

A Study of Coagulase-Negative *Staphylococci* in the Clinical Isolates of Tertiary Care Hospital of South India

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Abstract

Background: Coagulase Negative Staphylococci (CoNS) is becoming an important cause of nosocomial infections due to the increasing use of transient and permanent medical devices in seriously ill and immunocompromised patients. The aim of the current study is the CoNS isolates from the clinical samples.

Methods: Only CoNS isolates from positive cultures were selected. Phenotypic identification of an isolate was confirmed based on the coagulase reaction and a series of biochemical reactions from the API Staph system (BioMérieux, France). Antimicrobial susceptibility testing was performed on all isolates by the Kirby-Bauer Disc Diffusion method towards antibiotics following overnight incubation, the diameter of the inhibition zone was measured and interpreted as susceptible, intermediate, or resistant by referring to the current Clinical and Laboratory Standards Institute (CLSI) Guidelines 2015. **Results:** Urine samples yielded 60% of the total isolates whereas the isolation from blood, conjunctival swab, and pus were 13.63%, 6.36%, and 8.18% respectively. The overall antibiotic resistance pattern of the CoNS isolates was analyzed. The highest resistance was shown against penicillin (91.81%). No resistance was noted to linezolid and vancomycin. Teicoplanin resistance was seen in 4.54% of the isolates Clindamycin sensitivity was seen in 79.09% of the CoNS isolates. **Conclusions:** Coagulase-negative Staphylococci (CoNS) are increasingly becoming of clinical importance hence the identification should be done till species level. *S. hemolyticus* and *S. epidermidis* were the commonly isolated CoNS species. *S. hemolyticus* species had a higher antibiotic resistance antibiotic profile than *S. epidermidis*. Methicillin resistance was noted in 68.18% of the isolates.

Keywords: Coagulase-negative Staphylococci (CoNS); Methicillin-Resistant CoNS (MRCoNS); *S. hemolyticus*, *S. epidermidis*.

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Introduction

Coagulase-negative *staphylococci* (CoNS) are normal inhabitants of human skin and mucous membranes. They have long been usually dismissed as culture contaminants, even in samples obtained from normally sterile sites.^[1] They were considered non-pathogenic and were rarely reported to cause severe infections.^[2] As a group, the *staphylococci* are among the most frequently isolated microorganisms in the hospital microbiology laboratory. Describing

CoNS is challenging because they represent a heterogeneous group within the genus *Staphylococcus* that is not based on phylogenetic relationships.^[3] Often, identification of the *staphylococci* is limited to a rapid screening test for *Staphylococcus aureus*, and non-*S. aureus* isolates are simply reported as coagulase-negative *staphylococci* (CoNS).^[4] CoNS are differentiated from the closely related but more virulent *Staphylococcus aureus* by their inability to produce free coagulase. Today, CoNS, as a typical opportunist, represents one of the major nosocomial pathogens, having a

substantial impact on human life and health. They are particularly associated with the use of indwelling or implanted foreign bodies, which are indispensable in modern medicine. [2] *Staphylococcus epidermidis* and *Staphylococcus hemolyticus* are the most isolated species from clinical samples among CoNS. [1, 5, 6] CoNS infections occur more commonly in patients on corticosteroid therapy, those undergoing hemodialysis, or those with implanted catheters or prosthetic valves. [7] numerous reports during the last several decades have demonstrated that certain nosocomial genotypes of CoNS have been established as opportunistic pathogens in healthcare settings. [8] After admission to the hospital and especially after exposure to multiple courses of antibiotics or surgical prophylaxis, patients become colonized with multi-drug resistant CoNS species such as *Staphylococcus haemolyticus*. [9] CoNS are the most frequent cause of late-onset sepsis among newborn infants in neonatal intensive care units (NICU) worldwide. Bloodstream isolates are frequently antibiotic-resistant, similar to CoNS isolates from NICU personnel and NICU sites. [10] CoNS are the most common cause of nosocomial bloodstream infection, responsible for 30% to 40% of these infections. Coagulase-negative bacteremia occurs because of long-term usage of indwelling central venous catheters, administration of parenteral nutrition and previous antibiotics, co-morbid conditions in the patient including malignancy other predisposing factors like intensive-care unit stay, non-adherence to infection control practices like hand washing by medical staff. [6] With this background, we in the current study tried to study the CoNS isolates from the clinical samples of our tertiary care hospital.

