*ejpmr, 2020,7(7), 698-703*

SJIF Impact Factor 6.222

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF COAGULASE-NEGATIVE STAPHYLOCOCCAL ISOLATES FROM A TERTIARY CARE CENTER

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**Article Received on 12/05/2020** **Article Revised on 02/06/2020** **Article Accepted on 23/06/2020**

## ABSTRACT

**Background:** Over past decade Coagulase-negative staphylococci (CoNS) have transformed from innocuous microbe to offensive pathogen causing hospital acquired infection. This study was carried out to ascertain antimicrobial susceptibility profile of CoNS isolated from clinical specimens of patients attending a tertiary care teaching hospital. **Method:** Consecutive clinical samples were processed for conventional culture from Jan 2019 to Dec 2019. Identification of CoNS up to species level and antimicrobial susceptibility testing was performed by VITEK 2 compact system. The gene implicated in resistance to cefoxitin (*mec A*) was identified by conventional PCR. **Results:** A total of 300 CoNS were isolated during study period of which *S. epidermidis* was predominant (43.3%). Methicillin resistance was seen in 86% of the isolates. A total of 64.7% of MR-CoNS(methicillin resistant coagulase negative staphylococci) showed presence of *mec A* gene. **Conclusion:** The study indicate emergence of multi drug resistance in CoNS isolates. Multiple genotypic resistance targets apart from *mec A* gene needs to be analysed to detect resistance in CoNS.

**KEYWORDS:** Methicillin resistant coagulase negative staphylococci, *mecA* gene.

## INTRODUCTION

Coagulase negative staphylococci (CoNS), belong to the family Staphylococcaceae and includes 30 species, most important ones being *S.epidermidis, S. saprophyticus, S.haemolyticus, S.capitis*. Originally part of the normal flora, CoNS have assumed great pathogenic potential and is recognized as agents of hospital and community acquired infections. Previously dismissed as contaminants, these pathogens have now established their role in infections of blood stream, urinary tract, surgical sites, prosthetic devices and shunts.[1] Ability to produce biofilm over indwelling devices and increase in resistance to routinely used antibiotics makes CoNS an important pathogen causing hospital acquired infections. Treatment of such infections was dependent on penicillin and later, on synthetic penicillinase resistant penicillin. Emergence of methicillin resistant coagulase negative staphylococci (MR CoNS) has rendered penicillin and synthetic penicillinase resistant penicillin group of antibiotics ineffective.[2] MR CoNS have acquired and integrated into their genome the Staphylococcal Cassette Chromosome gene (*SCC* gene) which carries the methicillin resistance gene (*mecA*) and other antibiotic resistance determinants. As a result of this, binding site for the drug gets altered. This renders the drug unable to bind and inhibit the cell wall synthesis of the bacteria. Certain CoNS species have acquired resistance not only to methicillin but also to entire group of Beta Lactam antibiotics by means of production of Beta Lactamases enzyme mediated by *blaZ* gene. This enzyme cleaves the Beta Lactam ring and inactivates the molecule. This not only limits the treatment options but also enables transfer of these resistance elements to other staphylococci.[3]

Antibiotic resistant CoNS has emerged as a major cause of morbidity and mortality in the hospital settings during the last decade.[4] Prior study from community based setting from southern India have indicated 60-80% of the isolates to be MR CoNS, of which 85% were found to be MDR.[5] Also, due to cumbersome identification of the CoNS species, most of the time the associated infections go missed and are underestimated. It is imperative that the molecular characterization of resistant species of CoNS be elucidated to understand the mobilization and evolution of these genetic elements in our health care settings. Therefore our primary objective of the study

was to characterize antibiotic resistance in CoNS isolated from clinical specimens submitted to microbiology lab of tertiary care center. In addition we also explored presence of *mecA* gene among CoNS isolates.

## MATERIALS AND METHODS

This cross-sectional study was carried out in the department of microbiology of a tertiary care hospital in western Maharashtra over a period of 12 months. A total of 300 non repeat, consecutive isolates of CoNS were collected from various specimens.

Gram stain and culture on Blood agar, MacConkey agar, CLED agar and Chocolate agar was performed.

