ORIGINAL RESEARCH PAPER

Clinical Microbiology

STUDY OF SPECIES IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF VARIOUS CLINICAL ISOLATES OF COAGULASE –NEGATIVE STAPHYLOCOCCI

KEY WORDS: MRCoNS; Methicillin- resistant Coagulase negative Staphylococci , MDR- Multi Drug Resistant , Staphylococcus. epidermidis

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ABSTRACT

BACKGROUND- Interest in CoNS is increasing because of their role as pathogens in certain clinical conditions and their marked resistance to antibiotics. Frequent isolation of CoNS from the blood, other normally sterile body fluids, intravenous catheters and various tissues presents a recurrent interpretative challenge to both the clinician and microbiologist. The ubiquity of these organisms does present problems when one is faced with making a decision as to whether their isolation in clinical laboratory represents true infection or merely contaminant. AIM-The aim of this study was to isolate, identify and speciate coagulase negative staphylocci(CoNS) from clinical specimens,along with to find their resistance to antimicrobial agents with reference to their methicillin resistance .**MATERIAL & METHOD** - Various clinical specimens like blood, pus, wound swab, ascitic / synovial fluid, urine, cerebro spinal fluid were processed in laboratory following the standard protocol for isolation, identification and speciation of coagulase negative staphylococci. RESULT- Present study found, S. epidermidis to be most prevalent species of coagulase negative staphylocci from clinical isolates with 47.58% strains, followed by S. haemolyticus 26.06%, and S. lugdunensis 8.54%. S. saprophyticus was the most common of CoNS species from urinary tract infection cases. Methicillin resistant CoNS (MRCoNS) were detected by cefoxitin disk diffusion method Out of 234 strains of CoNS, 153 strains (65.38%) were detected as MRCoNS. Most of the MRCoNS were found to be resistant to penicillin (100%), ceftazidime (99.35%), amoxicillin-clavulanic acid (98.69%), cotrimoxazole (92.81%), gentamicin (88.23%).Susceptibility to vancomycin and pristinamycin was 86.93 % and 99.35% respectively while all the MRCoNS isolates were susceptible to linezolid.CONCLUSION- Vancomycin is the mainstay of therapy in MDR MRCoNS infections and should be used judiciously. Looking at the possibility of emergence of resistance to the drug, newer agents like linezolid and pristinamycin might provide a valuable option for the treatment of MRCoNS infections. There is a need to have the knowledge and monitoring of antibiotic sensitivity pattern and formulation of definite antibiotic policy to improve the empirical approaches to the therapy of serious infections caused by CoNS.

INTRODUCTION

Micro-organisms are still the important factors which are worsening the living conditions of man despite decades of dramatic progress in the prevention and treatment of diseases. To add to the misery of mankind, even the commensals are now being increasingly identified as the cause of diseases in special conditions like decreased host resistance. One such group of commensals is coagulase negative staphylococcus (CoNS).[1]

Staphylococcus is the second most commonly isolated microorganism from the clinical specimens in the microbiology laboratory with the exception of enterobacteriacae. Coagulase positive staphylococcus i.e. Staphylococcus aureus is well equipped with variant of virulence factors and is by far the most important pathogen amongst the staphylococci. Historically, S. aureus has been regarded as opportunistic pathogen whereas CoNS have been generally regarded as non pathogens.[2].

In hospital microbiology laboratory, staphylococcus isolation is often limited to coagulase test for S. aureus while non S. aureus isolates are simply reported as CoNS.[3] CoNS are one of those amongst the commonly isolated organisms in the clinical microbiology laboratory. Interest in CoNS is increasing because of their role as pathogens in certain clinical conditions and their marked resistance to antibiotics. Frequent isolation of CoNS from the blood, other normally sterile body fluids, intravenous catheters and various tissues presents a recurrent interpretative challenge to both the clinician and microbiologist.[1] The ubiquity of these organisms does present problems when one is faced with making a decision as to whether their isolation in clinical laboratory represents true infection or merely contaminant[4]. Being the normal flora of various parts of skin, respiratory system and gastrointestinal system, they are rarely significant when isolated from skin, sputum and nasal swabs but may well be significant when isolated from wound swabs, pus, body fluids or blood cultures especially if foreign material is present.[3] Over the past four decades, these agents have become recognized as important agents of human diseases.[5] CoNS are reported to be the third common causative agent of nosocomial infections and the most common cause of nosocomial blood stream infections.[6,7]

Clinically significant CoNS should be identified to the species level, because of their increasing importance. Due to the growing recognition that CoNS are of medical importance, numerous studies were initiated in an attempt to classify these organisms.[2] At present CoNS includes 41 recognized taxons of which 21 types represent etiological agents in the human diseases.[8] S. epidermidis is by far the most frequently recovered organism, accounting for 50 to 80 % isolates. S. haemolyticus is the second common isolate. Other isolates are S. saprophyticus, S. cohnii, S. hominis, S. capitis, S. lugdunensis, S simulans, S. warneri, S. xylosus etc.[5]

The determination of antimicrobial susceptibility of clinical isolates is often crucial for optimal therapy of infected patients. This is particularly important considering the increase of resistance and emergence of multidrug resistant organisms.[9] CoNS strains are commonly multi-resistant to various groups of antibiotics. In reports from different parts of Europe, methicillin resistance in CoNS varies between 70 to 80 % which is similar to that of U.S, Canada and Latin America.[10-12]

Thus it has become important to identify CoNS to species level and to determine their antimicrobial susceptibility for effective therapeutic intervention.