Materials and Methods

This cross-sectional study was conducted in the Department of Microbiology, Kakatiya Medical College, and MGM Hospital, Warangal, Telangana State. Institutional Ethical Committee Permission was obtained for the study. Ethical clearance was obtained for the study. N=110 samples were collected during the study period. CoNS isolates from positive cultures were selected. CoNS isolates with untraceable clinical records, duplicate CoNS samples from the same patient, and isolates inoculated from blood

originating from other sterile sites, such as cerebrospinal fluid and peritoneal dialysis fluid blood, were excluded from this study. Phenotypic identification of an isolate was confirmed based on the coagulase reaction and a series of biochemical reactions from the API Staph system (BioMérieux, France). Antimicrobial susceptibility testing was performed on all isolates by the Kirby-Bauer Disc Diffusion method towards antibiotics. Following overnight incubation, the diameter of the inhibition zone was measured and interpreted as susceptible, intermediate, or resistant by referring to the current Clinical and Laboratory Standards Institute (CLSI) Guidelines 2015. In this study, the zone of inhibition for vancomycin disc was interpreted according to the 2008 CLSI Guidelines. Nosocomial acquired was defined as isolates that were positive from blood cultures taken 48 hours or more after hospital admission, the species identification, antimicrobial susceptibility testing results, as well as data collected from the proforma, were entered into the Statistical Program for Social Sciences (SPSS) version 22 for analysis.

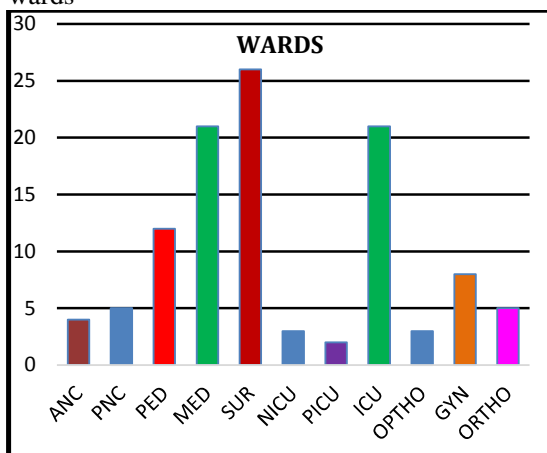
Results

A total of n=110 CoNS isolates were obtained during the study period from various clinical samples. Sex-wise distribution of the patients showed that n=78(70.9 %) of the CoNS isolation were from female patients and n=32(29.09%), were from male cases. Among the various age groups, the age group of 21- 30 yrs showed the highest isolation of CoNS n=26(23.63%) followed by 41-50 yrs n=18(16.36%), n=17(15.45%) in 11 – 20 years age group, n=15(16.36%) in 1 – 10 years age group, n=12(10.91%) in 31 – 40 and 51 – 60 years age groups and n=10(9.09%) in 61 – 70 years age group. Urine samples yielded 60% of the total isolates whereas the isolation from blood, conjunctival swab, and pus were 13.63%, 6.36%, and 8.18% respectively (Table 1). Among the CoNS isolated inwards the most common was surgery wards n=26(23.63%) cases, followed by n=21(19.09%) in the Medical ward and ICU ward. Pediatric wards had n=12(10.9%) and Gynec ward n=8(7.27%) and other areas depicted in figure 1.

Table 1: Distribution of different CoNS species in various clinical samples

Sample	<i>S. hemolyticus</i>	<i>S. epidermidis</i>	<i>S. warneri</i>	<i>S. schleiferi</i>	<i>S. capitis</i>	<i>S. cohnii</i>	<i>S. saprophyticus</i>
Urine	41	10	3	5	1	2	4
Blood	7	4	1	1	2	0	0
Conjunctival swab	1	3	2	1	0	0	0
Endotracheal tip	4	0	0	0	0	0	0
Pus	5	3	1	0	0	0	0
Central line	1	0	0	0	0	0	0
HVS	2	0	0	0	0	0	0
Cervical swab	1	0	0	0	0	0	0
Nasal swab	3	0	0	0	0	0	0
Vault swab	1	0	0	0	0	0	0
UVC tip	1	0	0	0	0	0	0
Total	67 (60.90%)	20 (18.18%)	7 (6.36%)	7 (6.36%)	3 (2.73)	2 (1.81%)	4 (3.64%)

