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Original Research Article

PREVALENCE AND ANTIBIOTIC RESISTANCE OF COAGULASE-NEGATIVE STAPHYLOCOCCI IN A TERTIARY CARE HOSPITAL SETTING

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Abstract

Introduction: Coagulase-negative Staphylococci (CoNS) are emerging as significant contributors to nosocomial infections, primarily due to the escalating utilization of both temporary and permanent medical devices among critically ill and immunocompromised patients. This study aims to investigate CoNS isolates obtained from clinical samples.

Methodology: Only CoNS isolates from cultures showing positive results were included. Confirmation of isolate identification was conducted through phenotypic methods, including coagulase reaction and a series of biochemical assays using the API Staph system (BioMérieux, France). Antimicrobial susceptibility testing was carried out for all isolates using the Kirby-Bauer Disc Diffusion method with various antibiotics. Following an overnight incubation period, the diameter of the inhibition zone was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines of 2015.

Results: Among the clinical samples, urine specimens yielded 60% of the total isolates, while blood, conjunctival swabs, and pus samples contributed 13.63%, 6.36%, and 8.18% of isolates, respectively. Analysis of the overall antibiotic resistance pattern revealed the highest resistance to penicillin (91.81%), while no resistance was observed against linezolid and vancomycin. Teicoplanin resistance was detected in 4.54% of the isolates, and clindamycin sensitivity was noted in 79.09% of CoNS isolates.

Conclusion: CoNS are increasingly recognized for their clinical significance, emphasizing the need for species-level identification. S. hemolyticus and S. epidermidis were the most commonly isolated CoNS species, with S. hemolyticus exhibiting a higher antibiotic resistance profile compared to S. epidermidis. Methicillin resistance was observed in 68.18% of the isolates.

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

Introduction

Coagulase-negative staphylococci (CoNS) typically inhabit human skin and mucous membranes and have historically been disregarded as culture contaminants, even in samples from normally sterile sites [1]. Initially perceived as non-pathogenic, CoNS were infrequently associated with severe infections [2]. Staphylococci, including CoNS, rank among the most frequently encountered

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microorganisms in hospital microbiology laboratories, presenting a challenge in description due to their heterogeneous nature within the genus Staphylococcus, not delineated by phylogenetic relationships [3]. Often, staphylococcal identification is limited to a rapid test for Staphylococcus aureus, with non-S. aureus strains being labeled as CoNS [4]. CoNS are distinguished from the more virulent Staphylococcus aureus by their inability to produce free coagulase. Presently, CoNS, as typical opportunists, constitute significant nosocomial pathogens, exerting a notable impact on human health and well-being, particularly in conjunction with indwelling or implanted medical devices, integral to modern healthcare [2].

Staphylococcus epidermidis and Staphylococcus hemolyticus emerge as the predominant species isolated from clinical samples among CoNS [1, 5, 6]. CoNS infections are prevalent among patients undergoing corticosteroid therapy, hemodialysis, or possessing implanted catheters or prosthetic valves [7]. Numerous reports spanning several decades underscore the establishment of specific nosocomial genotypes of CoNS as opportunistic pathogens within healthcare environments [8]. Following hospital admission, particularly after exposure to repeated antibiotic courses or surgical prophylaxis, patients often harbor multi-drug resistant CoNS strains such as Staphylococcus haemolyticus [9]. CoNS are the leading cause of late-onset sepsis among newborns in neonatal intensive care units (NICUs) globally, with bloodstream isolates commonly displaying antibiotic resistance akin to those from NICU personnel and sites [10]. Accounting for 30% to 40% of nosocomial bloodstream infections, CoNS bacteremia is frequently associated with prolonged use of central venous catheters, parenteral nutrition, prior antibiotic therapy, patient comorbidities including malignancy, and other predisposing factors such as ICU stays and lapses in infection control practices like medical staff hand hygiene [6]. Against this backdrop, our current study endeavors to examine CoNS isolates from clinical samples obtained at our tertiary care hospital.