Limited treatment options and prolonged course of infection due to these CoNS species could have severe consequences for patients. Full and accurate identification of CoNS isolates

in clinical samples is therefore of great importance for epidemiological purposes and infection control measures.[13] However scant data are available on CoNS responsible for infections in developing countries.[14,15]

AIMS AND OBJECTIVES

The present study was undertaken with an aim to identify the species of coagulase negative staphylococci isolated most commonly from clinical specimens, to find their resistance to antimicrobial agents with reference to their methicillin resistance . This aim was achieved by the following objectives:

1) Isolation, genus identification and species identification of infection/colonization strains of coagulase negative staphylococci by conventional test methods.
2) Correlation of antimicrobial resistance with the site of isolation of infection/colonization strain of coagulase negative staphylococci.
3) Correlation of antimicrobial resistance with the species of isolated infection/colonization strain of coagulase negative staphylococci
4) Detection of methicillin resistance in the isolated species of coagulase negative staphylococci.

MATERIAL and METHODS

The present study was carried out in Department of Microbiology, at our tertiary care hospital during Jan 2022 to Dec 2022.

All properly collected, well labeled samples of the indoor patients coming to the department for microbiological investigations were included in our study.

I] COLLECTION, TRANSPORTATION AND PROCESSING OF SPECIMEN IN THE LABORATORY

Clinical specimen i.e. urine, blood, pus, CSF and body fluids received in microbiology laboratory of our tertiary care center were collected aseptically in accordance with the standard recommendations from the patients admitted in various wards of L.N..Medical College and Hospital.

The labelled specimens were transported immediately, along with the requisition form, to the Microbiology laboratory for processing.

The specimens received in the Microbiology laboratory were processed in accordance with the recommended procedures for the isolation and identification of bacteria.[16] After initial scrutiny and screening process the samples used for final processing were blood (bacterial endocarditis, septicaemia), pus, urine (significant bacteriuria), cerebrospinal fluid, fluids(pleural, ascitic, drain-microscopically significant), catheter tips and aspirates.

II] ISOLATION, PRESUMPTIVE IDENTIFICATION AND STORAGE OF ISOLATES / STRAINS

1) Isolation

In accordance with the recommended procedures, the received samples were screened by microscopy and specimen showing gram positive cocci in cluster were inoculated on Blood agar and Mac Conkey agar.[5]

2) Presumptive Identification

The growth was identified by colony morphology, gram staining and biochemical reactions:

a) Blood agar: Large white / cream / yellow, 1 - 3 mm diameter, smooth, low convex / dome shaped , with entire edges, opaque, showing beta-hemolysis / no hemolysis, butyrous in consistency on blood agar plates were studied by Gram stain.

b) Mac Conkey agar: very small colonies, < 0.5 mm in diameter, opaque, lactose fermenting/ non lactose fermenting.

Gram stain:

Gram positive cocci uniform in size, appearing characteristically in groups, but also seen singly and in pairs were further identified by the scheme described for the identification of the gram positive cocci arranged in clusters using tests given below.

3) Storage

All the isolates /strains, presumptively identified were stored at minus 70^{0}C in Tryptic soy broth for future retrieval to perform various tests, slime production testing and antibiotic sensitivity testing. All strains were sub cultured at monthly intervals. Subsequently, all the 234 cultures presumptively identified as coagulase negative staphylococci were retrieved for testing.[16]

III] Identification scheme of Coagulase Negative Staphylococci

1) Genus identification :

Staphylococcal colonies were subjected to

a) Catalase test[5]

This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. Staphylococcus and Micrococcus are catalase positive.

A part of isolated colony was picked up with a sterile clean glass rod or wooden applicator stick and inserted into a small clean test tube containing 3% hydrogen peroxide solution. Immediate production of effervescence indicated positive catalase test.

Positive control- Staph aureus
Negative control- Streptococci

b) Modified Oxidase test;[5]

Staphylococcus are modified oxidase negative whereas Micrococcus is modified oxidase positive.

Principle:

The cytochrome oxidase system of Micrococcus contains cytochrome C, which yields a colored end product in presence of modified oxidase reagent.

Procedure:

Few drops of reagent were added to the filter paper strip. A colony from nutrient agar plate was smeared onto the reagent zone of the filter paper.

Interpretation:

Positive organism turned blue - purple within 30 seconds and negative remained colorless.
Positive control- Staphylococci
Negative control- Micrococci

c) Coagulase test:[5]

Staphylococcal coagulase is a protein of unknown chemical composition that has prothrombin-like activity, which can convert fibrinogen into fibrin, forming a visible clot. Both slide and tube coagulase tests were done in every staphylococcal isolate with positive & negative controls.

i) Slide coagulase test:[5]

Principle: Slide coagulase determines the bound coagulase (clumping factor). Clumping factor is attached to bacterial cell wall and is not present in culture filtrates. Fibrin strands are formed between the bacterial cells when suspended in plasma (fibrinogen), causing them to clump into visible aggregates. The test is falsely negative in 5-10 % Staphylococcus aureus strains which are otherwise positive

by tube coagulase test.

Procedure: Two drops of saline were placed in two separate circles drawn on the slide with a wax pencil. Colony material from the organism to be identified was gently emulsified in saline in each circle. After checking for absence of autoagglutination, a drop of undiluted heparinised plasma was added to the emulsion in one of the circles and mixed well. A drop of saline was added to another circle as a control. The slide was rocked back and forth, and observed for agglutination of the test suspension.

Interpretation: Appearance of coarse clumps within 10-15 seconds indicates the positive test. The test was considered as negative if no agglutination was observed after two minutes. The saline control should remain smooth and milky. If the control suspension agglutinates as well, the test was as un-interpretable.

All isolates were further processed by tube coagulase test.
Positive control- Staph aureus
Negative control- Staph epidermidis

2) Identification Of different species of coagulase negative staphylococcus
After the confirmation of genus, and performing slide coagulase and tube coagulase test those which are negative by both these test were subjected to following biochemical tests.[17]

i) Ornithine decarboxylase test [18,5]
The production of Ornithine decarboxylase identifies S. lugdunensis which, alone among staphylococci, shows this property.

A) Principle:
Decarboxylase are a group of substrate specific enzymes that are capable of reacting with carboxyl (COOH) portion of amino acids, forming alkaline reacting amines. This reaction, known as decarboxylation, forms carbon dioxide as a second product. Each decarboxylase enzyme is specific for an amino acid which on decarboxylation produces specific amine. Ornithine decarboxylates to produce putrescine.

