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Original Research Article

Species distribution and antibiogram of coagulase negative Staphylococci isolated from various clinical specimens in a tertiary care hospital

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ABSTRACT

Background: Coagulase Negative Staphylococci (CoNS) are common inhabitants of skin and mucous membrane, may act as pathogens causing fatal infections especially in immunocompromised patients. CoNS mainly cause infections involving biofilm on implanted biomaterials. Increase in antimicrobial resistance causes difficulties to treat life threatening infections. Despite their growing importance, their speciation is rarely done. Therefore, the present study is undertaken to identify CoNS to the species level and to know their antibiotic susceptibility pattern along with rate of MRCoNS.

Materials and Methods: 250 isolates from various clinical specimens were considered in this study. The isolates were identified by colony morphology, Gram staining, catalase, slide and tube coagulase test. Speciation was done by Novobiocin resistance, urease activity, ornithine decarboxylase, pyrrolidonylarylamidase and aerobic acid production from mannose. The antimicrobial susceptibility was performed by Kirby-Bauer's disc diffusion method as per CLSI guidelines.

Results: Among 250 CoNS isolates, commonest species identified was *S.epidermidis*(59.2%) followed by *S.haemolyticus*(19.6%) and *S.saprophyticus*(12.4%). They were commonly isolated in the age group 21 to 30 years (26.8%) and among males(58%). Total of 33.2% were isolated from pus followed by blood(21.6%). Majority species expressed resistance towards nalidixic acid(97.2%) followed by Penicillin(94%), 74.4% to Amoxicillin-Clavulanic acid and 66.4% to Cotrimoxazole. All the isolates were sensitive to Vancomycin. Methicillin resistance among CoNS was 73.2%.

Conclusion: The increased recognition of pathogenic potential in CoNS and emergence of drug resistance among them demonstrates the need to adopt simple laboratory methods to identify the species and determine the antibiotic resistant patterns to help the clinicians in treating the infections caused by CoNS.

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1. Introduction

Staphylococci are Gram positive non-motile, non-spore forming, usually non-capsulated and most species are facultative anaerobes. They are indigenous micro flora of the skin and mucous membranes of humans. The presence or absence of coagulase, an enzyme that clots plasma, divides the Staphylococcus species into two broad groups:

the coagulase positive Staphylococci and coagulase negative Staphylococci (CoNS).¹

As of 2014, the genus Staphylococcus consists of 47 species and 23 subspecies that are validly described. Of these, 38 fulfill the categorization of a coagulase-negative species.²

Coagulase negative *Staphylococci* are among the bacteria routinely isolated at clinical Microbiology departments. Although long considered non-pathogenic, since they are components of human skin, respiratory mucosa and gastro-

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intestinal mucosal tissue, they are turning as significant etiological agents causing nosocomial infections, mainly in link with the presence of foreign bodies in the human beings.³ CoNS are associated with a myriad of disease like urinary tract infections (UTI), catheter related infections, shunt infections, pneumonia, endophthalmitis surgical wound infections, breast abscess, osteomyelitis, and native valve endocarditis.⁴ Generally CoNS present as indolent rather than acute infections and they are associated with various diseases, both community and hospital acquired. Most of the infections are associated with medical devices because of their propensity to form a biofilm.⁵ Perhaps the same type of biofilm may be active in devitalized tissue like the diabetic foot infection which may often be associated with isolates of CoNS.⁶

Regardless of species specificity, treatment of infections caused by CoNS has become increasingly difficult due to the high prevalence of antibiotic-resistant strains. Widely used antibiotics including penicillins, particularly semi-synthetic penicillins, cephalosporins, macrolides, aminoglycosides, and tetracyclines, have proven to be ineffective in inhibiting several prevalent species of CoNS, thus, augmenting the need for new and effective antimicrobials.^{7,8} Therapeutic options for the treatment of CoNS are limited because the vast majority of clinically recovered isolates are methicillin resistant (MRCoNS). Vancomycin resistance has also been emerged among the CoNS species.² Multiple antibiotic resistances are characteristics of hospital strains of CoNS.⁹

Along with the colony morphology and pigment, the simple conventional tests, rapid and molecular identification systems are being used for identification of CoNS. These include various enzyme activities, hemolysins, oxygen requirements, products of glucose metabolism, utilization of substrates, and production of acid from carbohydrates and intrinsic resistance to certain antibiotics.^{1,10}

CoNS identification which is still difficult for most clinical laboratory, is necessary in order to establish epidemiological trends, confirm treatment failure or determine the cause of specific infections. Therefore the present study has been undertaken to speciate CoNS and to know their drug susceptibility patterns and rate of MRCoNS.