Material and Methods

This prospective study was done in the Department of Microbiology for the identification of the fungal strains in Kakatiya Medical College and MGM Hospital, Warangal, Telangana State. Institutional ethical approval was obtained for the study. Written consent was obtained from all the participants of the study after explaining the nature of the study in vernacular language. A total of 110 samples were collected during the study period. Coagulase-negative staphylococci (CoNS) isolates from positive cultures were included in the analysis, with exclusions made for CoNS isolates lacking identifiable clinical records, duplicates from the same patient, and isolates derived from blood samples taken from non-blood sterile sites, such as cerebrospinal fluid and peritoneal dialysis fluid. Isolate identification was confirmed through phenotypic methods, including the coagulase reaction and a series of biochemical tests using the API Staph system (BioMérieux, France). Antimicrobial susceptibility testing was conducted on all isolates using the Kirby-Bauer Disc Diffusion method with various antibiotics. After an overnight incubation period, the diameter of the inhibition zone was measured and interpreted as susceptible, intermediate, or resistant following the Clinical and Laboratory Standards Institute (CLSI) Guidelines from 2015. Additionally, vancomycin disc inhibition zone interpretations were based on the 2008 CLSI Guidelines. Nosocomial acquisition was defined as isolates obtained from blood cultures taken 48 hours or more after hospital admission. Data including species identification, antimicrobial susceptibility testing results, and proforma-collected information were entered into the Statistical Program for Social Sciences (SPSS) version 22 for analysis.

Statistical analysis: All the available data was analyzed and uploaded to an MS Excel spreadsheet and analyzed by SPSS version 19 in Windows format. The continuous variables were represented as mean, standard deviations, and percentages. The categorical variables were represented as p values obtained by application of Chi-square test and values of (<0.05) were considered as significant.

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Results

A total of 110 CoNS isolates were obtained during the study period from various clinical samples. Analysis by gender revealed that 78 (70.9%) of the CoNS isolates were from female patients, while 32 (29.09%) were from male patients. Across different age groups, the highest number of CoNS isolations occurred in the 21-30 age group, comprising 26 (23.63%) cases, followed by 18 (16.36%) cases in the 41-50 age group, 17 (15.45%) cases in the 11-20 age group, 15 (16.36%) cases in the 1-10 age group, and 12 (10.91%) cases each in the 31-40 and 51-60 age groups, with 10 (9.09%) cases in the 61-70 age group. Urine samples accounted for 60% of the total isolates, while blood, conjunctival swabs, and pus samples contributed 13.63%, 6.36%, and 8.18%, respectively (Table 1). Among the CoNS isolates, the highest frequency was observed in surgery wards with 26 (23.63%) cases, followed by 21 (19.09%) cases in medical wards and ICU wards. Pediatric wards accounted for 12 (10.9%) cases, while gynecological wards contributed 8 (7.27%) cases, with other areas represented as depicted in Figure 1.

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

Sample	*S. hemolyticus*	*S. epidermidis*	*S. warneri*	*S. schleiferi*	*S. capitis*	*S. cohnii*	*S. saprophyticus*
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%)