B) Media and Reagents:
a. Moeller decarboxylase broth base
Peptone 5gm
Beef extract 5gm
Bromocresol purple 0.01gm
Cresol red 0.005gm
Glucose 0.5gm
Pyridoxal 0.005gm
Distilled water 1 lit
Final p H = 6.0
b. Ornithine
10gm (final concentration of 1%) of the levo-form was added.

C) Quality control;
Positive control- Proteus mirabilis
Negative control – Klebsiella pneumoniae

D) Procedure;
From a well isolated colony of test organism, two tubes of Moeller decarboxylase medium, one containing the ornithine to be tested and the other to be used as a control tube devoid of ornithine were inoculated. Both the tubes were overlaid with sterile mineral oil to cover about 1cm of the surface. Tubes were incubated at 350 C for 18 - 24 hours.

E) Interpretation:
Conversion of control tube to a yellow colour indicated that organism was viable and the pH of the medium was lower sufficiently to activate the decarboxylase enzyme. Reversion of the tube containing ornithine to the blue purple colour indicated a positive test.

ii) Phosphatase test [19]
A) Media and reagents:
Muller Hilton agar buffered at pH 5.6 to 5.8
P- nitrophenyl phosphate (0.495 mg/ml)
B) Procedure;

The medium was spot inoculated with the organism and read after 18 to 24 hours of incubation.

C) Interpretation:
The presence of bright yellow color under and around the inoculums was a positive test result.

iii) **Novobiocin resistance test** [5]
A) Principle:
Coagu!ase negative staphylococci can be divided into novobiocin susceptible and novobiocin resistant species. Amongst the novobiocin resistant species. S. saprophyticus is the one commonly recovered from humans as a cause of urinary tract infection.

B) Media and reagents:
Novobiocin disc 5 µg
Muller Hinton Agar plate

C) Quality control
Positive control - S. saprophyticus
Negative control - S. epidermidis

D) Procedure
Suspension of organism was prepared in nutrient broth equivalent in turbidity to 0.5 McFarland standards. With a sterile swab suspension was spread over Muller Hinton agar plate. Novobiocin disc (5 µg) was applied aseptically on inoculated area and incubated for 18 to 24 hours at 35°C.

E) Interpretation
Zone of inhibition 6 mm to 12 mm from the centre of the disc was considered to be resistant and 16 mm or more as susceptible.

ii) Tube Coagulase test : [5]
Principle: Tube coagulase test determines the free coagulase. Free coagulase is a thrombin - like substance present in culture filtrates. When a suspension of coagulase - producing organism is prepared in plasma in a test tube, a visible clot is formed as the result of coagulase reacting with a serum substance, CRF (coagulase -reacting factor) to form a complex that, in turn, reacts with fibrinogen to produce fibrin clot.

Procedure:
Tube coagulase test was performed by any of the following procedures:

a. 1 in 6 dilution of plasma saline was prepared and 1 ml volume of diluted plasma was placed in small tubes. A colony of Staphylococcus under test / loopful of overnight broth was emulsified in a tube of diluted plasma.

b. A small amount of colony growth of the organism was emulsified in a tube containing 0.5 ml of coagulase plasma. Along with the positive and negative control, a tube of unseeded plasma was included to confirm that it did not clot spontaneously. The tubes were incubated at 37^{0} C for up to 4 hours and examined at 1, 2 and 4 hrs for clot formation by tilting the tube through 90^{0}. If negative, tubes were left at room temperature overnight and re-examined.

Interpretation: Any degree of clotting noted was considered as positive tube coagulase test.

Positive control- Staph aureus
Negative control- Staph epidermidis

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility testing was performed as per the CLSI guidelines (2012) by modified Kirby-Bauer method.

1)Medium:

Mueller-Hinton agar (Hi-media laboratories Pvt, Ltd. Mumbai) was prepared from dehydrated base as per manufacturer's recommendations.

2)Inoculum:

The inoculum was prepared from the primary culture plate by touching with a straight wire the top of the 3-5 colonies of similar appearance of the organism to be tested. This growth was transferred to a tube of sterile saline. The tube was compared with the 0.5 McFarland turbidity standard, and the turbidity of test suspension was adjusted to that of the standard.

3)Procedure:

The plates were inoculated by dipping a sterile swab into the inoculum. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60^0 after each application. Finally, the swab was passed around the edge of the agar surface.

4)Antibiotic disks:

Commercially available antibiotic disks (Hi-media laboratories Pvt. Ltd. Mumbai) with proper diameter and potency were used. All the strains were tested for their sensitivity to antimicrobial drugs using recommended CLSI guidelines (2012) combined with institutional antibiotic policy and hospital formulary practices for the purpose of reporting to the clinician.

- Penicillin 10 IU
- Amoxicillin-clavulanic acid 30 µg
- Cefoxitin 30 µg
- Ceftazidime 30 µg
- Erythromycin 15 µg
- Vancomycin 30 µg
- Linizolid 30 µg
- Pristinomycin 15 µg
- Ofloxacin 5 µg
- Gentamycin 10 µg
- Cotrimoxazole 25 µg
- Nitrofurantoin 300 µg

5)Application of antibiotic disks on the inoculated plate:

The above mentioned antibiotic disks were placed on the agar surface of inoculated plates. A maximum of six disks were placed on 9 cm plate. Each disk was gently pressed down to ensure even contact with the medium which were then incubated at 37°C for 18-24 hours aerobically and observed for zone of inhibition.

6)Reading and Interpretation:

Diameter of the circular zone of inhibition including the antibiotic disk was measured. Interpretation as sensitive, intermediate or resistant was done with reference to CLSI.

METHICILLIN RESISTANCE : In this study methicillin resistance was tested by cefoxitin disk diffusion method as per CLSI guidelines [20]

30 µg cefoxitin disk was placed on the agar surface of inoculated plates, then plate was incubated at 37°C for 18-24 hours aerobically and observed for zone on inhibition.CLSI guidelines (2012).

