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Original Research Article

Speciation and Antimicrobial Susceptibility Pattern of Clinically Significant Coagulase Negative Staphylococci in a Tertiary Health Care Centre

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Abstract: Coagulase negative staphylococci (CoNS) were once considered as skin commensals and dismissed as culture contaminants, are now being recognized as significant nosocomial pathogens. CoNS are also associated with resistance to several commonly used antimicrobial agents. It is important to identify CoNS up to the species level, as the epidemiology, the pathogenecity and drug resistance varies from species to species. Study was carried for period of 12 months. CoNS isolates from various clinical samples, collected from different sites were subjected to biochemical characterization. Antimicrobial susceptibility test were done by using Kirby Bauer disc diffusion method. Out of eighty six isolates *Staphylococcus epidermidis* 29(33.7%) was most common isolate, followed by *S.hemolyticus* 23(26.7%), *S.saprophyticus* 11(12.8%), and *S.hominis* 9 (10.5%). Other CoNS isolates were *S.lugdunensis* 6(7.0%) *S. schleiferi* 4(4.7%), *S.warneri* 2(2.4%), *S.cohnii* 1(1.2%). Antibiotic susceptibility testing showed maximum resistance to Penicillin and Amoxy-clav with 80%-90% and sensitivity to Gentamicin, Linezolid and Vancomycin (80%-100%). Methicillin resistance was detected in 27(37%) of isolates Due to emergence of multiple drug resistance among CoNS isolates, there is a need to adopt simple laboratory procedures to identify, speciate and determine the prevalence and antibiotic resistant patterns of CoNS.
Keywords: Coagulase-negative Staphylococci, Commensal, Speciation, antimicrobial susceptibility test

INTRODUCTION

Coagulase negative staphylococci (CoNS) were once considered as harmless skin commensals and dismissed as culture contaminants, but in recent years, they are increasingly being recognized as significant nosocomial pathogens, partly due to the growing appreciation of this group of organisms as opportunistic pathogens or due to increase in the use of transient or permanent medical devices in seriously ill and immunocompromised patients [1,2].

CoNS are one of the most frequent causes of nosocomial infections and are reservoirs of multiple antimicrobial resistant determinants [3]. CoNS are also associated with resistance to several commonly used antimicrobial agents and even multidrug resistant strains are common[4]. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections[6]. The organisms are known pathogens in urinary tract infection, prosthetic valve endocarditis, patients with central line catheters and other indwelling foreign devices [4,5]. They are also common opportunistic pathogens in patients who are immunocompromised [5]. However, there are a number of recent reports which state that CoNS are the most common pathogens of bloodstream infections [6,7].

In a routine microbiology laboratory *Staphylococcus aureus* is identified by a rapid screening test (Coagulase test) and all non *S. aureus* isolates are reported as CoNS [8,9]. It is important to identify CoNS up to the species level, as the epidemiology, the pathogenecity and drug resistance varies from species to species [10]. Ubiquitous member of normal flora makes this species the most commonly encountered in clinical specimens, usually as a contaminant and can be difficult

to establish clinical significance [11]. This study was carried out to identify the incidence and speciation of coagulase negative Staphylococci with their antibiogram from clinically relevant specimens coming to microbiology laboratory in a tertiary care hospital in Dakshina Kannada district.

MATERIALS AND METHODS

Eighty six CoNS isolated from various samples like exudates, urine, blood and ET secretions, central line tip at Yenepoya medical college hospital laboratory during the period of June 2016 to June 2017 were included in the study. Only those CoNS isolates were included in study, which were isolated from repeated sample for the second time. All the CoNS isolates were processed in the microbiology laboratory for identification by using conventional methods with standard procedures and precautions.

For speciation of CoNS, we followed the scheme from Kloos and Schleifer scheme, Mackie and Mc Cartney and Koneman *et al*. [2,12-14] (Table-1), that are simple, user friendly and economical. This scheme involved a two-step procedure (Table-1), first step aimed to identify species group by combined slide and tube coagulase with Novobiocin resistance, test for urease activity, Ornithine decarboxylase and aerobic acid from mannose. If further identification was required additional tests; a maximum of two tests were selected as shown in Table-1.

