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Microbiology

THE STUDY OF ANTIMICROBIAL SENSITIVITY PATTERN OF COAGULASE-NEGATIVE STAPHYLOCOCCI (CONS) ISOLATED FROM DIFFERENT CLINICAL SPECIMENS IN TMMCH, MORADABAD, UP.

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ABSTRACT

Background: In now days Coagulase negative staphylococci (CoNS) have been reported as a major public health problem worldwide. Low percentage in the antimicrobial susceptibility against CoNS has become a growing problem in developing countries.
Objective: To determine the incidence and antimicrobial sensitivity pattern of coagulase negative staphylococci.
Material and Methods: All the isolates were identified as per standard protocols and antimicrobial sensitivity pattern was determined by Kirby-Bauer disc diffusion method as par CLSI (Clinical and Laboratory Standards Institute) guidelines.
Results: A total of 53 (13.25%) coagulase-negative Staphylococci (CoNS) isolated among 400 Staphylococcal isolates. CoNS shows maximun sensitivity against Vancomycin (100) and minimum susceptibility against Penicillin-G (0%).
Conclusion: It is concluded that to preserve the value of vancomycin for the treatment of life threatening CoNS infections in future, there is need of regular surveillance of methicillin resistance among CoNS to avoid the use of powerful antibiotics for initial infections.

KEYWORDS : CoNS, Nosocomial infection, Vancomycin

INTRODUCTION

Staphylococci comes under the category of gram positive cocci, occurring singly, in pairs, short chains and in grape-like clusters. They are catalase-positive and also differentiated based on their ability to produce coagulase, causing visible agglutination on slide and tube coagulase test[1].

Coagulase-negative staphylococci (CoNS) previously considered as avirulent commensals have emerged as an important prevalent pathogen, especially as a cause of nosocomial infection[2]. CoNS are the major causes of blood Stream lnfections (BSIs) with increased morbidity and mortality[3].

Coagulase-negative staphylococci (CoNS) are found as normal flora on the skin and mucous membrane of humans[4]. It is commonly reported among very-low-birth-weight neonates, immune-compromised patients and individuals with indwelling intravascular devices or implanted medical devices[5].

Methicillin resistant coagulase negative Staphylococci (MRCoNS) has becoming a source of growing concern. One of the reasons is the ability of CoNS to form biofilms on foreign bodies (such as prosthetic heart valves, prosthetic joints and intravenous catheters) as well as on native structures such as heart valves[6,7].

The spread of multi-drug resistant CoNS strains has been promoted by the use of antibiotics in hospitals which has provided a reservoir of antimicrobial resistant strains. MRCoNS are becoming resistant to most of the antibiotics in clinical use. Multi drug resistance in CoNS is carried on a Staphylococcal chromosome cassette (SCC) which always includes the *mec*A gene for resistance to semi-synthetic penicillins (SCC*mec*)[8,9,10].

Clinical diagnosis of CoNS bacteremia involves a positive blood culture, a medical picture of health deterioration and an acute phase inflammatory marker usually C-reactive protein[11]. The treatment of infections caused by Coagulase-negative staphylococci has become very complicated due to the increasing resistance to various antibiotics.

The present study has been aimed to isolate methicillin resistance among CoNS with antibiotic sensitivity test so as to prevent morbidity and mortality caused by it.

MATERIAL AND METHODS
Study design:
The study was conducted in Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Moradabad from March 2015 to February 2016.

Inclusion criteria:-
The study included those patients from whom Staphylococci have been isolated among different clinical samples submitted to Microbiology Laboratory for culture and sensitivity.

Exclusion criteria:-
The study excluded those patients from whom other than Staphylococci and mixed growth have been isolated among different clinical samples submitted to Microbiology Laboratory for culture and sensitivity.

Isolation and Identification of Clinical Specimens
A total of 400 Staphylococci isolates were obtained from various clinical specimens including pus, blood, urine, high vaginal swab and CSF.

All the clinical specimens were processed according to Standard protocols and the antimicrobial sensitivity were determined according to CLSI (Clinical Laboratory Standard Institute) guidelines.

BacT/Alert culture bottles were used for the collection of blood and body fluids that are loaded in BacT/Alert 3D system according to the manufacturer instructions.

Different collected specimens and positively detected samples from BacT/Alert 3D system were inoculated on their respective culture plates and incubated aerobically at 37°C for 24 hrs. Further identification was carried out by colony

characteristics, Gram staining, catalase test, slide and tube coagulase test, growth on mannitol salt agar.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility pattern of all the confirmed CoNS were determined by Kirby Bauer disc diffusion method against the following antibiotics as per CLSI guidelines: Penicillin (10µg), Erythromycin (15µg), Clindamycin (2µg), Co-trimoxazole (25µg), Gentamycin (10µg), Vancomycin (30µg), Linezolid (15µg), Ciprofloxacin (5µg), Cefalexin (30µg), Ampicillin (10µg), Amoxy/Clavulanic acid (20/10µg), Teicoplanin (30µg), Amikacin (30µg), Tobramycin (10µg), Amp/Sulbactam (10/10µg).

