



## Medical Microbiology

## PREVALENCE OF GLYCOPEPTIDE RESISTANCE IN COAGULASE NEGATIVE STAPHYLOCOCCI IN A TERTIARY CARE MULTISPECIALITY CENTRE

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**ABSTRACT** **Background:** Coagulase Negative Staphylococci (CoNS) were once thought to be non-pathogenic skin contaminants when recovered from culture media, but the usage of intravenous and implantable devices and increase in the number of immune - compromised patients in hospitals, they have now emerged as a major contributor of hospital acquired infections. The number of CoNS with enhanced resistance to glycopeptides (especially vancomycin) has been observed to be on the rise in the recent decades. Therefore, this research was done to recognize the clinical isolates of CoNS and to recognize various drug resistance with distinct mention to Vancomycin. **Aim:** This study aimed to isolate Coagulase Negative Staphylococci from different clinical specimens and to determine antimicrobial susceptibility pattern of all the isolates. **Methods:** From January 2021 to December 2021, this study was conducted in the Department of Microbiology at the School of Medical Sciences & Research, SMSR, Sharda University, Greater Noida. Total 341 CoNS were recovered in 1 year duration time from various clinical samples. Descriptive statistics like frequency table, mean, proportions were used. Analysis was done by SPS software. **Results:** A total of 341 isolates of coagulase negative staphylococci (CoNS) were obtained from various specimens out of which 150 (44%) were found to be clinically significant. Maximum number of clinically significant CoNS were recovered in the age group 0-9 years 29 (19.3%) followed by age group,  $\geq 60$  years: 27 (18%). Clinically significant CoNS were isolated in maximum number from blood samples (n=51, 34%) followed by pus (n=35, 23.3%). Samples received from patients housed in the neonatal intensive care unit yielded the maximum number of clinically significant CoNS isolates, 31 (20.7%). *Staphylococcus epidermidis* was found to be the most commonly associated clinically significant CoNS with infection 72 (48%) followed by *Staphylococcus haemolyticus* 42 (28%). MIC by microbroth dilution and E test was also done on all clinically significant isolates of CoNS for vancomycin, all isolates were within the susceptible range with none of the isolates being resistant to vancomycin (with MIC  $\leq 4\mu\text{g/ml}$ ). **Conclusions:** It is thus concluded that isolation of CoNS and their antibiotic susceptibility pattern should be regarded with all seriousness in clinical practice and clinical epidemiology because by being resistant to multiple antibiotics, (methicillin resistant CoNS in particular), their prevalence not only limits the treatment options but also acts as a reservoir of drug-resistant genes.

**KEYWORDS :** CoNS -MRCoNS -Vancomycin resistance**INTRODUCTION :**

Term *Staphylococci* is deciphered from the Greek word- "staphyle" meaning grapes in a bunch. Sir Alexander Ogston in 1883, who was Scottish surgeon, devised the term as these organisms arranged in clusters. All *Staphylococci* belong to family Micrococcaceae. Depending on whether the coagulase enzyme is present, *Staphylococcus* can be classified as either coagulase-positive *Staphylococcus aureus* or Coagulase-Negative CoNS. CoNS makes up a sizable portion of the typical commensal flora, of the anterior part of nasal cavity, external auditory canal and skin of human beings.

There are 32 species of CoNS. Of which important ones are as follows.

- *S. epidermidis*:- most common species present on skin and mucus membrane, constituting 65-90% of all the CoNS isolated. .
- *S. haemolyticus* and *S. hominis* :- are present abundantly in apocrine glands like axilla, inguinal area and perineal region.
- *S. lugdunensis* and *S. warneri* :- are present in the skin but comprise the small population of CoNS.
- *S. saprophyticus* :- it is a pathogen causing UTI in sexually active females.
- *S. xylosum*, *S. cohnii*, *S. simulans*, *S. schleferi* :- are present on the skin but are less common.
- *S. intermedius* :- recovered from infected dog bites.
- Notable ungulate dwellers are *S. hyicus*, *S. chromogens*, *S. sciuri*, *S. lentus* and *S. vitulus*.
- *S. auricularis* :- present in ear canal.

They were once thought to be non-pathogenic skin contaminants when recovered from culture media, but the usage of intravenous and implantable devices and increase in the number of immune - compromised patients in hospitals, they have now emerged as a major contributor of hospital acquired infections.