Figure 1: CoNS isolated in patients of different wards



The clinical picture of patients with culture-positive CoNS, the majority of cases were isolated from Surgical, Medical, and ICU wards. Most of these patients have abnormal temperatures of 38 °C. The white blood cell counts showed 40% had abnormal counts (leucocytosis or leucopenia) 28% of patients were found with thrombocytopenia. These parameters indicate true infection and will help in interpreting the significance of blood culture positivity rates. It was found that the majority of patients with culture-positive CoNS had underlying risk factors that predisposed them to infection. The overall antibiotic resistance pattern of the CoNS isolates was analyzed. The highest resistance was shown against penicillin (91.81%). No resistance was noted to linezolid and vancomycin. Teicoplanin resistance was seen in 4.54% of the isolates (Table 2) Clindamycin sensitivity was seen in 79.09% of

the CoNS isolates. Erythromycin-induced clindamycin resistance was noted in 7% and constitutive clindamycin resistance was seen in 16% of the isolates. The antibiotic resistance pattern of various species was compared and almost all species showed the highest resistance to penicillin. Teicoplanin resistance was seen in *S. epidermidis* and *S. hemolyticus* Resistance was low to amikacin in all species except *S. capitis* (100%) and *S. schleiferi* (40%).

Table 2: Antibiotic sensitivity pattern Coagulase-negative Staphylococci

Antibiotic	concentration (µg/ml)	Sensitive N (%)	Resistant N (%)
Cotrimoxazole	2	59 (53.63)	51 (46.36)
Penicillin	8	09 (8.18)	101 (91.81)
Amoxycillin Clavulanic acid	20/10	43 (39.09)	67 (60.9)
Amikacin	8	98 (89.09)	12 (10.9)
Gentamycin	8	74 (67.27)	36 (32.73)
Chloramphenicol	30	85 (77.27)	25 (22.72)
Ciprofloxacin	2	51 (46.36)	59 (53.63)
Clindamycin	2	87 (79.09)	23 (20.9)
Erythromycin	4	43 (39.09)	67 (60.6)
Cefoxitin	4	35 (31.81)	75 (68.18)
Teicoplanin	30	105 (95.45)	5 (4.54)
Linezolid	30	110 (100.0)	0 (0.00)
Vancomycin	8	110 (100.0)	0 (0.00)

Of the n=25 clinically significant isolates n=22(88%) were methicillin-resistant CoNS (MRCoNS) depicted in table 3. Statistical analysis found a significant association between the significant bloodstream infections and MRCoNS (p=0.0241). Methicillin resistance was high in all species but comparatively less in *S. saprophyticus* and *S. epidermidis*. Of the n=25 cases n=14(56%), were diagnosed with sepsis and n=8(32%) with catheter-related bloodstream infection an n=3(12%) with nosocomial pneumonia.

Table 3: Association between MRCoNS and significant infection

CoNS isolates	N=110	Significant infection		p-value
		Yes	No	
MRCoNS	75(68.18%)	22	53	0.0214*
MSCoNS	35(31.81%)	3	32	

* significant

Discussion

Coagulase-negative *Staphylococci* (CoNS) have assumed a great pathogenic potential and are being recognized as important agents of nosocomial infection. [11] They are responsible for high morbidity and mortality mainly in hospitalized patients and usually show multidrug resistance. [12] The cause for this should be thought of in the ability of CoNS to create biofilm whereby they pose a particular threat for people with valve prostheses and the ones with implants or catheters. [13] The clinical significance of CoNS species continues to increase as strategies in medical practice lead to more invasive procedures. Hospitalized patients that are immunocompromised and/ or suffering from chronic diseases are most vulnerable to infections by coagulase-negative *staphylococci*. Since CoNS are widespread on the human body and can produce very large populations, distinguishing true pathogens from contaminating flora is a serious challenge. [14] In the present study CoNS, isolation was more from female patients (70.9 %). Usha et al., [5] have found coagulase-negative *staphylococci*, the isolates from the male patients constituted 59 %. In another study by Sheikh et al., [15] from Africa 57.5% of the CoNS isolates belonged to males. One of the reasons for female predominance in this study could be because of a larger number of urine samples from the female patients which were collected. The highest number of isolation of CoNS n=26(23.63%) was from 21 – 30 years followed by 41-50 yrs n=18 (16.36%) cases. Usha et al., [5] in their study found out that CoNS bloodstream infections were common in neonates whereas the other infections predominated in the age group between 30 yrs and 40 yrs. In the present study, Urine samples yielded 60% of the total isolates whereas the isolation from blood, conjunctival swab, and pus were 13.63%, 6.36%, and 8.18% respectively.