Table-1: Identification scheme of Coagulase negative Staphylococcus

Group/Species	Clumping factor	Tube coagulase	decorboxylase	urease	Novobiocin (5µg)	Mannose	Species/ Subspecies	Trehalose	Mannitol	Acetoin	Lactose	Anaerobic	Xylose
S.epidermidis group	-	-	+	+	S	+	*S.epidermidis*	-	-				
							S. caprae	+	-				
							S. capitis	-	+				
S.hemolyticus group	-	-	-	-	S	-	S hemolyticus			+			
							S auricularis			-	-		
							S caseolyticus			-	+		
S. saprophyticus	-	-	-	+	R	-	subspp saprophyticus	+					
S. warneri group	-	-	-	+	S	-	S warneri					+	
							S hominis	-					
S. lugdunensis	-	-	+	±	S	+							
S. schleiferi													
subspp schleiferi	+	-	-	-	S	+							
subspp coagulans	-	+		+	S	+							
S. capitis	-	-	-	-	S	+							
S. cohinii group	-	-	-	+	R	+							
subspp cohini	-	-	-	-	R	±	S xylosus						+
S simulans	-	-	-	+	S	±							

Kirby Bauer disc diffusion method was performed for antimicrobial susceptibility testing. The following antibiotic discs were tested: Penicillin [P] (10units), Amoxyclav [AMC] (20µg amoxycillin and 10µg clavulanic acid), Cefoxitin [CX] (30µg), Ciprofloxacin [CIP] (5µg), Gentamicin [G] (10µg), Erythromycin [E] (15µg), Cotrimoxazole [COT] (1.25/23.75µg), Linezolid [LZ] (30µg), and Vancomycin [VA] (30µg).

Methicillin resistance was screened using disc diffusion method. Cefoxitin (30µg, Micro express India Ltd) was used to identify Methicillin resistant coagulase negative staphylococci (MRCoNS). All antibiotic results were interpreted using CLSI guidelines **[**15**]**. Quality control was done using ATCC strain *Staphylococcus aureus* 25923.

RESULTS

Out of 86 isolates, the most common isolates were *S.epidermidis* 29(33.7%), and *S.hemolyticus*

21(26.7%), followed by *S.saprophyticus* (12.8) *S.hominis* 7(11.6%), *S.lugdunensis* 6(10.0%), and *S.schleiferi* 4(4.7%), *S. cohinii* and S. warneri were isolated 2(2.3%) each.

Table-2: **Different Species of CoNS isolated from various clinical samples**

Species	No. (%)	Pus	Blood	Urine	ET Secretions	Central Line tip
S.epidermidis	29(33.7)	16	4	3	4	2
S.hemolyticus	23(26.7)	4	4	12	1	2
S.saprophyticus	11(12.8)	4	1	4	2	-
S.hominis	9(10.5)	3	3	2	1	-
S.lugdunensis	6(7.0)	1	4	1	-	-
S.schleiferi	4(4.7)	-	2	2	-	
S. cohinii	2(2.3)	1	-	1	-	-
S. warneri	2(2.3)	-	1	1	-	-
Total	86	29	19	26	8	4