RESULTS

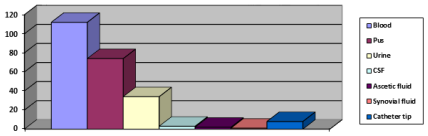
The study was conducted during the period from November 2012 to October 2014. Isolates of Coagulase Negative Staphylococci from the various clinical specimens received in the diagnostic bacteriology laboratory from inpatient and outpatient departments were included in this study.

We processed a total of 12358 clinical samples in our laboratory and were able to isolate 234 Coagulase Negative Staphylococcial strains from these specimens

As shown in table no 6 Maximum 112/234 (47.86%) isolates of CoNS were from blood. This was followed by pus/wound swab 74 (31.62%), urine 34 (14.52%), catheter tip 08 (3.41%), CSF and ascetic fluid 03(1.28%) each, synovial fluid 01 (0.85%).

Table no. 1 Frequency of Clinically significant CoNS from different sample

Sample	Number(234)	Percentage
Blood	112	47.86%
Pus	74	31.62%
Urine	34	14.52%
CSF	3	1.28%
Ascetic fluid	2	0.85%
Synovial fluid	1	0.42%
Catheter tip	8	3.41%



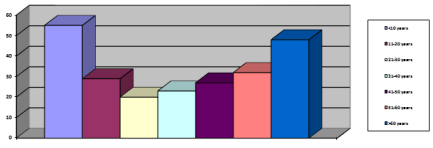
Graph-1 ,showing Frequency of Clinically significant CoNS from different samples

Because slide test is less sensitive and may remain negative in up to 5 - 10% S. aureus, only tube coagulase negative strains were selected for the study.

Table no.2 shows maximum number of samples were from patients in extreme of the age group, i. e. 0-10 years 55 (23.5%) followed by 60 years and above age group 48 (20.51%) isolates.

Table no.2, Age wise distribution of CoNS isolates

Age group	Number(234)	Percentage
<10 years	55	23.50%
11-20 years	29	12.39%
21-30 years	20	8.55%
31-40 years	23	9.82%
41-50 years	27	11.54%
51-60 years	32	13.68%
>60 years	48	20.51%

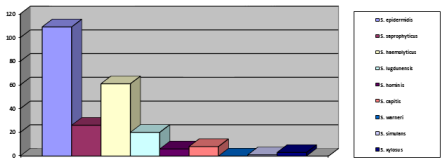


Graph-2, Age wise distribution of CoNS isolates

With the identification scheme employed in the present study, all the 234 strains were speciated on the basis of test results shown by them. Table 3 shows S. epidermidis to be most prevalent species from clinical isolates of CoNS in this study with 109 (47.58%) strains, followed by S. haemolyticus 61 (26.06%), S. saprophyticus (11.11%) and S. lugdunensis 20 (8.54%). Their was single isolate of S. simulans and S. warneri was not isolated from any sample.

Table no. 3, Species distribution of clinically significant isolates of CoNS

Species	Number	Percentage
S. epidermidis	109	46.58%
S. saprophyticus	26	11.11%
S. haemolyticus	61	26.06%
S. lugdunensis	20	8.54%
S. hominis	6	2.56%
S. capitis	8	3.41%
S. warneri	0	0%
S. simulans	1	0.42%
S. xylosus	3	1.28%
TOTAL	234	100%



Graph-3, showing Species distribution of clinically significant isolates of CoNS

Table no. 4 Sample wise species distribution of CoNS isolates

Species	Pus	Blood	Urine	Cather Tip	CSF	Ascitic Fluid	Synovial fluid	TOTAL
S. epidermidis	38	59	4	4	2	1	1	109
S.saprophyticus	4	0	22	0	0	0	0	26
S.haemolyticus	26	27	3	3	1	1	0	61
S.lugdunensis	4	12	4	0	0	0	0	20
S. hominis	0	5	1	0	0	0	0	6
S. capitis	1	6	0	1	0	0	0	8
S. warneri	0	0	0	0	0	0	0	0
S. simulans	0	1	0	0	0	0	0	1
S. xylosus	1	2	0	0	0	0	0	3
TOTAL	74	112	34	8	3	2	1	234

Table no. 5 shows there were total 136 (58.12%) CoNS isolates, that showed resistance to 6 or more than 6 drugs of which 57 (51.29%) were S. epidermidis and 51 (83.61%) were S. haemolyticus. There were total 88 (37.61%) CONS isolates which showed resistance to 7 or more than 7 drugs of which 32 (29.36%) were S. epidermidis and 43 (70.49%) were S. haemolyticus. 42 (17.95%) isolates of CoNS showed resistance to 8 or more than 8 drugs of which 11(10.09%) were S. epidermidis and 26(42.62%) were S. haemolyticus. 20(8.54%) isolates of CoNS showed resistance for 9 or more than 9 of which 7(6.42%) were S. epidermidis and 11 (18.03%) were S. haemolyticus. 7(2.99%) isolates showed resistance to 10 or more than 10 drugs of which 3 were S. epidermidis (2.75%) and 4(6.56%) were S. haemolyticus. There were 3(1.28) isolates of CoNS which showed resistance to 11 drugs all belong to S. haemolyticus.

Table no. 5 MULTI DRUG RESISTANCE IN CONS ISOLATES

Species	Minimum 6 drugs	Minimum 7 drugs	Minimum 8 drugs	Minimum 9 drugs	Minimum 10 drugs	Minimum 11 drugs
S. epidermidis	57	32	11	7	3	0
S. saprophyticus	13	5	1	1	0	0
S. haemolyticus	51	43	26	11	4	3
S. lugdunensis	9	5	3	1	0	0
S. hominis	2	1	0	0	0	0
S.capitis	4	2	1	0	0	0
S. warneri	0	0	0	0	0	0
S. simulans	0	0	0	0	0	0
S. xylosus	1	0	0	0	0	0
Total	136 (58.12%)	88 (37.61%)	42 (17.95%)	20 (8.54%)	7 (2.99%)	3 (1.28%)

In this study, methicillin resistance among CoNS isolates was identified by cefoxitin disk diffusion method. Table no. 6 shows that out of 234 strains of CoNS, 153 strains (65.38%) were detected as Methicillin resistant(MRCoNS) and 81 strains(34.62%) as Methicillin sensitive(MSCoNS) by cefoxitin disk diffusion method.