The VITEK 2 compact system, a fully automated system that performs bacterial identification by biochemical analysis using calorimetry was used for species identification.

CoNS isolates were subjected to Cefoxitin disc diffusion test using 30µg cefoxitin disc(Hi Media) for the detection of methicillin resistance by means of Kirby Bauer disc diffusion method using 0.5 Mc Farland suspension of the colony. The plates were incubated at 37ºC overnight and then were examined to confirm that a confluent lawn of growth obtained. Diameter of zone of inhibition was measured in mm. interpretation was done as per CLSI 2018.[6]

Molecular detection of *mecA* gene that encodes protein PBP2A (penicillin-binding protein 2A) was performed by polymerase chain reaction as described by Duran et al. The primers and cycling conditions are as under. The final product of 314bp was detected by agarose gel electrophoresis.[7]

| Gene | Oligonucleotide sequence(5'-3') | Size of amplified product (bp) |
|---|---|---|
| *mecA* – Fwd | CCTAGTAAGCTCCGGAA | 314 |
| *mecA* – Rev | CTAGTCCATTCGGTCCA | |

Positive control: ATCC MRSA 43300
Negative control: ATCC MSSA 29213

Phenotypic and Genotypic results were then assessed using statistical software SPSS Statistics 20.0).

## RESULTS

During the study period of 12 months a total of 300 samples were collected, of which 31.3% were from females. The age of the patient ranged from 14 days to 90 years. The details are shown in figure 1.

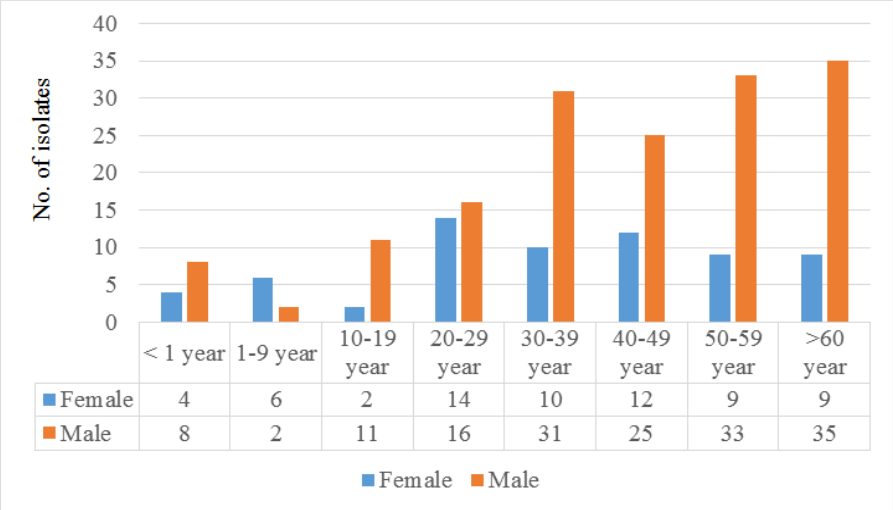


**Fig. 1: Age and Gender wise distribution of MRCoNS.**

**Table 1: Distribution of various samples.**

| Nature of sample | No. of sample | Percentage of isolates |
|---|---|---|
| Blood | 159 | 53% |
| Pus and Wound swabs | 90 | 30% |
| Urine | 22 | 7.3% |
| Semen | 8 | 2.6% |
| High vaginal swab | 4 | 1.3% |
| Tracheal Aspirate | 3 | 1% |
| Conjunctival swab | 11 | 3.6% |
| Tissue | 3 | 1% |
| Total | 300 | 100% |

Out of the total 300 samples, majority were *S. epidermidis* i.e. 43.3%, followed by *S.hemolyticus* (31.6%) and *S. hominis* (11.3%)