Sheikh et al., [15] found in their study that most of the CoNS were isolated from urine samples (51.5%) followed by blood (25.4%). Sharma et al., [14] also obtained a majority of their study isolates from urine samples (36%) followed by blood samples (27%) which is like the present study. Goyal et al., [16] study showed an isolation rate of 38.2% from wound infections, 28.4% from urine samples, and 14.7% from blood samples which is quite different from our study. In the present study, *S. hemolyticus* (67.90%) and *S. epidermidis* (18.18%) were the commonly isolated species of CoNS from various clinical samples followed by *S. warneri* and *S. schleiferi* in (6.36%) each. Chaudary et al., from Andhra Pradesh in their study, also found out *S. hemolyticus* as the predominant CoNS isolate which is like our study. [17] Goyal et al., [16] reported *S. epidermidis* (41%) as the predominant isolate followed by *S. hemolyticus* (14.7%). In a study from Saudi Arabia also the majority of the isolates from the blood of neonates was *S. epidermidis* but the frequency of isolation was much higher (75.8%). [12] Higher isolation of *S. epidermidis* (82.3%) was shown in a study by Mohan et al., [18] In the present study highest resistance was shown to penicillin (91.81%). Sheikh et al., [15] found the resistance against ampicillin, penicillin, and amoxicillin were 88.1%, 83.6%, and 64.9% respectively which shows the penicillin resistance is similar to the present study. [15] Resistance to the amoxicillin-clavulanic acid combination was noted in 60.9% of the isolates in our study which is comparable with the study by Veena et al., [19] where it was 69.77 Mohan et al., [18] on the other hand observed a lower (28.6%) resistance to amoxicillin-clavulanic acid in their study. Other studies by Usha et al., [5] and Sharma et al., [14] showed a higher resistance rate of 89% and 85% respectively. Among aminoglycosides, amikacin showed a lower resistance of 10.9% which is similar to the studies by Usha et al., [5] (21%) and Mohan et al., [18] (16.6%). Gentamicin resistance was higher (30.73%) in the present study as well as others. [5, 14, 18] Ciprofloxacin resistance was shown by 53.63% of the CoNS isolates in the present study. Similar results were obtained by Mohan et al., [18] (51%) whereas Sharma et al., [14] noted a slightly higher resistance rate (76%). Resistance

to cotrimoxazole was 46.36% in the present study which correlates with the studies by Veena et al., [19] and Usha et al., [5] whereas Sharma et al., [14] in their study recorded a resistance rate of 85.73%. Erythromycin resistance was noted in 60.6% isolates in the present study whereas resistance rates to erythromycin were as high as 84% and low as 27.9% were obtained in other studies. [14, 18] Teicoplanin resistance was seen in 4.54% of the study isolates in the present study. In a study from China comparing the antibiotic resistance pattern of CoNS over a period of 2004 to 2009, there was an increase in the teicoplanin resistance from no resistance noted till 2007 and 6.7% resistance in 2009. [20] Teicoplanin resistance of 2.7% was observed in Begum et al., [9] study but no teicoplanin resistance was noted in a study from Amritsar and in a Turkish study. [6] Methicillin-resistant coagulase-negative staphylococci are emerging nosocomial pathogens and act as a reservoir of drug resistance genes. [11] Methicillin resistance was seen in 68.18% of the isolates in our study which is similar to the observations of Chaudary et al., [17] and Koksai in their studies. [5] In the study by Sheikh et al., [15] maximum resistance was shown against oxacillin (94%) whereas Usha et al., [5] noted a comparatively lower methicillin resistance in their study (56%). In addition, the methicillin-resistant CoNS isolates also revealed higher resistance to the other groups of antibiotics when compared with their methicillin-sensitive counterparts. This is similar to the findings of Koksai et al., [6] and Sharma et al., [14]

Conclusion

Coagulase-negative *Staphylococci* (CoNS) are increasingly becoming of clinical importance hence the identification should be done till species level. *S. hemolyticus* and *S. epidermidis* were the commonly isolated CoNS species. *S. hemolyticus* species had a higher antibiotic resistance antibiotic profile than *S. epidermidis*. Methicillin resistance was noted in 68.18% of the isolates. The increase in the methicillin resistance and multidrug resistance among CoNS should be viewed with critical importance because the therapeutic options for multidrug-resistant CoNS are reducing.

Conflict of Interest: None

Source of support: Nil

Ethical Permission: Obtained

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