Table no. 6 Methicillin resistance pattern of CoNS isolates

Isolates	NUMBER	PERCENTAGE
MRCoNS	153	65.38%
MSCoNS	81	34.62%
TOTAL	234	100%

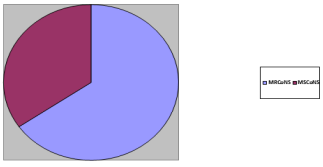


Fig-4 , showing Methicillin resistance pattern of CoNS isolates

Table no. 7, shows that all the MRCoNS were resistant to penicillin 153 (100%) and 152 (99.35%) were found to be resistant to ceftazidime, 151 (98.69%) to amoxicillin-clavulanic acid, 142 (92.81%) to cotrimoxazole, 135 (88.23%) to gentamicin, 127 (79.74%) to ofloxacin, 115 (75.16%) to erythromycin and 73 (47.71%) to rifampacin. 11 (55%) of the MRCoNS isolates from urine were resistant to nitrofurantoin. Least resistance was noted to pristinomycin 20 (13.07%) and single isolate (0.65%) of MRCoNS was found resistant to vancomycin. No isolate were resistant to linezolid.

Table no. 7, Resistance pattern of MRCoNS

ANTIBIOTIC	NUMBER	PERCENTAGE
Penicillin	153	100%
Amoxicillin-clavulanic acid	151	98.69%
Ceftazidime	152	99.35%
Erythromycin	115	75.16%
Vancomycin	1	0.65%
Linizolid	0	0%
Pristinomycin	20	13.07%
Ofloxacin	127	79.74%
Gentamycin	135	88.23%
Cotrimoxazole	142	92.81%
Rifampacin	73	47.71%
Nitrofurantoin *	11	55.00%

(*Nitrofurantoin was tested in only urinary isolates of CoNS.)
Table no. 8 shows that maximum resistance in MSCoNS was seen to penicillin 76 (93.83%) isolates, followed by resitance to amoxicillin-clavulanic acid in 36 (44.44%), ofloxacin 30 (37.03%), cotrimoxazole 29 (35.80%), gentamicin 19

(23.80%). Lees resistance was noted to, erythromycin 14 (17.28%), ceftazidime 12 (14.81%) and rifampacin 9 (11.11%). 2 (14.28%) isolates of the MSCoNS isolates from urine were resistant to nitrofurantoin. No MSCoNS isolate were resistant to linezolid, vancomycin and pristinomycin.

Table no. 8, Resistance pattern of MSCoNS

ANTIBIOTIC	NUMBER	PERCENTAGE
Penicillin	76	93.83%
Amoxicillin-clavulanic acid	36	44.44%
Ceftazidime	12	14.81%
Erythromycin	14	17.28%
Vancomycin	0	0%
Linizolid	0	0%
Pristinomycin	0	0%
Ofloxacin	30	37.03%
Gentamycin	19	23.46%
Cotrimoxazole	29	35.80%
Rifampacin	9	11.11%
Nitrofurantoin*	2	14.28%

(*Nitrofurantoin was tested in only urinary isolates of CoNS.)

DISCUSSION

Coagulase negative staphylococci (CoNS) were generally regarded to be the contaminants, having little clinical significance in the past.[5] Formerly regarded as harmless inhabitants of the skin and mucous linings, CoNS are now recognized as a major cause of nosocomial infections in critically ill patients especially in intensive care units, which leads to morbidity and even mortality.[21]

Ohman et al[22] mentioned the data from various studies dignifying CONS to be the common cause of nosocomial infections and the most Common cause of nosocomial bloodstream infections. In the period 1992 to 1998, surveillance conducted in Medical-surgical units revealed that CoNS most common cause of primary bloodstream infections and the second commonest cause of post surgical infections.[23]

Most developed countries have reported an increase in colonization and infection in hospitalized patients by CoNS, which are resistant to methicillin and other antibiotics. However, scant data is available on CoNS responsible for infections in the developing countries.[14,15]

In our study a total number of 234 clinically significant CoNS strains (1.89%) were isolated from 12,358 processed clinical specimens. Among the 234 CoNS isolates, maximum strains, 112 (47.86%) were isolated from the Blood.

Various other workers, Singhal et al[14] and Alcaraz et al[24] also found Blood to be the commonest specimen in which CoNS were isolated. Singhal et al[14] isolated 54.2 % CoNS from Blood in 2006 and Alcaraz et al[24] 31.1% in 2003. Our findings match with Singhal et al and Alcaraz et al, for the blood to be the most common source of CoNS. But Goyal et al[25] isolated less number i.e. 14.7% of CoNS isolates from blood.

CoNS, the most frequent blood culture isolates, are predominantly blood culture contaminants, but they are also a significant cause of bacteramia.[26]

In the present study, pus was the second commonest sample from which 74 (31.62%) CoNS were isolated. Singhal et al[12] also reported pus to be the second commonest sample (30.1%). Other workers isolated CoNS in various percentages from pus sample. Kleeman et al[27] reported 12.4% in 1993, Goyal et al[25] reported 38.2% in 2006 and ArsIan and Ozkardes[28] reported 17.1% CoNS in 2007 from pus sample.

In present study CoNS isolation from urine was 14.52%. Goyal et al[37] isolated 28.7% CONS from urine in 2006 and Tan et al[171] isolated 14% in 2007.