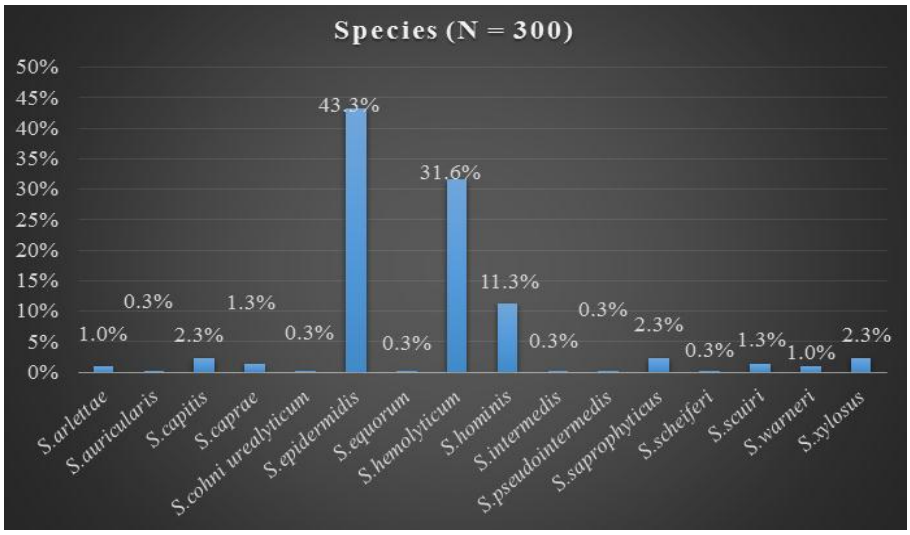


**Fig. 2: Percentage distribution of the CoNS species.**

Out of these 300 isolates, 86%were methicillin resistant, which were detected using cefoxitin disc(30µg).

Antibiotic sensitivity pattern for these CoNS isolates is shown in figure 3. All isolates were susceptible to glycopeptides.

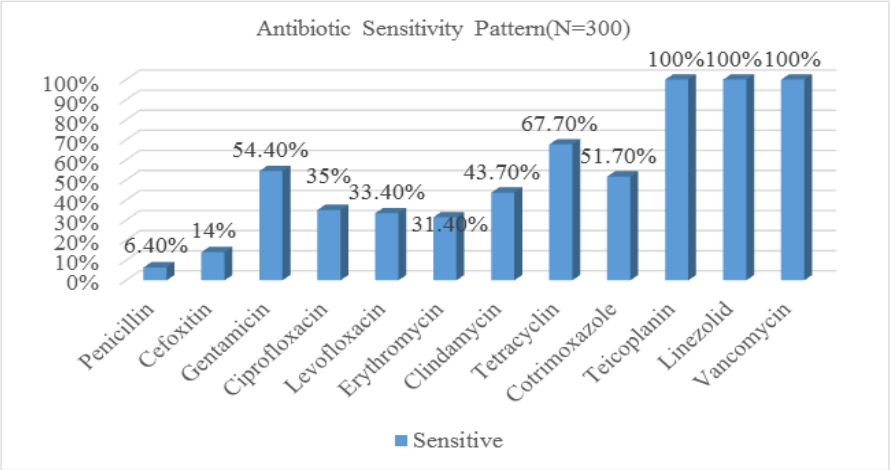


**Fig. 3: Percentage of sensitivity to recommended antibiotics.**

PCR was carried out for all the CoNS isolates using primers targeting *mecA* gene with positive and negative controls.

It was found that 179 isolates showed the presence of *mecA* gene, of which 167 isolates also showed the resistance to methicillin phenotypically.

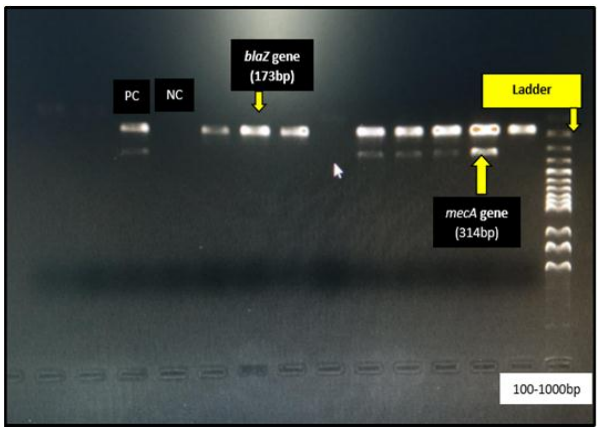


**Fig. 4: Gel Documentation showing ladder, *mecA* and *blaZ* genes (5, 6 being positive control, PC and negative control, NC).**

