55 (40.7)
S. haemolyticus	16 (61.5)	9 (35)	0	0	0	0	1 (3.8)	0	26 (19.3)
S. hominis	10 (62.5)	5 (31.3)	1 (6.2)	0	0	0	0	0	16 (11.9)
S. xylosus	7 (70)	2 (20)	1 (10)	0	0	0	0	0	10 (7.4)
S. saprophyticus	8 (100)	0	0	0	0	0	0	0	8 (6.0)
S. schleiferi	7 (100)	0	0	0	0	0	0	0	7 (5.2)
S. simulans	4 (67)	0	0	0	0	0	0	2 (33)	6 (4.4)
S. warneri	0	4 (100)	0	0	0	0	0	0	4 (3.0)
S. lugdunensis	1 (100)	0	0	0	0	0	0	0	1 (0.7)

S. capitis	1 (100)	0	0	0	0	0	0	0	1 (0.7)
S. cohnii	1 (100)	0	0	0	0	0	0	0	1 (0.7)
Total	88 (65.2)	37 (27.5)	3 (2.2)	2 (1.5)	1 (0.7)	1 (0.7)	1 (0.7)	2 (1.5)	135 (100)

[Table/Fig-4]: Distribution of CoNS species in various clinical specimens.

Maximum number of samples was urine (65.2%) samples followed by blood (27.5%) and the distribution of individual species of CoNS varied in different samples is shown in [Table/Fig-4]. Majority of *S. epidermidis* (19/55 or 34.55%) and *S. haemolyticus* (8/26 or 30.77%) were observed in age groups of 20-29 years as showed in [Table/Fig-5]. The maximum number of isolates was resistant to penicillin 114 (84.5%), followed by erythromycin 80 (59.3%), ciprofloxacin 57 (42.2%), cotrimoxazole 48 (35.5%), clindamycin 36 (26.7%), nitrofuratoin 14 (10.4%), and amikacin 11 (8.2%) as displayed in [Table/Fig-6]. All the 135 isolates remained between the MIC of 0.016 µg/mL and 2 µg/mL. 41 isolates had shown MIC of 0.064 µg/mL followed by 34 isolates of 0.032 µg/mL to vancomycin as shown in [Table/Fig-7]. The MSCoNS was detected in 79 (58.5%) isolates and MRCoNS in 56(41.5%) isolates. All the isolates of MRCoNS were found to be resistant to penicillin (100%) and least to vancomycin and linezolid [Table/Fig-8].

Age groups in years / CoNS isolates	<10 (n=33)*	10-19 (n=13)	20-29 (n=39)	30-39 (n=20)	40-49 (n=10)	50-59 (n=9)	60-69 (n=8)	70-79 (n=0)	≥80 (n=3)	Total
S. epidermidis	13	5	19	6	5	3	3	0	1	55
S. haemolyticus	6	2	8	2	2	4	0	0	2	26
S. hominis	5	2	2	4	2	1	0	0	0	16
S. xylosus	4	0	1	1	1	0	3	0	0	10
S. saprophyticus	0	3	3	2	0	0	0	0	0	8
S. schleiferi	1	1	3	2	0	0	0	0	0	7
S. simulans	0	0	1	2	0	1	2	0	0	6
S. warneri	4	0	0	0	0	0	0	0	0	4
S. lugdunensis	0	0	0	1	0	0	0	0	0	1
S. capitis	0	0	1	0	0	0	0	0	0	1
S. cohnii	0	0	1	0	0	0	0	0	0	1
Total	33	13	39	20	10	9	8	0	3	135

[Table/Fig-5]: Distribution of CoNS isolates among the different age groups.
*n: No. of isolates

Antimicrobials / *CoNS	P (%)	AK (%)	CIP (%)	E (%)	CD (%)	COT (%)	NIT (%)	LZ (%)	TIG (%)
S. epidermidis (n=55)	45 (82)	1 (1.8)	25 (45.5)	29 (52.7)	13 (23.6)	27 (49)	6 (10.9)	0	2 (3.6)
S. haemolyticus (n=26)	24 (92.3)	4 (15.3)	15 (57.6)	17 (65.3)	6 (23)	8 (30.7)	2 (7.69)	0	1 (3.8)
S. hominis (n=16)	14 (87.5)	0	6 (37.5)	8 (50)	1 (62.5)	4 (25)	1 (62.5)	0	0
S. xylosus (n=10)	10 (100)	4 (40)	4 (40)	9 (90)	8 (80)	2 (20)	5 (50)	0	0
S. saprophyticus (n=8)	6 (75)	1 (12.5)	2 (25)	4 (50)	0	0	0	0	0
S. schleiferi (n=7)	7 (100)	1 (14.3)	3 (42.8)	5 (71.4)	2 (28.5)	2 (28.5)	0	0	0
S. simulans (n=6)	4 (66.7)	0	2 (33.3)	4 (66.7)	2 (33.3)	3 (50)	0	0	0
S. warneri (n=4)	2 (50)	0	0	2 (50)	2 (50)	1 (25)	0	0	0
S. lugdunensis (n=1)	1 (100)	0	0	1 (100)	1 (100)	0	0	0	0
S. capitis (n=1)	1 (100)	0	0	1 (100)	1 (100)	1 (100)	0	0	0
S. cohnii (n=1)	0	0	0	0	0	0	0	0	0
Total (135)	114 (84.5)	11 (8.2)	57 (42.2)	80 (59.3)	36 (26.7)	48 (35.5)	14 (10.4)	0	3 (2.2)

