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Isolation, identification and antibiotic susceptibility pattern of coagulase –negative *staphylococci* (cons) in various clinical specimensJaspreet Kaur¹, Kiranjeet Kaur^{1,*}¹Dept. of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India

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ABSTRACT

Background: Coagulase Negative *Staphylococci* (CoNS) have been recognized as an important agent of human infection since the past five decades. Significance of CoNS in human infection is increasing these days. CoNS are the indigenous flora of the skin and mucous membrane. They have long been considered as non pathogenic and were rarely reported to cause severe infections. However, as a result of the combination of increased use of intravascular devices and an increase in the number of hospitalized immunocompromised patients, CoNS have become the major cause of Nosocomial blood stream infections and they account for 9% of nosocomial infections.

Purpose: This study has been done to identify clinically significant CoNS associated with different human infections and also study their antibiotic susceptibility pattern isolated from clinical samples.

Materials and Methods: The present study was conducted in a teaching hospital of Punjab, India. A total 52 strains of CoNS isolated as a sole agent with a significant growth from various clinical samples were included in the study. The speciation of CoNS was done based gram staining, catalase test, coagulase test and VITEK – 2 Compact system.

Results: Out of 52 sample, 61.5% is male and 38.5% female coming. The most common CoNS species isolated were *S. hemolyticus* 28(54%), *S. epidermidis* 8(15%), *S.hominis* 8(15%) and *S. saprophyticus* 8(15%).

Conclusions : The current study shown that pathogenic CoNS cause infection-related signs and symptoms. CoNS was discovered to be isolated from blood, urine, and pus, and *S. hemolyticus*, *S. epidermidis*, *S. homoinis*, and *S. saprophyticus* were the most often found species in patients.

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1. Introduction

Gram-positive *Staphylococci* are non-sporing, non-motile cocci that are arranged in clusters that resemble grapes. On the skin and mucous membranes, they often form a normal part of the flora. The most famous member of the family and the most popular coagulase-positive strain is *S.aureus*.¹ Coagulase-positive and Coagulase-Negative *Staphylococcus* (CoNS) species are commonly distinguished. Species of *Staphylococcus* that do not

produce an enzyme coagulase are known as coagulase -negative *Staphylococci*. CoNS are the type of bacteria that frequently live on a person's skin and mucous membranes and are known to be a major cause of human infection.² About 40 different species of bacteria make up the heterogeneous CoNS group, and several of these have been identified as potential human pathogens.³ *S. epidermidis*, *S. hemolyticus*, *S. saprophyticus* and *S. hominis* are the most often isolated species from human specimens that cause disease. Other species like *S.lugdunensis*, *S. capitis*, *S. simulans*, *S. cohnii*, *S. saccharolyticus*, and *S. xylosus* have been regarded as important opportunistic pathogens but are

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rarely isolated.^{4,5} Currently, CoNS, as typical opportunists, is one of the most dangerous nosocomial, posing a serious threat to human life and health and being responsible for between 27% and 32% and 50% of nosocomial infections in adult and paediatric patients, respectively.⁶ The risk of contracting CoNS infection increases for surgical patients and hospitalised individuals.⁷ People most vulnerable to CoNS infections are those with weakened immune systems. This includes those who have cancer, are elderly, are very young, or have autoimmune diseases and those who have a urinary catheter inserted.⁸

About 75 % of all clinical isolates of CoNS are caused by *Staphylococcus epidermidis* and its emergence as a multiple drug resistance determinant makes patient medical management more challenging.⁹ *Staphylococci* are becoming more and more resistant to antimicrobial treatments. Methicillin resistance in CoNS varies in India from 22.5% to 64.8%.^{10–12}

2. Objectives

1. To study the prevalence of CoNS in clinical samples of patients in ICUs.
2. To identify various species of CoNS isolated from clinical samples.
3. To study the antimicrobial susceptibility pattern of CoNS.