Muller-Hinton agar used to perform all antimicrobial susceptibility tests and the interpretation criteria were taken according to NCCLS (National Committee for Clinical Laboratory Standard).

Detection of Methicillin-resistance among CoNS
Cefoxitin (30µg) disc diffusion test

The isolated samples were subjected to cefoxitin disc diffusion test by using 30µg discs. A suspension, equivalent to 0.5 McFarland standard was prepared from each strain. Then a swab was taken and dipped into the suspension and lawn culture was done on MHA plate after that plate was incubated at 37°C for 18-24 hours and zone of inhibition was measured.

An inhibition zone diameter of ≤ 21mm was considered as Cefoxitin resistant reported as Methicillin-resistant and ≥ 22mm was reported as Cefoxitin sensitive indicating Methicillin-sensitive.

RESULTS

In our study, 400 Staphylococcal isolates were isolated from various clinical specimens among IPD and OPD patients in Teerthanker Mahaveer Medical College and Research Centre, Moradabad, Uttar Pradesh, India.

Out of 400 Staphylococcal isolates tested, 347 isolates (86.75%) were coagulase positive *staphylococcus aureus* and 53 isolates (13.25%) were coagulase negative staphylococci, tested by both slide and tube coagulase test (Table-1).

Table 1: Distribution of Staphylococcal isolates among Coagulase- positive S. aureus and Coagulase-negative Staphylococci.

Total Staphylococcal isolates	400	100%
Staphylococcus aureus	347	86.75%
CoNS	53	13.25%

Among 53 coagulase negative *staphylococci*, methicillin resistant strains were 28 (52.83%) and methicillin sensitive strains were 25 (47.17%) (Table-2).

Table 2: Distribution of Coagulase-negative staphylococci among MRCoNS and MSCoNS.

CoNS	53	100%
MRCoNS	28	52.83%
MSCoNS	25	47.17%

Among 28 MRCoNS obtained from different clinical samples, highest percentage was obtained from blood 46.42% (13/28), followed by pus 25% (7/28), urine 25% (7/28), and HVS 3.57% (1/28). Out of 25 MSCoNS maximum number were obtained from both pus 32% (8/25) and blood 32% (8/25) followed by urine 24% (6/25) (Table-3) or (Figure-1).

Table 3: Isolation of Coagulase-negative staphylococci from various clinical samples.

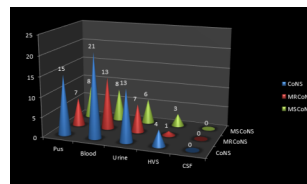| Clinical Specimens	Number of CoNS	MRCoNS (n,%)	MSCoNS (n,%)
Pus	15	7 (25)	8 (32)
Blood	21	13 (46.42)	8 (32)
Urine	13	7 (25)	6 (24)
HVS	4	1 (3.57)	3 (12)
CSF	0	0	0
Total	53	28 (52.83%)	25 (47.17%)

Figure 1: Isolation of Coagulase-negative staphylococcal isolates among various clinical specimens.

Out of 28 MRCoNS isolates, 9 (32.14%) were from male patients and 19 (67.85%) isolates belong to female patients. In both sexes maximum isolates of MRCoNS was belong to the age group of 0-10 years and minimum from the age group of 71-80 years. Out of 25 MSCoNS isolates, 13 (52%) were from male patients and 12 (48%) isolates belong to female patients. Among 13 MSCoNS isolated from male patients, maximum 4 (30.70%) belonged to (11-20) age group, and minimum 1 (7.60%) from (41-50), (51-60), (61-70) age group. Among 12 MSCoNS isolated from female patients, maximum 3 (25%) belonged to (21-30) age group and minimum 1 (8.33%) from (31-40), (51-60), (71-80) age group (Table-4).

Table 4: Age and sex separation of MRCoNS and MSCoNS isolates.

Age group	MRCoNS			MSCoNS		
	Male	Female	Total	Male	Female	Total
0-10	3	6	9	2	2	4
11-20	1	2	3	4	2	6
21-30	2	3	5	2	3	5
31-40	0	2	2	2	1	3
41-50	1	2	3	1	2	3
51-60	1	2	3	1	1	2
61-70	0	1	1	1	0	1
71-80	1	1	2	0	1	1
Total	9	19	28	13	12	25

Antimicrobial sensitivity pattern of CoNS shows highly sensitivity to Vancomycin, Linezolid and Teicoplanin and minimum sensitivity to Penicillin-G and Co-trimoxazole (Table-5

Table 5: Antimicrobial sensitivity pattern of Coagulase negative staphylococci.