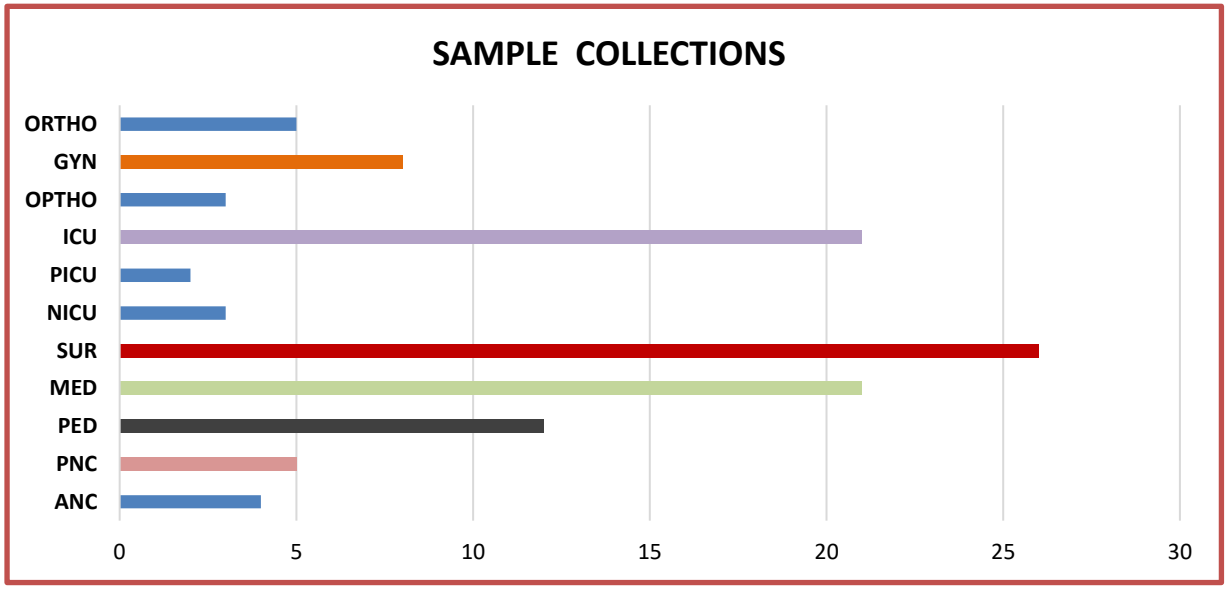

Figure 1: Frequency of CoNS isolated from different wards in the study

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In patients with culture-positive CoNS, the clinical presentation predominantly originated from Surgical, Medical, and ICU wards. A significant proportion of these patients exhibited abnormal temperatures, with the majority registering at 38°C. White blood cell counts indicated abnormalities in 40% of cases, displaying either leucocytosis or leucopenia, while 28% of patients were identified with thrombocytopenia. These clinical parameters signify genuine infection and aid in interpreting the significance of blood culture positivity rates. Notably, a majority of patients with culture-positive CoNS harbored underlying risk factors predisposing them to infection. An analysis of the overall antibiotic resistance pattern of CoNS isolates revealed considerable resistance to penicillin, reaching 91.81%. Conversely, no resistance was observed against linezolid and vancomycin. Teicoplanin resistance was detected in 4.54% of the isolates (see Table 2). Sensitivity to clindamycin was observed in 79.09% of CoNS isolates. Erythromycin-induced clindamycin resistance was noted in 7%, while constitutive clindamycin resistance was evident in 16% of the isolates. A comparative assessment of the antibiotic resistance patterns among various species revealed a predominant resistance to penicillin across almost all species. Teicoplanin resistance was identified in S. epidermidis and S. hemolyticus. Resistance to amikacin was generally low across all species except for S. capitis (100%) and S. schleiferi (40%).

Table 2: Antibiotic sensitivity pattern Coagulase-negative *Staphylococci*

Antibiotic	*Concentration(µg/ml)*	*Sensitive (%)*	*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)

Out of the 25 clinically significant isolates, 22 (88%) were identified as methicillin-resistant CoNS (MRCoNS), as shown in Table 3. Statistical analysis revealed a significant correlation between significant bloodstream infections and MRCoNS (p=0.0241). Methicillin resistance was prevalent across all species, albeit relatively lower in S. saprophyticus and S. epidermidis. Among the 25 cases, 14 (56%) were diagnosed with sepsis, 8 (32%) with catheter-related bloodstream infections and 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 emerged with notable pathogenic potential and are increasingly recognized as significant contributors to nosocomial infections (reference [11]). They

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are associated with considerable morbidity and mortality, particularly among hospitalized patients, often exhibiting multidrug resistance (reference [12]). This propensity for resistance may be attributed to the ability of CoNS to form biofilms, presenting a particular threat to individuals with prosthetic valves, implants, or catheters (reference [13]). The clinical importance of CoNS species is on the rise, paralleling the expansion of invasive medical procedures. Hospitalized patients, particularly those immunocompromised or afflicted with chronic illnesses, are at heightened risk of CoNS infections. Given the widespread presence of CoNS in the human body and their capacity to proliferate extensively, distinguishing true pathogens from contaminating flora poses a significant challenge (reference [14]). In our present study, CoNS isolation was more prevalent among female patients (70.9%). This observation contrasts with findings by Usha et al. (reference [5]), where CoNS isolates from male patients constituted 59%, and a study by Sheikh et al. (reference [15]), reporting 57.5% of CoNS isolates belonging to males. A plausible explanation for the female predominance in our study could be the higher number of urine samples collected from female patients. The highest number of CoNS isolations (n=26, 23.63%) was observed in the 21–30 age group, followed by 18 (16.36%) cases in the 41–50 age group. Usha et al. (reference [5]) found that CoNS bloodstream infections were common in neonates, while other infections predominated in the age group between 30 and 40 years. In our study, urine samples yielded 60% of the total isolates, with blood, conjunctival swabs, and pus samples contributing 13.63%, 6.36%, and 8.18%, respectively.