2. Materials and Methods

2.1. Source of data

CoNS isolates from various clinical samples submitted to diagnostic microbiology Laboratory, Karnataka Institute of Medical Sciences, Hubballi

2.2. Study period

From January 2015 to December 2015

2.3. Study design

Cross sectional, Prospective study

During the study period, a total of 250 consecutive, non-duplicate CoNS species were isolated from various clinical specimens from different patients visiting and admitted to the Karnataka institute Medical Sciences, Hubballi, tertiary care hospital in North Karnataka. The Specimens were inoculated onto Chocolate agar and the isolates were initially identified by colony morphology, Gram staining, catalase test, slide and tube coagulase test. [Figures 1 and 2] CoNS speciation was done by Novobiocin resistance test, urease activity, ornithine decarboxylase, pyrrolidonylarylamidase and aerobic acid production from mannose. [Table 1] The antimicrobial susceptibility test was performed by Kirby-Bauer's disc diffusion method as per CLSI guidelines with the following panel of antibiotics.¹¹

Penicillin (10U), Amoxicillin/Clavulanic acid (20/10µg), Gentamicin (10µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), Trimethoprim/Sulphamethoxazole (1.25/23.75µg), Linezolid (30µg), Vancomycin (30µg), Teicoplanin (30µg), Nitrofurantoin (300µg) and Norfloxacin (10µg). Methicillin resistance was detected using cefoxitin (30µg) disc.¹¹

3. Results

Among 250 CoNS isolates, maximum number of CoNS isolated was from the age group of 21 to 30 years (26.8%) followed by neonates (13.6%) (Table 2). Male to female ratio is 1.36:1. (Graph 1) Maximum number of CoNS species was isolated from the samples received from various wards (52%) followed by ICUs (26%) among which samples from NICU (13.6%) account for the majority. 19.2% of CoNS were isolated from the samples of patients attended OPD clinics. Labour room and Cath-lab samples accounted for 2% and 0.8% respectively. (Table 3)

Present study speciated 250 CoNS isolates into ten different species. Of which commonest species identified was *S. epidermidis* (59.2%) followed by *S. haemolyticus* (19.6%) and *S. saprophyticus* (12.4%). Among other species of CoNS, 2.8% of each *S. warneri* and *S. schleiferi subsp. schleiferi* are isolated. *S. simulans* accounted for 1.2% of all CoNS. two isolates of *S. lugdunensis*, one species of each *S. cohnii subsp. cohnii*, *S. capitis subsp. capitis* and *S. hominis subsp. novobiosepticus* are also isolated. (Graph 2)

Maximum number of CoNS species isolated from Pus samples (33.2%) then from blood culture (21.6%). Urine and ear discharge samples accounted for 16% and 13.2% respectively followed by sputum and CSF samples. Vaginal and cervical swabs also yielded the CoNS species. Two CVP tips and one ICD drain tip were considered among devices. Ascetic fluid, pleural fluid and throat swab yielded one CoNS species each. (Table 4)

Among antibiotics, maximum resistance is shown against Nalidixic Acid (97.2%) followed by Penicillin (94%), Amoxicillin-Clavulanic Acid (74.4%), Cotrimoxazole (trimethoprim-sulfamethoxazole 66.4%), Norfloxacin (62.8%), Ciprofloxacin (61.6%) and Erythromycin (60.8%), then Clindamycin (53.2%) and Gentamycin (55.2%). Nitrofurantoin (5.6%), linezolid (1.2%) and Teicoplanin (0.4%) showed least resistance. None of the 250 isolates were resistant to Vancomycin. Methicillin resistance among CoNS was observed to be 73.2%. (Table 5)