In our study, CONS isolation from CSF was 1.28%. Goyal et al [25], reported 3.9% and Alcaraz et al[24] reported 0.2% isolation from CSF.

In our study, CoNS isolation from ascitic fluid was 0.85% and synovial fluid was 0.42%. Gaikwad and Deodhar[30] reported 9.62%, Goyal et al[37] 3.8% and Singhal et al[12], 1.2% isolation of CoNS from body fluids.

In our study, CoNS isolation from catheter tip was 3.41%. Goyal et al [25] reported 10.7% CoNS isolation from catheter tip.

INFECTIONS CAUSED BY CLINICALLY SIGNIFICANT CoNS

In the present study, most common infection caused by CoNS was septicemia i.e. 112 (47.86%) cases. Singhal et al[14] reported 45 (54.2%) cases of CoNS septicemia. Data by Emori and Gaynes[31] shows that CoNS accounted for 31% of the nosocomial septicemia. CoNS are reported to be the most common cause of nosocomial blood stream infections.[6,7] Although most CoNS septicemia is due to intravascular catheters, bacteraemia and septicemia can also occur in absence of such devices. It is widely believed that CoNS on the skin adjacent to catheter entry sites or surgical incisions are often the source of catheter-related infections, post surgical infections and infected implanted prostheses.[23]

In the present study,74 (31.62%) cases of abscesses and wound infections were found, which were the second commonest infection caused by the CoNS. Singhal et al[14] also found 25 (30.1%) causes of abscesses and wound infection to be the second commonest infection by CoNS. Goyal et al[25] reported 39 (38.2%) cases of abscesses and wound infections by CoNS. Tan et al[29] mentioned 48 cases (47.05%) of abscesses and wound infections by CoNS. Data by Emori and Gaynes[31] shows that 14% of surgical wound infections are caused by CoNS in United States annually.

The present study revealed 34 (14.52%) patients with urinary tract infections by CoNS. Tan et al[29] reported 10 (9.8%) cases and Singhal et al[14] reported 3 (3.6%) cases of urinary tract infections due to CoNS. Data by Emori and Gaynes[31] determined 4% of urinary tract infections annually in United States. S. saprophyticus, one of the important species of CoNS is repoted to be the true urinary pathogen which is found to be the second most common cause of urinary tract infection after E. coli in females. The reason for the association of S. saprophyticus with urinary tract infections in young women remain unclear, but may relate to carriage of the organism in the rectum or introitus.[32] Rupp and colleagues[33] found rectum (40%) to be the most common site of colonization by S. saprophyticus followed by urethra (30%) and cervix.

In the present study, 2 (0.85%) cases of ascitis were caused due to CoNS. Goyal et al[25] reported 2(1.9%) cases of ascites due to CoNS. Bobadilla et al[34] repoted 2 cases and Chow et al reported 28 cases of ascitis due to CoNS.

In the present study, a 3 (1.28%) case of neonatal meningitis was observed due to CONS. All the three case were in neonates. An Australian study[35] reported 5 (0.4%) cases of CONS meningitis. A British pediatric surveillance unit[36] reported 1.1% incidence of CoNS meningitis. The role of CoNS in meningitis is equally Controversial. When cases of neonatal CoNS meningitis are described in the absence of foreign material, clinical picture consistent with meningitis, and either a positive CSF culture or elevated CSF white cell count (> 100/µl) in association with a positive blood culture holds true infection.

AGE AND SEX WISE DISTRIBUTION OF CoNS INFECTIONS

In the present study, CoNS infections were found in both the sexes and also in different age groups. It was observed that CoNS infections were more common in males i.e. 138 (58.97%) cases. Capell et al[37] reported males to be more common in CONS related catheter sepsis. Huang et al[21] also reported males (54.1%) to be mostly affected in CoNS related septicemia.

In present study, the CoNS infection was most commonly found in extremes of ages. In the 0-10 years age group, CONS infections were seen in 55 (23.50%) cases while in elderly age group of more than 60 years, 48 (20.51%) cases were infected by CoNS. CONS are considered to be important opportunistic pathogens in premature neonates, pediatric group patients and elderly persons with serious underlying diseases[5]. Bodonaik and Moonah[38] reported maximum patients to be adults with mean age of 61 years.

SPECIES DISTRIBUTION OF CoNS

Particular species of CoNS are associated with distinct types of infections and patterns of antimicrobial susceptibility.[39] In addition to the value of having an exact etiological diagnosis, identification of species increases the knowledge of the pathogenicity of the various species of CoNS, provides useful epidemiologic information, and contributes to the predictive value of an isolate being of clinical significance versus being a contaminant.[1,40] Because of increasing clinical significance of CoNS, accurate species identification of CONS is highly desirable to permit a more precise determination of host-pathogen relationship of CoNS.[41] Therefore, species identification of CoNS is increasingly of clinical and epidemiological interest to clinicians.[42]

In the present study, the commonest species isolated in clinically significant CoNS was S. epidermidis (46.58%). S. epidermidis represents about 50-70% isolates amongst CoNS. The reason of their appearance may be their sheer number all over the skin and mucous membrane and are possessed with the virulence which other CoNS may lack.[43] The other species isolated were S. haemolyticus (26.06%) followed by S. saprophyticus(11.11%), S.lugdunesis (8.54%), S. capitis (3.41%), S. hominis (2.56%), S.xylosus (1.28%), S. simulans(0.42%).