In their investigation, Sheik et al. [15] discovered that a majority of CoNS isolates originated from urine samples (51.5%), followed by blood samples (25.4%). Similarly, Sharma et al. [14] found a predominant isolation rate from urine samples (36%), followed closely by blood samples (27%), which aligns with our present study. In contrast, Goyal et al. [16] reported differing isolation rates, with 38.2% from wound infections, 28.4% from urine samples, and 14.7% from blood samples, diverging from our findings. In our study, the most frequently isolated species of CoNS from various clinical samples were S. hemolyticus (67.90%) and S. epidermidis (18.18%), followed by S. warneri and S. schleiferi, each comprising 6.36%. Correspondingly, Chaudary et al. [17] found S. hemolyticus to be the predominant CoNS isolate, consistent with our study. However, Goyal et al. [16] reported S. epidermidis (41%) as the predominant isolate, followed by S. hemolyticus (14.7%). Regarding antibiotic resistance, our study revealed the highest resistance to penicillin (91.81%). Similar findings were reported by Sheikh et al. [15], where resistance rates against ampicillin, penicillin, and amoxicillin were 88.1%, 83.6%, and 64.9%, respectively, indicating comparable penicillin resistance. Additionally, our study noted a resistance rate of 60.9% to the amoxicillin-clavulanic acid combination, consistent with the study by Veena et al. [19], where it was 69%. However, Mohan et al. [18] observed a lower resistance (28.6%) to amoxicillin-clavulanic acid in their study. Furthermore, our study showed a lower resistance rate to amikacin (10.9%), similar to findings by Usha et al. [5] (21%) and Mohan et al. [18] (16.6%), but a higher resistance rate to gentamicin (30.73%), consistent with other studies [5, 14, 18].

Ciprofloxacin resistance was observed in 53.63% of CoNS isolates in our study, resembling findings by Mohan et al. [18] (51%), while Sharma et al. [14] noted a slightly higher resistance rate (76%). Resistance to cotrimoxazole was 46.36% in our study, correlating with studies by Veena et al. [19] and Usha et al. [5], whereas Sharma et al. [14] recorded a higher resistance rate (85.73%). Erythromycin resistance was observed in 60.6% of isolates in our study, with varying rates reported elsewhere [14, 18]. Teicoplanin resistance was detected in 4.54% of isolates in our study, consistent with other reports [9, 20], while methicillin resistance was noted in 68.18% of isolates, similar to findings by Chaudary et al. [17] and Koksal et al. [5]. In contrast, Sheikh et al. [15] reported maximum resistance against oxacillin (94%), whereas Usha et al. [5] noted a lower methicillin resistance rate

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(56%). Moreover, methicillin-resistant CoNS isolates exhibited higher resistance to other antibiotic groups compared to their methicillin-sensitive counterparts, corroborating findings by Koksal et al. [6] and Sharma et al. [14].

Conclusion

In conclusion, Coagulase-negative Staphylococci (CoNS) are increasingly recognized as clinically significant pathogens, underscoring the importance of species-level identification. S. hemolyticus and S. epidermidis emerge as commonly isolated CoNS species, with S. hemolyticus displaying a higher antibiotic resistance profile than S. epidermidis. Methicillin resistance was prevalent among the isolates, emphasizing the critical need for effective therapeutic strategies in the face of rising multidrug resistance among CoNS.