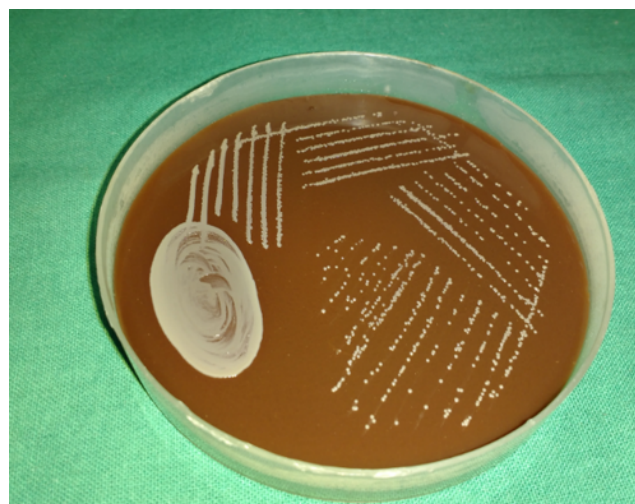
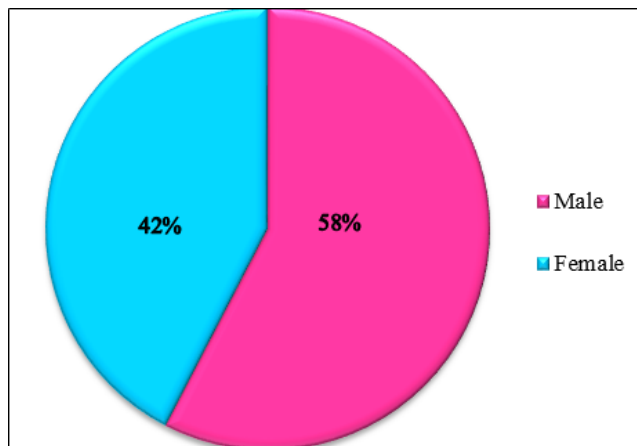


Fig. 1: Colony morphology on Chocolate Agar



Graph 1: Gender-wise distribution of CoNS species (n=250)

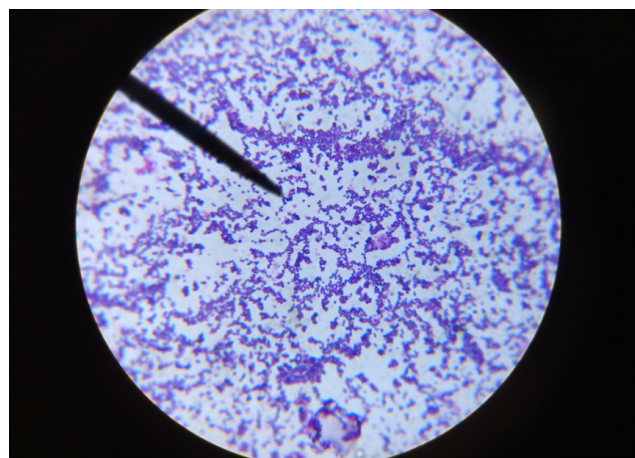
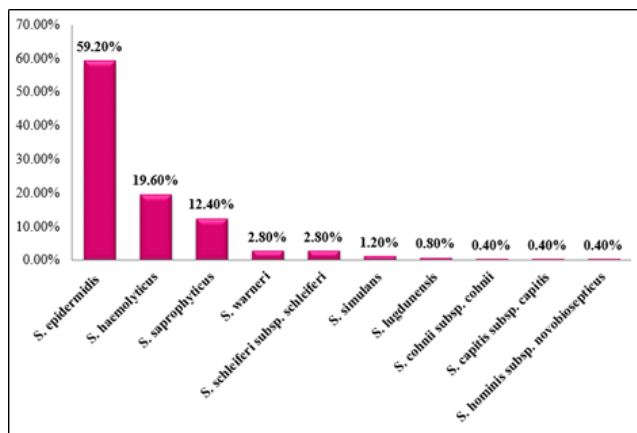


Fig. 2: Gram Stain Morphology



Graph 2: Frequency of CoNS species isolated in the present study

4. Discussion

CoNS form a part of the normal flora of human body. Moreover, if isolated along with another organism, its pathogenic potential may be totally neglected. Hence it is necessary to speciate CoNS and understand the pathogenic potential of individual CoNS species. CoNS

species formerly known as contaminant bacteria, but are now as important possible pathogens with augment in number of sternly incapacitated patients.¹ During the last decade, CoNS (CoNS) have emerged as a major cause of nosocomial infections.