The discordance in the phenotypic and the genotypic findings were further analysed statistically, and was expressed in terms of the "p value" as follows:

**Table 2: *p- value* for various species.**

| | Isolate | Cx | *mecA* | | *p*-value |
|---|---|---|---|---|---|
| | | | Neg | Pos | |
| 1 | *S.epidermidis* | Sen | 0 | 4 | 0.137 |
| | | Res | 55 | 71 | |
| 2 | *S.hemolyticus* | Sen | 6 | 0 | 0.001 |
| | | Res | 23 | 66 | |
| 3 | *S.hominis* | Sen | 10 | 4 | 0.001 |
| | | Res | 0 | 20 | |
| 4 | *S.saprophyticus* | Sen | 0 | 0 | NA |
| | | Res | 3 | 4 | |
| 5 | *S.caprae* | Sen | 4 | 0 | NA |
| | | Res | 0 | 0 | |
| 6 | *S.capitis* | Sen | 3 | 0 | NA |
| | | Res | 4 | 0 | |
| 7 | *S.xylosus* | Sen | 0 | 0 | NA |
| | | Res | 3 | 4 | |
| 8 | *S.scuiri* | Sen | 0 | 4 | NA |
| | | Res | 0 | 0 | |
| 9 | *S.arlettae* | Sen | 3 | 0 | NA |
| | | Res | 0 | 0 | |
| 10 | *S.warneri* | Sen | 2 | 0 | NA |
| | | Res | 0 | 0 | |
| 11 | *S.pseudointermidis* | Sen | 0 | 0 | NA |
| | | Res | 1 | 0 | |
| 12 | *S.scleiferi* | Sen | 0 | 0 | NA |
| | | Res | 1 | 0 | |
| 13 | *S.intermidis* | Sen | 1 | 0 | NA |
| | | Res | 0 | 0 | |
| 14 | *S.equorum* | Sen | 0 | 0 | NA |
| | | Res | 0 | 1 | |
| 15 | *S.cohini urealyticum* | Sen | 0 | 0 | NA |
| | | Res | 0 | 1 | |
| 16 | *S.auricularis* | Sen | 0 | 0 | NA |
| | | Res | 1 | 0 | |

NA= not applicable, p-value <0.05 (Significant) Chi-Square and Fisher's exact test used

## DISCUSSION

Coagulase-negative staphylococci (CoNS) are normal inhabitants of the human skin and mucous membranes, but can also cause a variety of infections i.e. can act as opportunistic pathogens particularly in immunocompromised patients and those with implanted medical device.[8] Until recently, CoNS were considered as the non pathogenic members of the genus *Staphylococcus* and thus were not of much interest to the research community. However, due to their implication in infections in both humans and animals, research interest in CoNS has increased over the past decade. In addition, CoNS have, over the last decade, developed resistance to multiple antibiotics, making their study worthwhile, especially, since they are known commensals and could be prevalent in most environments.[9]

The present study was carried out at the department of microbiology of a medical college and its affiliated tertiary care hospital with an aim to study the characterization and antimicrobial susceptibility pattern of coagulase negative staphylococcal isolates among the samples received in the microbiology laboratory.

As a result of the patient- and procedure-related changes in the current medical practice scenario, CoNS now represent one of the major nosocomial pathogens, with *S. epidermidis* and *S. haemolyticus* being the most significant species. Therapeutically also, CoNS have come up as one of the most challenging group of microorganisms due to the large proportion of methicillin-resistant strains and increasing numbers of isolates with less susceptibility to other antibiotics recommended in CLSI and EUCAST guidelines.[10] The increasing rates of antibiotic resistance and multidrug resistance among pathogenic, non-pathogenic, commensals, and opportunistic bacteria call for increased report on the distribution (prevalence) of these organisms and their antibiotic profiles.[11]

CoNS as a group, being a part of the natural flora on the human body, makes it greatly challenging and difficult to distinguish the etiologic agent(s) from contaminating normal flora. This has come up as a serious challenge to the clinical laboratory. This can be inferred by proper specimen collection and recording adequate clinical details of each case including hospital stay, indwelling devices, co-morbidities. Involvement of clinical microbiologist as infectious disease clinician during history taking and sample collection would be useful in differentiating CoNS as commensal or causative agent of infection. Isolation and identification of the colonies obtained on culture should proceed to the species and strain levels. A confident and fruitful opinion can be reported for a specific etiologic agent if the same strain is repeatedly isolated from a series of specimens than if different strains of one or more CoNS species are isolated.