Sr. No.	Antibiotic	MRCoNS (n=28)	MRCoNS (%)	MSCoNS (n=25)	MSCoNS (%)
1	Penicillin-G	0	0	6	24
2	Co-trimoxazole	0	0	8	32
3	Amoxycillin	3	10.71	12	48
4	Ampicillin	3	10.71.	13	52
5	Amoxy/Clavulanic acid	5	17.85	15	60
6	Ciprofloxacin	7	25	16	64
7	Erythromycin	4	14.28	11	44
8	Gentamycin	12	42.86	20	80
9	Amp/Sulbactam	4	14.28	19	76
10	Clindamycin	8	28.57	16	64
11	Tobramycin	5	17.85	14	56
12	Amikacin	13	46.43	22	88
13	Teicoplanin	26	92.86	25	100

| 14 | Linezolid | 26 | 92.86 | 25 | 100 |
| 15 | Vancomycin | 28 | 100 | 25 | 100 |

DISCUSSION

CoNS is a group of an opportunistic pathogen that causes a wide range of diseases in hospitalized patients[12] with increased morbidity and mortality[13].

In the present study out of 400 Staphylococcal isolates, 347 isolates (86.75%) were coagulase positive *staphylococcus aureus* and 53 isolates (13.25%) were coagulase negative staphylococci which is correlated with the study done by Mir BA et al[14] but not correlate with the studies done by Praveen I et al[15] and Kumar S et al[16].

The present study reported that, of the 53 CoNS, MRCoNS were 28 (52.83%) which is almost similar to the study done by Begum ES et al[17] which reported a resistance of 48% but not correlated with the study done by Kumar S et al[16] reported a resistance of 23.2% which is low as compare to our study and Mehdinezad M et al[18] which reported a resistance 62.8% which higher as compare to our study.

This variation in prevalence may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

In the present study occurrence of CoNS isolates, noted variable among different clinical specimens. Out of 53 CoNS, the highest percentage of CoNS obtained from blood which was 39.62% (21/53) followed by pus, 28.30% (15/53) which is accordance to the findings reported by Sharma V et al [19] according to which maximum number of CoNS isolated from blood 46.33% (139/300) followed by pus, 24.33% (73/300) but does not correlates with the study done by Kumar S et al[16] according to which maximum number of CoNS isolated from pus 80.67% (167/207) followed by HVS 8.69%(18/207).

In the present study CoNS represent maximum sensitivity to vancomycin and linezolid while minimum sensitivity to penicillin which is accordance with the study done by Praveen I et al[15] and Sharma V et al [19].

This variation in the occurrence of methicillin resistance among coagulase negative staphylococci is due to the various factors as the availability of healthcare services in a hospital, and monitoring of infection management guidelines, implementation, antimicrobial therapy that play an important role among hospitals.

Most of MRCoNS show multidrug resistance that makes difficult to treat the infection. So it is necessary to evaluate the drug resistant pattern against MRCoNS for controlling the nosocomial infections in an effective manner.

CONCLUSION:

The present study concluded that CoNS shows higher antimicrobial resistance against commonly used antibiotics that is worrisome in the present therapeutic scenario. Female child's in the age group of 0 to 10 years were more susceptible to infections caused by CoNS.

In present study, most of the MRCoNS shows multidrug resistant pattern. Although MRCoNS and MSCoNS shows no resistance to vancomycin. The continuous observation of MRCoNS and MSCoNS will also be helpful to identify the varying trends of antimicrobial susceptibility pattern in improving hospital antimicrobial strategy for selecting a suitable antibiotic to avoid the misuse of powerful antibiotic like vancomycin.

To reserve vancomycin for life threatening infections, we should avoid the use of vancomycin as first line treatment. Linezolid and Teicoplanin can be used as alternative drug.

As of multidrug-resistant nature of MRCoNS, the infection control committee of the hospital should follow continuous surveillance of hospital-associated infection.

The findings from this study have reached to the following conclusions:

1. Use of personal protective equipment specially masks by all Health care workers when they are in direct contact with patients.
2. It is important to eradicate MRCoNS colonization in both patients and Health care workers to prevent its spread to the community.
3. There is a need for a widespread screening program for MRCoNS among patients admitted in hospital, to know the exact occurrence in the hospital and also developing a program for isolation and treating MRCoNS for all patients & Health care workers.
4. Rational use of antibiotic based on anti microbial sensitivity report to prevent the development of bacterial resistance.

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