Present study has studied species distribution of 250 CoNS species isolated from various clinical samples and analysed the results by comparing with similar studies conducted all over the country and globe. Various other studies have isolated various species of CoNS in variable frequencies of incidence. Different materials and methods were used to identify and speciate CoNS species which includes conventional, automated and molecular methods. Among conventional methods different authors used different set of biochemical reactions for the speciation. The present study conducted in the tertiary care hospital has utilized minimum biochemical tests to speciate CoNS isolates.

Table 1: Scheme for identification of CoNS Species.

Species	Slide coagulase test	Urease test	Ornithine decarboxylase test	Mannose test	PYR Test	Novobiocin
<i>S. epidermis</i>	–	+	+	+	–	S
<i>S. haemolyticus</i>	–	–	–	–	+	S
<i>S. saprophyticus</i>	–	+	–	–	+	R
<i>S. warneri</i>	–	+	–	–	+	S
<i>S. schleiferi</i> subsp. <i>shleiferi</i>	+	–	–	+	+	S
<i>S. simulans</i>	–	+	–	+	+	S
<i>S. lugdunensis</i>	+	+	+	V	+	S
<i>S. cohnii</i> subsp. <i>Cohnii</i>	–	–	–	+	–	R
<i>S. capitis</i> subsp. <i>Capitis</i>	–	–	–	+	–	S
<i>S. hominis</i> subsp. <i>novobiocepticus</i>	–	+	–	–	–	R
<i>S. caprae</i>	–	+	–	+	V	S
<i>S. cohnii</i> subsp. <i>Ureolyticus</i>	–	+	–	+	V	R

– = negative reaction, + = positive reaction, V = variable reaction, R = resistant, S = sensitive.

Table 2: Age wise distribution of CoNS species isolated. (n=250)

Age group	Number of CoNS species	Percentage
Day 1 – Day 30	34	13.6%
1Month -1year	06	2.4%
2-10 year	19	7.2%
11-20 year	23	9.2%
21-30 year	67	26.8%
31-40 year	29	11.6%
41-50 year	24	9.6%
51-60 year	20	08%
61-70 year	19	7.2%
71-80 year	8	3.2%
81-90 year	01	0.4%

Table 3: Distribution of CoNS among hospital localities: (n=250)

Ward	No. of CoNS species isolated	Percentage
Wards	130	52%
	65	
	NICU 34	13.6%
ICU	OICU 8	3.2%
	PICU 8	3.2%
	SICU 8	3.2%
	MICU 7	2.8%
OPD	48	19.2%
Labor Room	5	02%
Cath-lab	2	0.8%

In the present study, maximum CoNS were isolated from male patients (57.6%) than females (42.4%) which are in par with the studies conducted by Mane P et al,¹² Sardar S A et al¹³ and Usha M G et al¹⁴ except Goudarzi et al¹⁵ in which maximum CoNS were isolated from female patients.

In the present study, maximum of CoNS were isolated from the patients belonging to the age-group between 21-30 years of age (26.8%) which is in par with the studies

conducted by, Mane P et al¹² (22.33%) and Goudarzi et al.¹⁵ Second most commonest age group in the present study is neonates and 31-40 years (13.6%) which is in par with the study conducted by Usha M G et al¹⁴ who isolated the maximum of CoNS from Blood stream infections of neonates. But contrasting results are seen in the study conducted by C. Roopa et al¹⁶ who isolated the maximum number of CoNS from the patients belonging to the age

Table 4: Sample wise Distribution of CoNS isolated in the study. (n=250)

Sample	No. of cons isolates	Percentage
Pus	83	33.2%
Blood	54	21.6%
Urine	40	16%
Ear discharge	33	13.2%
Sputum	13	5.2%
CSF	11	4.4%
Vaginal swab	6	2.4%
Cervical swab	4	1.6%
Device	3	1.2%
Throat swab	1	0.4%
Ascitic fluid	1	0.4%
Pleural fluid	1	0.4%

group of 61-70 years and Sardar S A et al¹³ where the commonest age group was 31-50 years (40%). Even though infections caused by CoNS are common in extremes of age, the present study showed only 13.6% of prevalence rate of CoNS infections in neonates. The age group 21-30 years has been shown to be most common which may be attributed to the higher incidence of UTI, post-operative wound infections in these age groups and blood stream infections in neonates.