References

1. Piette A, Verschraegen G. Role of Coagulase-Negative *Staphylococci* In Human Disease. Vet Microbiol 2009; 134(1-2): 45-75.
2. Asangi SY, Mariraj J, Sathyanarayan MS, Nagabhushan, Rashmi. Speciation Of Clinically Significant Coagulase Negative *Staphylococci* and Their Antibiotic Resistant Patterns In A Tertiary Care Hospital. Int J Biol Med Res 2011; 2(3): 735-739.
3. Becker K. Heilmann C, Peters G. Coagulase-Negative *Staphylococci*. Clin Microbiol Rev 2014; 27(4): 870 - 926.
4. Kleeman KT, Bannerman TL, Kloos WE. Species Distribution of CoagulaseNegative Staphylococcal Isolates at A Community Hospital and Implications for Selection of Staphylococcal Identification Procedures. J Clin Microbiol 1993; 31(5): 1318-21.
5. Usha MG, Shwetha DC, Vishwanath G. Speciation of Coagulase Negative Staphylococcal Isolates from Clinically Significant Specimens and Their Antibiogram. Indian J Pathol Microbiol 2013; 56(3): 258-260.
6. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative *staphylococcus* strains isolated from blood cultures of septicemic patients in Turkey. Microbiological Research 2009; 164: 404- 10.
7. May L, Klein EY, Rothman RE, Laxminarayan R. Trends in Antibiotic Resistance in Coagulase-Negative *Staphylococci* in the United States, 1999 to 2012. Antimicrob Agents Chemother 2014; 58(3): 1404–09.
8. Widerström M, Wiström J, Sjöstedt A, Monsen T. Coagulase-negative *Staphylococci*: update on the molecular epidemiology and clinical presentation, with a focus on *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Eur J Clin Microbiol Infect Dis 2012; 31: 7–20.
9. Begum ES, Anbumani N, Kalyani J, Mallika M. Prevalence and antimicrobial susceptibility pattern of Coagulase-negative *Staphylococcus*. Int J Med Public health 2011; 1(4): 59-62.
10. Hira V, Kornelisse RF, Sluijter M, Kamerbeek A, Goessens WH, de Groot R. Colonization Dynamics of AntibioticResistant Coagulase-Negative *Staphylococci* in Neonates. J Clin Microbiol 2013; 51(2): 595–597.
11. Sharma V, Jindal N, Devi P. Prevalence of methicillin-resistant coagulase-negative *staphylococci* in a tertiary care hospital. Iran J Microbiol 2010; 2(4): 185-188.
12. Khan MM, Faiz A, Ashshi AM. Clinically significant Coagulase Negative *Staphylococci* and their antibiotic resistance pattern in a tertiary care hospital. JPMA 2014; 64: 1171- 1174.
13. Otto M. *Staphylococcus epidermidis*- the "accidental" pathogen. Nat Rev Microbiol 2009; 7(8): 555-567.
14. Sharma P, Lahiri KK, Kapila K. Conventional and molecular characterization of coagulase-negative *staphylococcus* in hospital isolates. Indian J Pathol Microbiol 2011; 54(1): 85-89.
15. Sheikh AF, Mehdinejad M. Identification and determination of coagulase-negative

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Staphylococci species and antimicrobial susceptibility pattern of isolates from clinical specimens. African Journal of Microbiology Research 2012; 6(8): 1669- 1674.

16. Goyal R, Singh NP, Kumar A, Kaur I, Singh M, Sunita N, et al. Simple and economical method for speciation and resistotyping of clinically significant coagulase-negative *staphylococci*. Indian J Med Microbiol 2006; 24(3): 201-204.
17. Chaudhury A, Kumar AG**.** In vitro activity of antimicrobial agents against oxacillin-resistant *Staphylococci* with special reference to *Staphylococcus haemolyticus.* Indian J Med Microbiol 2007; 25(1): 50-52.
18. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase-negative *staphylococci* isolated from various clinical specimens. Indian J Med Microbiol 2002; 20(1): 45-46.
19. Veena M, Ramya TG, Spoorthi KU, Vishwanat G. Association between Biofilm Formation and Methicillin Resistance in Coagulase Negative *Staphylococci.* Int J Curr Microbiol App Sci 2015; 4(7): 153-159
20. Ma XX, Wang EH, Liu Y, Luo EJ. Antibiotic susceptibility of coagulase-negative *staphylococci* (CoNS): the emergence of Teicoplanin non-susceptible CoNS strains with inducible resistance to vancomycin. J Med Microbiol 2011; 60.