Table no. 9 Species distribution of CoNS

SPECIES	Kleeman et al 1993	Jarlov et al 1996	Singhal et al 2006	Present study 2022
S. epidermidis	64.5%	57.1%	33.7%	46.58%
S. saprophyticus	1%	0.8%	-	11.11%
S. haemolyticus	13.4%	12.2%	13.3%	26.06%
S. lugdunensis	2.8%	0.2%	13.3%	8.54%
S. hominis	7.4%	12.2%	2.4%	2.56%
S.capitis	3.6%	0.4%	9.6%	3.41%
S. warneri	4%	2.4%	1.2%	0%
S. simulans	2.4%	-	-	0.42%
S. xylosus	-	-	6%	1.28%

Singhal et al[14] reported S. epidermidis (33.7%) followed by S. haemolyticus (13.3%), S. lugdunensis (13.3%), capitis (9.6%), S. xylosus (6%), S. hominis (2.4%) and S. warneri (1.2%). Kleeman et al[44] reported S. epidermidis (64.5%) to be the commonest species causing infections followed by S. haemolyticus (13.4%), S. hominis (7.4%), S.warneri (4%), S. capitis (3.6%), S. lugdunensis (2.8%), S. simulans (2.4%), S. saprophyticus (1%) and S. cohnii (0.6%). Jarlov et al[45] isolated S. epidermidis (57.1%) to be the most common isolate followed by S. hominis (12.2%), S. haemolyticus (12.2%), S. warneri (2.4%), S. saprophyticus (0.8%), S. cohnii (1.4%), S. capitis (0.4%) and S. lugdunensis (0.2%). Mohan et al[46] showed that S. epidermidis (82.29%) was the most common species isolated from all clinical specimens followed by S. saprophyticus (15.62%) isolated mainly from urine. Only two other species of CoNS were identified S.cohnii(0.5%) and S. haemolyticus (1.6%). The slight variation in the number of species isolated may be due to variation of specimens, schemes applied for the identification and the species predominating in various setup.

In the present study, S. epidermidis (52.67%) was the commonest species to cause septicemia, followed by S. haemolyticus (24.11%), S. lugdunensis (10.71.%) and others. Kim et al[47] also found S. epidermidis (52.94%) to be the commonest species causing septicemia, followed by S. haemolyticus (11.8%), S. hominis (11.8%), S. warneri (11.8%) and others. Akpaka et al[48] reported S. epidermidis (42%) to be the commonest cause of septicemia, followed by S. haemolyticus (17%).

In the present study, abscesses and wound infections were most commonly caused due to S. epidermidis (51.35%) followed by S. haemolyticus (35.14%) ,S. lugdunensis (5.4%) and S. saprophyticus (5.4%). Tan et al[29] found S. lugdunensis (46.8%) to be the commonest isolate from the abscesses and wound infections followed by S. epidermidis (43.8%).

The present study revealed that urinary tract infections were most commonly due to S. saprophyticus (64.70%) followed by S. epidermidis (11.76%), S. lugdunensis (11.76%) and others. S. saprophyticus is determined to be the true urinary tract pathogen which is found to be the second most common cause of urinary tract infection after E. coil in females.[5] The reasons for the association of S. saprophyticus with urinary tract infections in young women remain unclear, but may relate to carriage of the organism in the rectum or introitus.[32] Other CoNS are rare cause of urinary tract infections, and about 70-80% of these infections are caused by S. epidermidis, which are usually catheter associated or associated with urological abnormalities.[5]

MULTIDRUG RESISTANCE IN CONS ISOLATES

Multidrug-resistant CONS could be under reported, remain largely unknown or uncharacterized because many laboratories do not identify CONS to the species level. Moreover, the majority of patients with superficial infections are treated in outpatient departments, which are not guided by microbiological analysis. The community could therefore serve as a reservoir of multi resistant CoNS[49]. Although community- acquired isolates are frequently susceptible to a wide variety of agents, strains isolated from hospitalized patients have been noted to be resistant to an increasing number of antibiotics.[1] Cases of multi-drug-resistant CoNS species in human infection have been reported by various studies.[13,50,51]

In the present study, there were total 136 (58.12%) CoNS isolates, that showed resistance to 6 or more than 6 drugs of which 57 (51.29%) were S. epidermidis and 51 (83.61%) were S. haemolyticus. There were total 88 (37.61%) CONS isolates which showed resistance to 7 or more than 7 drugs of which 32 (29.36%) were S. epidermidis and 43 (70.49%) were S. haemolyticus. 42 (17.95%) isolates of CoNS showed resistance to 8 or more than 8 drugs of which 11(10.09%) were S. epidermidis and 26(42.62%) were S. haemolyticus. 20(8.54%) isolates of CoNS showed resistance for 9 or more than 9 of which 7(6.42%) were S. epidermidis and 11 (18.03%) were S. haemolyticus.. 7(2.99%) isolates showed resistance to 10 or more than 10 drugs of which 3 were S. epidermidis (2.75%) and 4(6.56%) were S. haemolyticus. There were 3(1.28) isolates of CoNS which showed resistance to 11 drugs all belong to S. haemolyticus. Multidrug resistance in CONS is carried on a staphylococcal chromosome cassette (SCC) which almost always includes the mecA gene for resistance to semi synthetic penicillins (SCCmec), as in S. aureus. SCCmec

resides in the chromosome as several cassette variants I-V.[52] Archer[53] found that 67% of clinical isolates obtained from blood, cerebrospinal fluid, or heart valve tissue were resistant to six or more antibiotics. Singhal et al[14] also highlights a high prevalence of multi-drug resistant CoNS with clear dominance of resistance in S. haemolyticus.

Ohman et al[22] found that multi resistance was commonly seen in the CoNS isolates. CoNS isolates approximately 21 % were resistant to six tested antibiotics, 34% to at least five tested antibiotics and 59% were resistant to at least four of the seven tested antibiotics. Shittu et al reported multidrug resistance specifically in isolates of S. haemolyticus strains.