Maximum of CoNS are isolated from the patients admitted in the various wards (52%) which in par with the study conducted by C. Roopa et al.¹⁶ Second most common source of CoNS in the present study is ICU (26%) which is comparable with Jayanthi et al¹⁷ (25%). Among 26% of CoNS infected ICU patients, 13.6% were from NICU in the present study which is in correlation with the study done by Tayyar et al¹⁸ (11.2%). A total of 19.2% of CoNS are isolated from patients attending OPD clinics of various specialties in the present study. This explains the incidence of community acquired infections caused by CoNS like community acquired UTI, respiratory tract infections, chronic suppurative otitis media and others. About 02% of CoNS were isolated from the patients admitted in labour room which included the UTI among pregnant ladies.

In the present study *S. epidermidis* is the predominant species isolated (59.2%) which is in par with most of the previously conducted studies.^{14,19–23} The incidence of *S. epidermidis* isolated in the present study (59.2%) is close to the results of Manikandan et al²⁴ (57%), Jayanthi et al¹⁷ (56%) and Tayyar et al¹⁸ (54.7%). Second most common species isolated in the present study is *S. haemolyticus* which is in par with the other studies who also isolated *S. haemolyticus* as the second most common isolate.^{14,21,22,25} Present study isolated 19.6% of *S. haemolyticus* which in correlation with the studies conducted by Asangi et al¹⁹ (19.7%), De Paulis et al²⁵ (18.5%) and Goliya S et al²³ (20%). *S. saprophyticus* is the third most common CoNS species isolated in the present study which in par

with the studies who also observed the *S. saprophyticus* as the third most common isolate.^{17,21,25,26} Present study isolates 12.4% of *S. saprophyticus* among all CoNS. The values are in comparison with the studies conducted by Parashar S et al²⁶ (14.7%), Goyal et al²² (14.7%) and Karigoudar R et al²⁷ (15%). Other species of CoNS are also isolated but in very small proportion which explains the low incidence of such species in human infections in the present study setting. Similar scenario is seen in most of the studies.^{13,14,22,23,28} However contrasting results are observed in the studies conducted by Priya et al²⁰ which isolated 16% of *S. capitis* subsp. *capitis* which is the second most common isolates among all the CoNS isolated by them and Manikandan et al²⁴ who isolated 5.7% of *S. simulans* and *S. lugdunensis*. The difference may be due to the nature of samples considered in their study, as they isolated CoNS from ocular samples only.

In the present study CoNS species are isolated from various clinical samples. Maximum isolates are from Pus followed by blood, urine then ear discharge and small numbers of CoNS are isolated from other sterile body fluids, which is in par with the studies where the pus sample was the most common clinical sample.^{16,19,27} About 33.2% of the CoNS species are isolated from the pus samples which is in comparison with the results of the various other studies^{14,19,22} that included pus discharge from skin and soft tissue infections from surgical site infection, implant associated orthopaedic infections, infections of diabetic foot and others which explain the association of CoNS with the infections caused by endogenous skin commensals. CoNS is one of the most common causative agent of surgical site infections which is also showed in the study done by Tayyar et al¹⁸ who isolated 30% of CoNS from surgical site infections. 21.6% of CoNS were isolated from the Blood culture in the present study. Studies done by Karigoudar R et al,²⁷ Jayanthi et al¹⁷ also show the similar results. Positive Blood culture reveals the association of CoNS with neonatal sepsis, endocarditis and others. A total of 16% of CoNS are isolated from the urine samples in the present study

Table 5: Antibiotic resistance pattern of CoNS species: (values are in %)