3. Material and Methods

The Present Study entitled "Isolation, Identification, and Antibiotic Susceptibility Pattern of Coagulase –Negative *Staphylococci* (CoNS) in Various Clinical Specimens" was conducted in a teaching hospital of Punjab, India. Over six months from October 2021 to March 2022 after the approval from Institutional Research committee and Ethics Committee for Biomedical and Health Research. A total of 1200 various clinical specimens received from different infections from both males and females of all age groups patients admitted in ICU, CCU, NICU, and PICU were processed at the Bacteriology Laboratory of Microbiology department. Samples of blood, wound swabs, pus, drain fluid, tracheal aspirate, peritoneal fluid, pleural fluid, and urine were received. For blood and body fluid, BacT/ALERT 3 D System was used. Blood agar, MacConkey agar and CLED agar media was used as solid culture media for isolation of pathogen. Culture showing significant growth of organism were further processed for identification on the basis of colony characteristics. For all Gram positive cocci, catalase test was performed to differentiate *Staphylococci* from Streptococci. For all *Staphylococci*, slide coagulase was done using human plasma to differentiate between coagulase positive and coagulase negative. Coagulase negative *Staphylococci* were further identified to species level by Vitek 2 Compact system

using GP card. For antibiotic susceptibility testing P628 AST Panel was used to assess the antibiotic sensitivity by VITEK-2 compact system. Results were interpreted in accordance with recommendations made by the Clinical Laboratory Standards Institute (CLSI).¹³ Clinical data of patients was collected using proforma by taking relevant history and from record files.

4. Results

During the six-month period between 1 October 2021 to 31 March 2022, a total 1200 clinical sample were received from various intensive care departments (ICU,CCU,PICU,NICU) in bacteriology laboratory of microbiology department for culture and sensitivity. Among these 1200 clinical samples 216 showed significant growth with 52(23.7%) CoNS and 164(76.3%) others bacterial isolates. Isolation of CoNS were more from male patient 32(61.5%) then females 20(38.5%). Samples was distribution was as shown in Table 1.

5. Discussion

A total of 1200 clinical samples were received from various ICUS 52 CoNS were isolated. The prevalence of CoNS infection in present study was found to be 23.7% which is very high. A similar results was found by a Bora P (2018) reported that out of 120 samples 68 isolates were CoNS with 56% prevalence. A study by Kumar S. (2018) reported that out of 400 clinical samples, 60 isolates were as CoNS with 15% prevalence and a study by Ariel E Stella (2020) reported that out of 276 clinical samples, 58 isolated were coagulase negative *Staphylococci* with 19% prevalence which are less than the present study. The present study showed the CoNS infection isolates out numbered in males 32(61.5%) then females 20(38.5%) This may be due to more hospital admission of male patients. In the present study maximum isolation of CoNS was from blood 30(57.7%) followed by sputum 2(3.8%) urine 17(32.7%), pus 1(1.9%) body fluid 1(1.9%)(Table 1). A Study by Qadar.S et.al shows the result of sample collection as from urine 31%, pus 2.0%, sputum 2.8%, fluid 2.0% and from blood 55.5% which is almost similar to present study.¹⁴ A Study by Mila V.S et al and Ibrahim et al are less then the present study.

In our study *S. hemolyticus* was highest 54% followed while others like *S.saprophyticus*, *S.epidermitidis*, *S.hominis* (Table 2) A stella and mohan et.al showing similar studies but a study by Qadar et.al is less then to present study.

5.1. Antibiotic susceptibility pattern (Table 3)

According to the current study's analysis of CoNS species' susceptibility to antibacterial agents, *S.hemolyticus* is more susceptible to the antibacterial drugs tigecyclin 18 (65%)

Table 1: Distribution of CoNS among different clinical samples.

Sample	No. of sample	Percentage
Urine	17	32.7%
Pus	1	1.9%
ET	1	1.9%
Sputum	2	3.8%
Fluid	1	1.9%

Table 2: Speciation profile of coagulase negative Staphylococci:

Species	No. of isolate	Percentage (%)
<i>S.heamolytic</i>	28	54%
<i>S.saprophyticus</i>	8	15%
<i>S.hominis</i>	8	15%
<i>S. S.epidermitidis</i>	8	15%