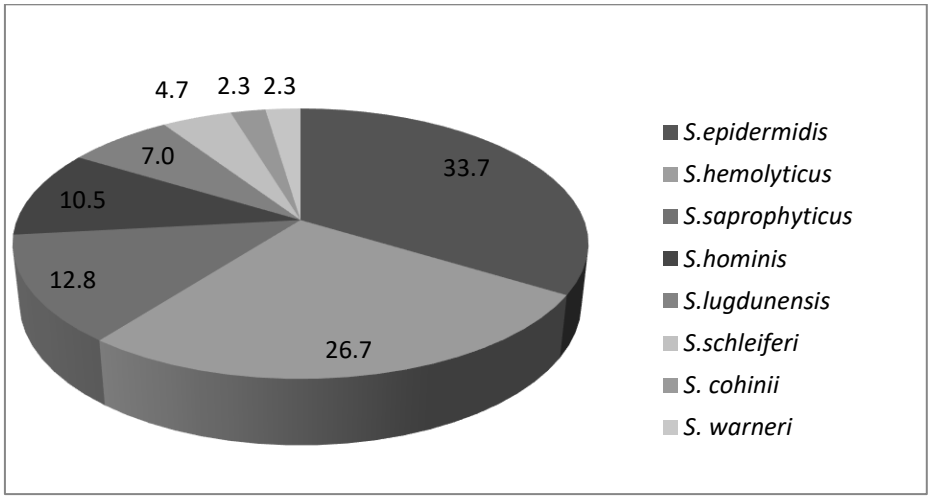

Fig-1: Species of CoNS isolated

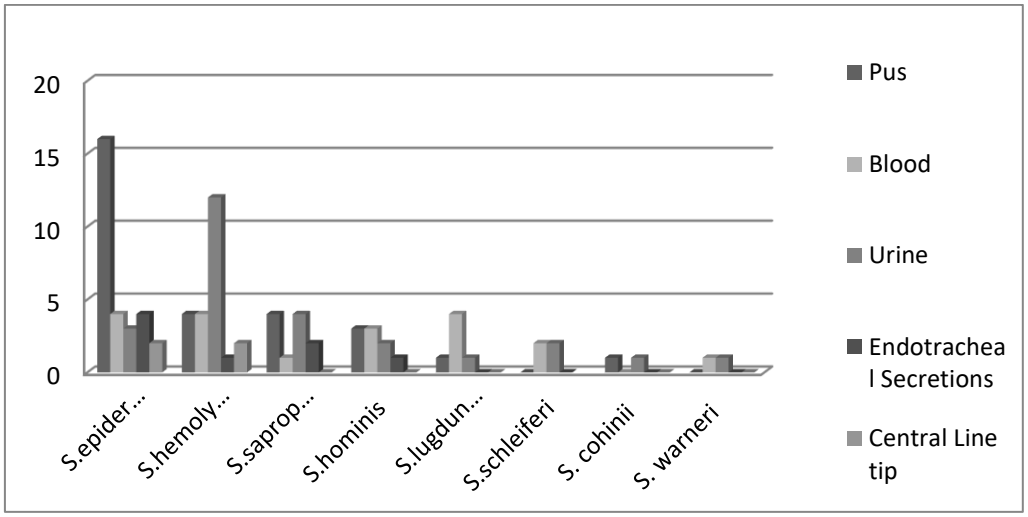

Fig-2: CoNS isolates from various clinical samples

CoNS were more commonly isolated from Pus 29(33.7%) and urine 26(30.23%) samples. The most common isolate in pus samples was, *S.epidermidis* (55%), while in blood cultures, 21% were *S.epidermidis*, *S.hemolyticus*, and *S.lugdunensis*, *S. hominis* (15%), were common. In urine samples *S. hemolyticus* (46%) was the most common species.

Table-3: **Antibiotic Resistance pattern of CoNS isolates**

Isolate	P	AMC	CX	CIP	G	E	LZ	COT	VA
S.epidermidis(29)	27(93.1)	26(89.6)	5(17.2)	15(51.7)	5(17.2)	12(41.3)	3(10.3)	9(31.0)	0(0)
S.hemolyticus(23)	22(95.6)	21(91.3)	6(26.0)	14(60.8)	6(26.0)	7(30.4)	5(21.7)	6(26.0)	0(0)
S.saprophyticus(11)	9(81.8)	10(90.9)	3(27.2)	6(54.5)	2(18.1)	7(63.6)	2(18.1)	2(18.1)	0(0)
S.hominis(9)	9(100)	7(77.7)	3(33.3)	4(44.4)	2(22.2)	5(55.5)	1(11.1)	2(22.2)	0(0)
S.lugdunensis(6)	5(83.3)	5(83.3)	4(66.6)	5(83.3)	1(16.6)	5(83.3)	1(16.6)	3(50)	0(0)
S.schleiferi(4)	3(75)	3(75)	1(25)	3(75)	1(25)	3(75)	1(25)	1(25)	0(0)
S. cohinii (2)	2(100)	2(100)	1(50)	0(0)	0(0)	2(100)	0(0)	0(0)	0(0)
S. warneri (2)	2(100)	1(50)	1(50)	0(0)	0(0)	1(50)	1(50)	0(0)	0(0)