CoNS strains with multiple resistance antibiotics have become a big threat in nosocomial infections. CoNS are frequently associated with blood stream infections, accounting for 30% to 40% of total of these

infections. Most CoNS blood stream infections caused by infected catheters, from CoNS living on skin and mucus membrane and then gain entry while inserting the intravascular catheters, and then advance into the blood stream.

Nosocomial isolates have up to 80% resistance to methicillin, and it is also associated with multidrug resistance. Methicillin resistance has been more prevalent in CoNS during the past several years, which has resulted in an utterly irrational overuse of vancomycin as a therapy for cases of methicillin resistant CoNS. Due to this, isolates that are significantly less sensitive to vancomycin and teicoplanin have started to emerge and have been described since 2006.

Few current reports have revealed a high level of resistance to methicillin, the development of biofilm communities, and a reduction in vancomycin susceptibility which is immense hazard. This also explains the choice of multidrug-resistant isolates in health care facilities environment and ineffective of antimicrobial treatment.

Therefore, this research was done to recognize the clinical isolates of CoNS and to recognize various drug resistance with distinct mention to Vancomycin.

**AIM & OBJECTIVES :**

1. To isolate *Coagulase Negative Staphylococci* from different clinical specimens.
2. To speciate different strains of *Coagulase Negative Staphylococci* isolated.
3. To determine antimicrobial susceptibility pattern of all the isolates.
4. To detect the minimum inhibitory concentration of isolates by microbroth dilution method and by E-test.

**MATERIALS & METHODS :**

From January 2021 to December 2021, this study was conducted in the Department of Microbiology at the School of Medical Sciences &

Research, SMSR, Sharda University, Greater Noida. Total 341 CoNS were recovered in 1 year duration time from various clinical samples. Age, sex, the history of any complaints the patient had, the history of any prosthetic implants (such as pacemakers, hip and knee replacements, CSF shunts, etc.), the length of the hospital stay, the history of treatment, including any antibiotics given, the history of prior admissions, and the history of sample collection for repeat tests (such as whether a blood culture was sent to the lab more than once, etc.), were all recorded.

Patients who were admitted to various wards, ICUs, NICUs, MICUs, and SICUs as well as those who were attending various OPDs including surgery, medicine, orthopaedics, gynaecology, ENT, and ophthalmology, from there various clinical samples such as blood, urine, pus, pleural fluid and ascitic fluid were taken.

Descriptive statistics like frequency table, mean, proportions were used. Analysis was done by SPS software.

All the clinical samples which came to microbiology laboratory were processed as per the standard procedures. Clinical samples were inoculated onto Nutrient agar to rule out *S.aureus* golden yellow pigmentation, Blood agar and MacConkey's agar plate. On CLED agar plate, urine samples were inoculated. Then these plates were then incubated under aerobic conditions at 37 °C for 18 to 24 hours.

The colonies resembling CoNS on culture media were selected for further characterization and confirmation. Isolates which were recovering repeatedly from infected site and also having history of any prosthetic implant were considered as clinically significant. These isolates were further recognized by colony morphology (pin head, circular, emulsifiable, white opaque, smooth, convex and entire edge) and further by following criteria, as per the standard procedures, which are as follows:-

1. Preliminary Identification Microscopy: Gram's staining was used to confirm the presence of violet-coloured, spherical Gram-positive cocci arranged in clusters about 1 µm in size.
2. Speciation of CoNS: Phenotypic identification CoNS were as per the procedures. Clinically significant *Staphylococcus* species were identified based on Kloos and Bannerman (1995), following tests were performed for speciation of various isolates of CoNS. Ambiguous strains were identified by automated Vitek 2 compact system.
3. Antibiotic Susceptibility Testing: It was done using Kirby-Bauer's disk diffusion method on MHA agar plate and using 0.5 McFarland's standard with appropriate controls. The panel of antibiotic disks used were commercially obtained from HIMEDIA. Methicillin resistance was detected using Cefoxitin 30µg disks. For quality control *Staphylococcus aureus* ATCC 25923 was used.
4. Interpretation: Measurement of zone diameter with the help of a ruler. The zones of growth of inhibition around each disks were measured. The diameter of zone of inhibition of each disk was compared with CLSI zone size interpretive chart.

Detection of vancomycin susceptibility was done by E-test & MIC was done by microbroth dilution method.