In this study, 300 CoNS isolates were identified up to species level with *S.epidermidis* being the commonest. Most of them were isolated from blood samples, which were 53% of the total samples (10% of which being clinically significant). The rest were obtained from samples like pus(30%), urine(7.3%), high vaginal swab(1.3%), tissue(1%), semen(2.6%), conjunctival swab(3.6%), tracheal aspirate(1%) The results were seen to be consistent with the previous similar studies as done by Tayyer et al.[12] Majority of the CoNS species were obtained from samples received from various wards(52.3%), followed by that from OPD(26%) and least from ICU(21.3%). This could potentially explain the wrong blood collection practices being more common in ward and OPD settings as compared to the ICU settings in cases where isolates are clinically not relevant. Increase use of central venous catheters (CVC) and other indwelling vascular devices in hospitals,

especially in ICU patients, create a potential for increased rates of infection. The possibility of colonization of these catheters and/or medical devices with CoNS as a result of skin contamination of patients and staff may explain the isolation of CoNS from ICUs. Due to these circumstances, CoNS species must be considered as an important causative agent of nosocomial bacteraemia and catheter related infection.[13]

Different antibiotics were used to evaluate the susceptibility pattern of isolated CoNS according to recommendations of CLSI 2018 guidelines for Staphyloccocal isolates.[6]

CoNS isolates showed high sensitivity to vancomycin, linezolid and teicoplanin. These antibiotics may play an important role in the treatment and prevention of nosocomial infections of CoNS. However, CoNS species showed remarkable resistance to penicillin(93.6%), cefoxitin(86%) and other types of antibiotics mentioned in results. While bacteria continue to acquire resistance to antibiotics, selection of the appropriate agents is of paramount importance. The results were consistent with the observations by other institutions and studies, as done by El Kholly et al.[14]

Also, the results of antibiotic susceptibility by disk diffusion method were compared with gene analysis results in staphylococcal isolates. The phenotypic expression of antimicrobial resistance has been reported to be influenced by various factors.[15]

Methicillin resistance was observed in 258 (86%) percent isolates when tested by cefoxitin disk diffusion method, whereas 179 (59.6%) per cent isolates had *mecA* gene, of which only 167 expressed it phenotypically. Phenotypically 12 methicillin susceptible isolates also carried the *mecA* gene. Comparison of conventional method and multiplex.

PCR assay did not show a good agreement. This discordance is explained based on the fact that there are other genes apart from *mecA*, namely *mec*C, B etc causing the isolate to express the phenotypic resistance against methicillin. Also, those having the *mecR* locus tend to be slow in the induced production of *mecA*. The *mecI mecR1* regulator genes were found in the *mecR* locus. Inactivation of *mecI* gene derepressed production of *mecA*, and induced expression of methicillin resistance. On the other hand, depressed *mecA* expression due to the intact *mecI* was considered as a precursor of MRCoNS, designated pre-MRCoNS (or phenotypically methicillin sensitive strains).[16] It is this group of strains, which if isolated from clinically significant samples are likely to show treatment failure as these would be treated with Beta Lactam antibiotics in view of there methicillin sensitivity status when tested phenotypically. Results about resistance to methicillin found in this study were higher than those in similar studies from Europe (25%) but were similar to those in Japan.[17]

Further, this discordance between the phenotypic and the genotypic findings were analyzed statistically by means of Chi-square test to calculate the p-value using the SPSS software version 20. The p-value was found to be statistically significant for *S.epidermidis* and *S.hemolyticus* and not for other species, which could be possibly because of sample bias as these two species were the majority in number in comparison to other species.

## CONCLUSION

To conclude our study indicate emergence of multi drug resistance in CoNS isolates, with resistance to beta lactam antibiotics. Molecular identification of *mecA* alone does not correlated well with methicillin resistance. Multiple genotypic resistance targets apart from *mec A* gene needs to be analyzed to confirm methicillin resistance in CoNS.

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