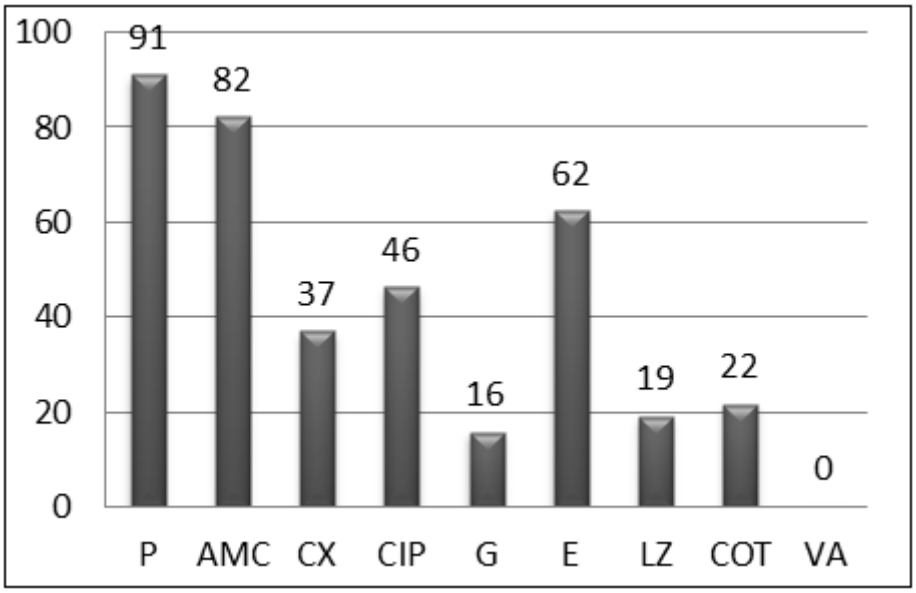

Fig-1: Species of CoNS isolated

Antibiotic susceptibility testing showed maximum resistance to Penicillin (91%) followed by Amoxy-clav (82%), CoNS isolates showed better susceptibility to Gentamicin (84%), Linezolid (81%), and Cotrimoxazole (78%) but resistance was more observed for commonly used drugs like Erythromycin (62%) and Ciprofloxacin (46%). All CoNS isolates were sensitive to Vancomycin. Methicillin resistance was observed in 27(37%) isolates. Methicillin resistance was found in all species, but more in *S.lugdunensis* (66.7%), all isolated from blood cultures.

DISCUSSION

CoNS are generally considered normal inhabitants of skin and nares & are capable of causing only opportunistic infections; so many clinical laboratories do not identify clinical isolates of CoNS to the species level. As CoNS is increasingly being implicated as significant nosocomial pathogen several reviewers has

emphasized the need for species identification, which is possible only by a simple, easily adaptable, inexpensive method. The species identification is important in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agent.

The present scheme conveniently identified the most frequently encountered clinical isolates in our hospital as *S.epidermidis* (33.7%), *S.hemolyticus* (26.7%), *S.saprophyticus* (12.8%), *S.hominis* (10.5%), *S.lugdunensis* (7.0%), *S.schleiferi* (4.7%), *S.cohinii* (2.3%), and *S. warneri* (2.3%).

In our study *S.epidermidis* (33.7%) was the most common isolate. This correlates with other studies by K. Punitha Valli *et al*. (32.3) [18], Saroj Golia *et al.* (46.3%) [19], C. Roopa *et al.* (50%) [20], S. S. Vijayasri Badampudi *et al.* (40%) [21]. The remaining species isolated were *S. haemolyticus* (26.27%), *S.saprophyticus* (12.8%), *S. hominis* (10.5%) and *S. lugdunensis* (7%). This prevalence is in concordance with various other studies [18-21].

The present study revealed maximum number of CoNS isolates from pus sample (33%), followed by urine (30%). This isolation frequency is also seen in other studies by K. Punitha Valli *et al.* [18] and S. Vijayasri Badampudi *et al.* [21].

CoNS isolate isolates in this showed maximum resistance to penicillin, Amoxy-clav and erythromycin (91%, 82%, and 62%). U Mohan *et al*. (90%) [11], Saroj Golia *et al*. (95.5%, 88%, and 71.6%) 19, S. Vijayasri Badampudi *et al.* (91%) [21], showed similar resistance pattern for these antibiotics. Sensitivity to other drugs like Gentamicin, Linezolid, and cotrimoxazole (84%, 81%, and 78%) was better in our study. Studies by Saroj Golia *et al.* [19], C. Roopa *et al.* [20], showed similar sensitivity results for these antibiotics.

Vancomycin resistance was not observed in our study. This in concordance with other studies by K. Punitha Valli *et al.* [18], Saroj Golia *et al.* [19], C. Roopa *et al.* [20], *S.* S. Vijayasri Badampudi *et al.* [21].

Methicillin resistance (MRCoNS) was detected in 37% of isolates in our study. C. Roopa *et al.* (33%) got similar results in their study but a higher prevalence of MRCoNS was reported in studies by, Saroj Golia *et al.* (66.4%) 19 and S. S. Vijayasri Badampudi *et al.* (72%) [21].

SUMMARY & CONCLUSION

CoNS have become the major cause of nosocomial infections. And they are often resistant to multiple antibiotics. Speciation of CoNS is helpful in interpreting the significance of it in clinical laboratory and elucidates the spectrum of diseases.

The study revealed S. epidermidis is the predominant CoNS from pus, urine and also blood samples. *S.hemolyticus* was isolated from endotracheal and central lining tips, *S.hominis* and *S.lugdunensis* and *S.schleiferi* were isolated mainly from blood cultures. Hence it is important to monitor the CoNS infection in ICU patients, patients on ventilator and with central lines and patients with post-operative wounds. The species identification is important in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agent.

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