[Table/Fig-6]: Overall antibiotic resistance pattern of CoNS isolates.
*CoNS: Coagulase-negative staphylococci; N: No. of isolates; P: Penicillin; AK: Amikacin; CIP: Ciprofloxacin; E: Erythromycin; CD: Clindamycin; COT: Co-trimoxazole; NIT: Nitrofurantoin; LZ: Linezolid; TIG: Tigecycline

CoNS isolates (n=No. of isolates)	MIC (µg/mL) of vancomycin										
	0.016	0.032	0.064	0.125	0.25	0.50	1	2	4	8	>16
S. epidermidis (n=55)	0	15	10	9	15	3	1	2	0	0	0
S. haemolyticus (n=26)	0	6	7	8	2	2	0	1	0	0	0
S. hominis (n=16)	0	5	3	3	0	4	1	0	0	0	0
S. xylosus (n=10)	0	3	6	1	0	0	0	0	0	0	0
S. saprophyticus (n=8)	1	2	4	1	0	0	0	0	0	0	0
S. schleiferi (n=7)	0	2	3	2	0	0	0	0	0	0	0
S. simulans (n=6)	0	0	4	2	0	0	0	0	0	0	0
S. warneri (n=4)	0	0	2	1	1	0	0	0	0	0	0
S. lugdunensis (n=1)	0	1	0	0	0	0	0	0	0	0	0
S. capitis (n=1)	0	0	1	0	0	0	0	0	0	0	0

S. cohnii (n=1)	0	0	1	0	0	0	0	0	0	0	0
Total 135	1	34	41	27	18	9	2	3	0	0	0

[Table/Fig-7]: Distribution of MIC of CoNS isolates (n=135) to vancomycin.
N: No. of isolates

Antimicrobials	MRCoNS (n=79) (%)	MSCoNS (n=56) (%)	p-value	Level of significance
Penicillin	79 (100%)	35 (62.5)	0.003	Significant
Amikacin	7 (8.9%)	4 (7.1)	0.012	Significant
Ciprofloxacin	42 (53.1%)	15 (26.8)	0.001	Significant
Erythromycin	50 (63.3%)	30 (25.8)	0.002	Significant
Clindamycin	20 (25.3%)	16 (25)	0.592	Not significant
Co-trimoxazole	27 (34.2%)	21 (37.5)	0.675	Not significant
Nitrofurantion	8 (10.1%)	6 (10.7)	0.007	Significant
Vancomycin	0	0	*	-
Tigecycline	3 (3.7)	0	0.011	Significant
Linezolid	0	0	*	-

[Table/Fig-8]: Resistant pattern of MRCoNs and MSCoNS.
N: No.of isolates
*p-value could not be determined

DISCUSSION

In the laboratory, identification of staphylococci is often limited to a screening test for *S. aureus*, while non *S. aureus* isolates are simply reported as CoNS. As the pathogenic significance of CoNS increases, it has become important to know regarding the epidemiology and pathogenic potential of individual species [20]. Therefore, rapid and accurate identification of CoNS species has gained importance in the recent few years.

In present study, majority of the isolates were obtained from urine (65.2%) followed by blood (27.5%). Alex AM et al., and Sharma P et al., reported that predominant isolates were from urine (62% and 36%, respectively) and blood (12.7% and 27%, respectively) [12,21]. A study by Sheik AF and Mehdinejad M showed a similar isolation rate from urine (51.5%) and blood (25.4%) [22]. The present study revealed that the predominant isolates were *S. epidermidis* (40.7%) followed by *S. haemolyticus* (19.3%), *S. hominis* (11.9%). These findings were correlated with the study done by Al Tayyar IA et al., in Jordan where *S. epidermidis* and *S. haemolyticus* were the most common species isolated from all specimens representing 54.7% and 23.4% of all CoNS species, respectively [10]. Comparative findings of CoNS isolates obtained from different studies are shown in [Table/Fig-9] [9-16]. The difference in the distribution of *CoNS* species among the various studies conducted in different parts of the country might be due to difference in geographical location and patient population. *S. epidermidis, S. haemolyticus, S. hominis* and *S. saprophyticus* were predominantly isolated from urine (60%, 61.5%, 62.5% and 100%, respectively) and blood (31%, 35%, 31.3% and 0, respectively). Nicolle LE et al., John JF Jr et al., Kumari N et al., and Asangi SY et al., obtained similar findings [23-26].