Table 3: Antibiogram of different species of CoNS

Antibiotics	<i>S.hemolyticus</i>		<i>S.epidermitidis</i>		<i>S.hominis</i>		<i>S.saprophyticus</i>	
	S	R	S	R	S	R	S	R
Benzylpenicillin	3(11%)	25(89%)	2(25%)	6(75%)	2(25%)	6(75%)	2(25%)	6(75%)
Oxacillin	7(25%)	21(75%)	5(63%)	3(37%)	2(25%)	6(75%)	5(63%)	3(37%)
Gentamicin	11(39%)	17(61%)	4(50%)	6(50%)	7(88%)	1(12%)	4(50%)	6(50%)
Ciprofloxacin	10(36%)	18(64%)	2(25%)	6(75%)	3(38%)	5(62%)	2(25%)	6(75%)
Levofloxacin	7(25%)	21(75%)	4(50%)	4(50%)	4(50%)	4(50%)	4(50%)	4(50%)
Erythromycin	7(25%)	21(75%)	6(75%)	2(25%)	6(75%)	2(25%)	6(75%)	2(25%)
Clindamycin	12(43%)	16(57%)	7(88%)	1(12%)	6(75%)	2(25%)	7(88%)	1(12%)
Cotrimazodole	14(50%)	14(50%)	8(99%)	-	7(87%)	1(13%)	8(99%)	-
Linezolid	16(57%)	12(43%)	8(99%)	-	7(87%)	1(13%)	8(99%)	-
Daptomycin	18(64%)	10(46%)	8(99%)	-	6(75%)	2(25%)	8(90%)	-
Teicoplanin	18(64%)	10(46%)	8(99%)	-	6(75%)	2(25%)	8(99%)	-
Vancomycin	18(64%)	10(46%)	8(98%)	-	7(87%)	1(13%)	8(98%)	-
Tetracycline	16(57%)	12(53%)	8(98%)	-	6(75%)	2(25%)	8(99%)	-
Tigecycline	16(57%)	12(53%)	8(99%)	-	1(12%)	7(88%)	8(97%)	-
Nitrofurantion	14(50%)	14(50%)	8(98%)	-	6(75%)	2(25%)	8(97%)	-
Rifampicin	11(50%)	11(50%)	4(50%)	-	1(12%)	7(88%)	4(50%)	50

Table 4: Species wise distribution reported by different studies comparative to present study.

Species	Present study	Mohan et al	Mila V.S et al	Qader et al
<i>S.hemolyticus</i>	(28)54%	52%	20%	35%
<i>S. saprophyticus</i>	(8)15%	18%	27%	29%
<i>S.epi dermitidis</i>	(8)15%	16%	40%	59%
<i>S.hominis</i>	(8)15%	11%	15%	9%

and vancomycin 18 (64%), while studies by Ahmed et al and Mila V.S. et al. found lower susceptibilities to these drugs than did the present study.

S.epidermitidis, which is very responsive to Daptomycin 8 (99%) and Tetracycline 8 (99%) as well as Gentamicin 90% and Daptomycin 85%, according to a research by Ahmed et al.¹⁵ A study by Mila V.S. et al. found that gentamicin is only 65 percent effective against penicillin-resistant bacteria.

In the current study, *S. hominis* is very sensitive to Vancomycin 6 (75 %), Daptomycin 7 (87 percent), and Tigecycline 6 (75%)(Table 3). In contrast, a study by

Prapti found that *S. hominis* is sensitive to Daptomycin 90%, Vancomycin 92 %, and Gentamicin 54% and study by Ibrahim et al which is 75% daptomycin and 80% vancomycin resistant.

In the current study *S. saprophyticus* sensitivity to Linezolid 8 (99%), Daptomycin 8 (99%)(Table 3) and a comparable study by Mila V.S et al. that is Daptomycin 98% and Linezolid 98% are all very toxic to *S. Saprophyticus*.¹⁶ According to a study by Ibrahim Ali et al, 46% of the sample was resistant to Gentamycin and 34% to Vancomycin.

6. Conclusion

The current study shown that pathogenic coagulase-negative *Staphylococci* cause infection-related signs and symptoms. CoNS was discovered to be isolated from blood, urine, and pus, and *S. hemolyticus*, *S. epidermidis*, *S. hominis*, and *S. saprophyticus* were the most often found species in patients.

The majority of the isolates of *S. hemolyticus* came from cultures. Most patients in CCUs who develop CoNS infection do so. Those, those with central lines and ventilators, and patients with post-operative wounds. Vancomycin and gentamycin are very efficient against a group of microorganisms that are very resistant to penicillin and amoxicillin.

7. Source of Funding

None.

8. Conflicts of Interest

There is no conflict of interest.

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