CoNS Species	<i>S. epidermidis</i>	<i>S. haemolyticus</i>	<i>S. saprophyticus</i>	<i>S. warneri</i>	<i>S. schleiferi subsp. schleiferi</i>	<i>S. simulans</i>	<i>S. lugdunensis</i>	<i>S. cohnii subsp. Cohnii, S. capitis subsp. Capitis and S. hominis subsp. novobiosepticus</i>	Total
NA	97.97	100	87.09	100	100	100	100	100	97.2
AMC	94.6	97.95	80.65	100	100	100	100	100	94
COT	77.03	87.76	38.71	57.14	71.43	100	100	100	74.4
NOR	72.3	87.76	19.36	42.86	71.43	33.33	50	0	66.4
CIP	64.87	87.76	35.48	28.57	42.86	0	50	100	62.8
GEN	64.19	89.8	29.03	28.57	42.86	0	50	0	61.6
E	60.8	81.63	19.36	0	14.29	0	50	0	55.2
CD	56.08	95.92	35.48	57.14	42.86	33.33	50	100	60.08
NF	62.84	61.23	19.36	28.57	14.29	0	50	0	53.2
LZ	7.43	4.08	0	0	14.29	0	0	0	5.6
TEI	0.68	4.08	0	0	0	0	0	0	1.2
VA	0	2.04	0	0	0	0	0	0	0.4
CX	0	0	0	0	0	0	0	0	0
	75.68	91.84	38.7	57.14	57.14	66.67	50	100	73.2

which is good in correlation with the other studies.^{17,26,27} Urine samples yielded the *S. saprophyticus* as the most common CoNS species followed by *S. epidermidis* and *S. haemolyticus* which once again proves that *S. saprophyticus* as one of the most common causative agent of community acquired urinary tract infection. 13.2% of CoNS isolated from the ear discharge samples in the present study which is in par with the study done by Goudarzi et al¹⁵ who isolated 12.3% of CoNS from ear discharge samples.

In the present study the isolated CoNS species have been challenged with the various antibiotic discs to study their antibiotic resistance pattern. Majority of CoNS expressed resistance towards nalidixic acid (97.2%) followed by Penicillin (94%), 74.4% to Amoxicillin-Clavulanic acid and 66% to Cotrimoxazole. Low level resistance was observed towards Nitrofurantoin (5.6%) and Linezolid (1.2%). All the isolates were sensitive to Vancomycin. Similar results are observed in various studies.^{15,18,20}

In the present study 73.2% of CoNS were Methicillin resistant. Such higher incidence of MRCoNS was observed in most of the studies.^{19,21,23}

However low level resistance against Amoxicillin-Clavulanic acid has been observed in the studies conducted by Goliya S et al²³ (18.7%) and C. Roopa et al¹⁶ (27.6%). Similarly study done by C. Roopa et al¹⁶ showed no resistance to Gentamicin, whereas 86.2% of resistance was observed in the study conducted by Goudarzi et al¹⁵ but in present study it is 55.2%. This kind of variable resistance pattern of CoNS against various antibiotics can be attributed to the geographical variation as well as empirical antibiotic therapy practiced in those health care settings.

The present study reveals that Penicillin is an outdated drug to treat the infections caused by CoNS and Amoxicillin-Clavulanic acid is also not the effective drug of choice in our setting. The lower level of resistance against Linezolid, Teicoplanin and no resistance against Vancomycin turns them to be the most effective antibiotics in the treatment of infections caused by CoNS.

5. Conclusion

Present study identifies the commonest CoNS species to be *S. epidermidis* and *S. saprophyticus* as commonest cause of UTI. Pus sample being the commonest source of CoNS isolates and productive age group is predominantly affected. Study also throws light on the Antibiotic susceptibility pattern of CoNS and observes that β -lactam agents are least effective against CoNS and incidence of MRCoNS is substantially very high and linezolid, Nitrofurantoin and Glycopeptides are shown to be effective agents in treating infections caused by CoNS

The increased recognition of pathogenic potential in CoNS and emergence of drug resistance among them demonstrates the need to adopt simple cost effective laboratory methods to identify the species and determine

the antibiotic resistant pattern that will help the clinicians in treating the infections caused by CoNS.

6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

7. Source of Funding

None.

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