In this study, isolates were recovered more in female (74.8%) than male patients (25.2%). Age group of 20-29 years showed highest isolation of CoNS (28.9%) while no isolate was recovered from the age group of 70-79 years. Similar parameters were reported by Alex AM et al., [12]. On the contrary, Asangi SY et al., and Baddour LM and David L found majority of the CoNS isolates in males and above the age group of 40 years [26,27]. However, Roopa C and Biradar S revealed maximum number of isolates in the age group of 61-70 years with no particular gender predominance [9].

Antibiotic susceptibility testing has shown variability and multidrug resistance with maximum resistance to penicillin (84.5%) and least to tigecycline (2.2%). No resistance to vancomycin and linezolid was seen. Usha MG et al., Asangi SY et al., Sharma V et al., Pedroso SHSP et al., and Gunti R et al., have shown maximum resistance to penicillin, erythromycin, ciprofloxacin and cotrimoxazole with over 80% which correlate with the present study [2,26,28-30]. Alex AM et al., and Jayakumar R et al., noted in their studies that all the isolates were uniformly susceptible to vancomycin and linezolid [12,13].

This study demonstrated that the MIC of vancomycin against the CoNS isolates ranged between 0.016 to 2 µg/mL. Paiva RM et al., and Center KJ et al., reported higher range of MIC of vancomycin (0.38 to 4 µg/mL and 0.25 to 4 µg/mL, respectively) [31,32]. The vancomycin MICs at which 50% and 90% (MIC50 and MIC90) of isolates were inhibited for the total population of CoNS in the present study were 0.064 and 0.5 µg/mL, respectively. Paiva RM et al., and Center KJ et al., revealed higher MIC50 (1.5 µg/mL, 1 µg/mL, respectively) and MIC90 (2 µg/mL in both) of vancomycin [31,32].

Present study revealed a prevalence of MRCoNS in 58.5% isolates, similar to the finding of Singh S et al., (57.6%) [8]. Prevalence of MRCoNS ranging from 48.2% to 66% has been previously reported [33]. However, the proportion of resistance to methicillin was very high in a study conducted at China by Cui J et al., where it ranged from 83.3%-100% [34]. Highest methicillin resistant was found in *S. haemolyticus*, supporting the findings of other centres where resistance rates as high as 90% have been reported by Barros EM et al., (88%) [35].

An overall high prevalence of resistance to all antibiotics was seen with MRCoNS showing higher resistance to non beta-lactam antimicrobials as compared to MSCoNS, difference being statistically

CoNS species	Roopa C and, Biradar S Karnataka, 2015 [9]	Al Tayyar IA et al., Jordan, 2015 [10]	Kashid RA and Kausalya R Karnataka, 2016 [11]	Alex AM et al., Kerala, 2017 [12]	Jayakumar R et al., Tamil Nasdu, 2018 [13]	Senthilsevan B et al, Tamil Nadu, 2019 [14]	Kulkarni M and Patil S Mumbai, 2020 [15]	Raina D et al, Dehrdun, 2020 [16]	Present study, 2022
S. epidermidis	50.8	54.7	4	26.8	57.4	53	10.7	11.67	40.7
S. haemolyticus	26.7	23.4	44	56.3	10.48	41	25	25	19.3
S. hominis	-	5.8	-	-	-	-	1.8	-	11.9
S. xylosus	-	0.9	-	-	1.6	-	3.6	-	7.4
S. saprophyticus	4.46	3.1	-	4.9	12.9	-	26.8	6.67	6.0
S. schleiferi	7.1	-	2	3.5	3.23	3	3.6	1.67	5.2
S. simulans	-	0.9	5	-	-	-	7.1	10	4.4
S. waneri	-	1.8	30	5.6	-	3	-	20	3.0
S. lugdunensis	10.7	4	1	-	13.71	-	12.5	1.67	0.7
S. capitis	-	3.6	14	1.4	-	-	-	8.3	0.7
S. cohnii	-	-	-	-	-	-	1.8	1.67	0.7

[Table/Fig-9]: Comparison of distribution of CoNS species among various studies [9-16].
Values given in %

significant for amikacin, erythromycin, ciprofloxacin, nitrofurantoin and tigecycline. The non beta-lactam agents, most active against MRCoNS were clindamycin, nitrofurantoin and tigecycline probably due infrequent use at our centre, resulting in low selection pressure. Amikacin still remained sensitive to MRCoNS isolates despite its rampant administration. However, all MRCoNS isolates were susceptible to vancomycin and linezolid.

The strength of this study was that speciation of CoNS species could be carried out using simple phenotypic characteristics such as scheme of Kloos and Shchleifer and most findings of this study were correlated with other previous studies which followed the same scheme of characterisation.

Limitation(s)

Advance molecular methods for molecular characterisation of CoNS at the subspecies level could not be accessed due to lack of infrastructure.

CONCLUSION(S)

The clinical significance of CONS is increasing day by day. Therefore, accurate identification to species level using simple and inexpensive methodology is needed. *S. haemolyticus*, *S. epidermidis* and *S. hominis* were the common species isolated in this study. Most isolates were resistant to penicillin and erythromycin. However, no resistance to vancomycin and linezolid was observed.

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