Ohman et al[54] studied the correlation of multi resistant CoNS strains and prolonged stay in the ICUs. The majorities of the isolates (69%) were considered multi resistant, i.e. expressed resistance to at least 4 of the tested antimicrobial agents. About 43% of the CoNS isolates were resistant to 5 or more than 5 antibiotics. The risk for being colonized by a multi resistant isolate was significantly higher for the long stayers group in the hospital. They gave several explanations for the bacterial dissemination among the long stayers. It was shown that nurses in the ICUs harbor the highest frequency of multi resistant CoNS compared to nurses at other wards in the hospital. Strains of multi resistant CoNS are known to survive easily in the surroundings for several days or weeks and become epidemic.[54]

4)COMPARISON OF SUSCEPTIBILITY PATTERN IN METHICILLIN RESISTANT AND METHICILLIN SUSCEPTIBLE CoNS

CoNS tend to be more resistant to antimicrobial agents than S. aureus, especially to methicillin.[55] Currently, more than 70% CoNS isolates worldwide are resistant to methicillin. In addition, those CoNS strains acquired in hospitals have become resistant to various other antimicrobial agents.[12]

In the present study, the total numbers of methicillin sensitive CoNS (MSCoNS) were 81 (34.62%) and methicillin resistant CoNS (MRCoNS) were 153 (65.38%).

Singhal et al[14] also determined 37.3% MSCoNS and 62.7% MRCoNS in total 83 CoNS. Mohan et al[46] found 71.7% MSCoNS and 28.3% MRCoNS isolates. Goyal et al[37] reported 75% MSCoNS and 25% MRCoNS. Azap et al[56] found that out of total 192 CoNS, 98 (51%) were MSCoNS and 94 (48.9%) MRCoNS. Delialioglu et al[57] demonstrated that out of total 291 CoNS, 111(38.1%) were MSCoNS and 180 (61.9%) were MRCoNS. Yilmaz et al[58] found 196 (24.4%) MSCoNS and 608 (75.6%) MRCoNS in total 804 CoNS.

Analysis of U.S key bloodstream bacterial isolates from 1995, 1996, and 1997 showed that among 43,789 CoNS, 42% were MSCoNS and 58% were MRCoNs. Cuevas et al in the study of the trends of resistance in clinical isolates of CoNS in Spain for five years period from 1986 to % in 2002 reported that MRCoNS rose steadily from 32.5% in 1986 to 61.3% in 2002.

Table No.10 : Percentage of Methicillin resistant(MR) and Methicillin sensitive(MS) CoNS

Study	MRCoNS	MSCoNS
Delialioglu et al 1995	61.9%	38.1%
Mohan et al 2002	28.3%	71.7%
Singhal et al 2006	62.7%	37.3%
Yilmaz et al 2007	75.6%	24.4%
Present study 2022	65.38%	34.62%

In the present study, we too compared the antibiotic susceptibility pattern of various antimicrobial agents in MSCoNS and MRCoNS. In the present study, a coexisting resistance to different antibiotics was significantly higher in MRCoNS as compared to MSCoNS. Resistance to various antibiotics was seen more in MRCoNS as compared to MSCoNS. 115 (75.16%) MRCoNS were resistant to erythromycin as compared to 14 (17.28%) MSCoNS.

Gentamicin resistance was seen in 135 (88.23%) MRCoNS as compared to only 19 (23.46%) in MSCoNS.

Resistance to ofloxacin was high in MRCoNS ie 127 (79.74%) as compared to only 30 (37.03%) MS CONS. Resistance to cotrimoxazole, pristinomycin, nitofurantoin, rifampacin and others was more in MRCoNS as compared to MSCoNS.

Singhal et al[14] found that MRCoNS showed higher level of resistance to all antimicrobials as compared to MSCoNS. Resistance to ciprofloxacin was shown by 78.8% MRCoNS and only 22.6% MSCoNS. Rifampicin resistance was seen in 40.4% MRCoNS and 12.9% MSCoNS. Also cotrimoxazole resistance was seen in 94.2% MRCoNS and 45.2% MSCoNS.

A database, SENTRY[12], was expanded to other countries during 1997-1999. Co-resistance patterns were reported for the five regions based on methicillin resistance. For example in the USA, cotrimoxazole resistance was about 17% in MSCoNS compared with nearly 57% in MRCoNS. Similar trends were seen for gentamicin, ciprofloxacin, clindamycin, and erythromycin both in US and global sites. A similar large study was conducted in which CoNS were collected from 20 regional health centers in several countries across Europe, Asia, and Latin America. Cross resistance was most common in MRCoNS. For example, rate of cotrimoxazole, ciprofloxacin and gentamicin resistance were 64%, 50.5% and 72.3% respectively.

Another study[59] tested susceptibility of only skin and soft tissue isolates of CoNS in 283 US hospitals and 301 hospitals in Europe. For MSCoNS, sensitivity for ciprofloxacin (90%), gentamicin (86.1-96.4%), cotrimoxazole (88.1%-92.7%) and erythromycin (75%). For MRCoNS, resistance for ciprofloxacin was 65.0%-66.7% across four European countries (France, Germany, Italy, and Spain) versus a range of 38.1%-47.0% in the US. Gentamicin resistance was higher in Europe (range of 50.2%-61.3%) than in the US (32.4%). No vancomycin resistance was detected.

CONCLUSIONS

From our study it can be concluded that

1. Maximum prevalence of CoNS infection was seen at extremes of age.
2. Most *CoNS* were isolated from, blood, pyogenic and urinary specimens.
3. The isolates comprised of only nine species i.e. S. epidermidis, S. saprophyticus, S. haemolyticus, S. lugdunensis, S. hominis, S. capitis, S. warneri, S. simulans, and S. xylosus
4. The percentage of MRCoNS among all CoNS isolates was 65.38%.
5. MRCoNS strains showed 100% resistance to Penicillin. MRCoNS were Multi-drug resistant (MDR) too.
6. Vancomycin is the mainstay of therapy in MDR MRCoNS infections and should be used judiciously. Looking at the possibility of emergence of resistance to the drug, newer agents like linezolid and pristinamycin might provide a valuable option for the treatment of MRCoNS infections.

The study documents the importance of *CoNS* as important Gram-positive pathogen special in hospital settings, resistant to commonly used antibiotics. There is a need to have the knowledge & monitoring of antibiotic sensitivity pattern and formulation of definite antibiotic policy to improve the empirical approaches to the therapy of serious infections caused by *CoNS*.

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