## RESULTS:

A total of 341 isolates of coagulase negative staphylococci (CoNS) were obtained from various specimens over a period of one year, out of which 150 (44%) were found to be clinically significant. Clinically significant CoNS were recovered equally from male and female patients, n-75 (50%) each as compared to non-significant CoNS which were recovered more from male patients. Maximum number of clinically significant CoNS were recovered in the age group 0-9 years 29 (19.3%) followed by age group, ≥ 60 years: 27 (18%).

Clinically significant CoNS were isolated in maximum number from blood samples (n=51, 34%) followed by pus (n=35, 23.3%). Samples received from patients housed in the neonatal intensive care unit yielded the maximum number of clinically significant CoNS isolates, 31 (20.7%). Significant isolation of clinically significant CoNS was seen from patients who had the presence of intravenous line catheters 115 (76.7%) whereas only 60 (31.4%) patients from whom non-significant CoNS were recovered had Intravenous line catheters.

Hospital stay of >1 week was found to be a significant risk factor in patients from whom clinically significant CoNS were recovered 90

(60%) though 61 (31.9%) patients from whom non-significant CoNS were isolated also had hospital stay of >1 week.

Prior use of B-lactam antibiotics and fluoroquinolones was more often noted in patients from whom significant CoNS were isolated as compared to those from whom non-significant CoNS were isolated. Significant number of patients from whom clinically significant CoNS were isolated were suffering from septicemia (70%).

*Staphylococcus epidermidis* was found to be the most commonly associated clinically significant CoNS with infection 72 (48%) followed by *Staphylococcus haemolyticus* 42 (28%). *Staphylococcus saprophyticus* was isolated from 4 urine specimens which were included in the study. All these specimens were from females in the sexually-active age group. (Table 1)

**Table 1: Clinically significant species of Coagulase Negative Staphylococci**

Organism isolated	Number of isolates (N=150)	Percentage (%)
<i>Staphylococcus epidermidis</i>	72	48
<i>Staphylococcus haemolyticus</i>	42	28
<i>Staphylococcus hominis</i>	24	16
<i>Staphylococcus saprophyticus</i>	4	2.6
<i>Staphylococcus auricularis</i>	2	1.3
<i>Staphylococcus chromogenes</i>	1	0.7
<i>Staphylococcus cohnii</i>	1	0.7
<i>Staphylococcus warneri</i>	1	0.7
<i>Staphylococcus scuiiri</i>	2	1.3
<i>Staphylococcus xylosus</i>	1	0.7

100% of the clinically significant CoNS (150) were found to be resistant to penicillin. Methicillin resistance was seen in 85 (56.7%) of these isolates. (Table 2)

**Table 2: Antimicrobial susceptibility profile of the recovered isolates of clinically significant CoNS**

Antibiotic	No. tested	Sensitive		Resistant	
		N	%	N	%
Penicillin	150	0	0	150	100
Methicillin	150	65	43.3	85	56.7
Clindamycin	150	40	26.7	110	73.3
Erythromycin	150	20	13.3	130	86.7
Co-trimoxazole	150	22	14.7	128	85.3
Tetracycline	150	45	30	105	70
Ciprofloxacin	150	15	10	135	90
Linezolid	150	145	96.7	5	3.3
Nitrofurantoin	4	0	0	4	100
Novobiocin	4	0	0	4	100

5 (3.3%) were found to be resistant to Linezolid. MIC by microbroth dilution was done on all clinically significant CoNS isolates for vancomycin. All isolates were well within the susceptible range with none of the isolates being resistant to vancomycin. MIC by E-test was also done on all clinically significant isolates of CoNS for vancomycin, all isolates were within the susceptible range with none of the isolates being resistant to vancomycin (with MIC = <4µg/ml). (Table 3)

**Table 3: Showing MIC by microbroth dilution method and E-test**

MIC (ug/ml)	Vancomycin (Microbroth dilution method)		Vancomycin (E-test)	
	N	%	N	%
0.125	8	5.3	0	0
0.25	12	8	8	5.3
0.5	70	46.7	12	8
1	40	26.7	70	46.7
2	20	13.3	60	40
4	0	0	0	0
8	0	0	0	0
16	0	0	0	0
32	0	0	0	0
64	0	0	0	0

## CONCLUSION:

It is thus concluded that isolation of CoNS and their antibiotic susceptibility pattern should be regarded with all seriousness in

clinical practice and clinical epidemiology because by being resistant to multiple antibiotics, (methicillin resistant CoNS in particular), their prevalence not only limits the treatment options but also acts as a reservoir of drug-resistant genes.

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