



## Original Article

# Characterization and Clinical Significance of Coagulase –Negative Staphylococci

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## Abstract

**Background:** *Coagulase-negative staphylococci are normal commensal of human skin. They are considered as non-pathogenic and rarely reported as to cause severe infections. But their important role as pathogen and the increased incidence of infections caused by them have been recognized in recent years. The present study is undertaken to characterize and determine the antibiogram of coagulase-negative staphylococci isolated from clinical specimen submitted to Department of Microbiology Government Medical College Kottayam.*

**Introduction:** *Coagulase- negative Staphylococci are indigenous microbial flora of human skin and mucous membrane. They are usually consider as contaminants when isolated from a clinical specimens. These organisms are becoming increasingly recognized as pathogenic agents in nosocomial infections. Virulence factor is not clearly established but role of factors such as bacterial polysaccharide components are involved in attachments and persistence of bacteria in foreign materials.*

**Aim of the study:** *The study was undertaken to identify the Coagulase negative staphylococci, their speciation and to determine the antibiotic resistance pattern.*

**Materials and Methods:** *A total of 63 isolates were collected from different samples and subjected to biochemical characterization and antimicrobial screening using conventional methods. The isolates were identified to species level by VITEK 2- compact.*

**Results:** *The common isolation was Staphylococcus epidermidis. Antibiotic susceptibility testing showed maximum resistance to Penicillin and 100% sensitivity to Vancomycin and Linezolid. The increased isolation of potential CoNS to cause infection and drug resistance among them leads to adopt laboratory procedures for identification and antibiotic pattern of CoNS, which will help the clinicians in treating the infection.*

**Keywords:** *Coagulase negative staphylococci, S.epidermidis, Erythromycin.*

## Introduction

Coagulase-negative staphylococci are part of the normal flora of the skin and considered as nonpathogenic but their role as pathogen and their

increasing incidence recognized in recent years and they exhibit a wide variability in their patterns of resistance.<sup>(1,2)</sup> Most of these infections are consequence of hospitalization.<sup>(3)</sup> Despite, there is

increased significance of infections attributed to coagulase-negative staphylococci, the organisms are not generally identified in medical microbiology laboratories. CoNS are the main colonizers in the NICU environment and are often drug resistant. The accurate identification of coagulase-negative staphylococci is necessary for the prediction of potential pathogenicity and /or antibiotic sensitivity. Identification of coagulase-negative staphylococci repeatedly isolated from patients with active infection is essential in the determination of strain similarity. Without the identification the clinical significance and their role as etiological agents of infectious diseases will not be developed and progressed.

### Objective of the Study

- To Identify species of Coagulase-negative staphylococci isolated from body fluids
- To determine the susceptibility of Coagulase-negative staphylococci to antimicrobial agents
- To assess the significance of the clinical isolates of Coagulase-negative staphylococci

### Materials and Methods

The study was carried out in the Department of Microbiology, Government Medical College Kottayam for a period of 6 months from December 2012 to June 2013. A total of 63 clinically significant CoNS isolates were collected from clinical samples and processed using conventional microbiological method and by VITEK 2 system. Strains were isolated from blood, urine and pus. The isolates were considered clinically significant when isolated in pure culture more than once. The isolates were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase test and anaerobic acid formation from mannitol in standard of medium.<sup>(4,5)</sup> Coagulase test were done in slide and tube method as per standard text books. Tube coagulase was read at 1,2,4 hours for clot formation, then tubes were left at room temperature overnight and re-examined.<sup>(4)</sup> Simple and easy test to perform were selected from the scheme of Kloos and Sheleifer to identify CoNS species.<sup>(6,7,8,9)</sup> Confirmation of the isolated organisms was made with Rapid Identification of Staphylococci using VITEK 2 Card.

**Table: 1** Identification of CoNS by simple scheme(6)

Species	Clumping factor	Tube coagulase test	Ornithine decarboxylase test	Urease activity	Manose fermentation	Novobiocin sensitivity (5 g)
<i>S.epidermidis</i>	-	-	+	+	-	S
<i>S.saprophyticus</i>	-	-	-	+	-	R
<i>S.haemolyticus</i>	-	-	-	-	-	S
<i>S.lugdumensis</i>	-	+	+	+	+	S
<i>S.cohnii</i>	-	-	-	+	+	R
<i>S.warneri</i>	-	-	-	+	-	S

The antimicrobial susceptibility profile of all isolates were done by Disc diffusion method using Muller-Hinton agar. Sensitivity to novobiocin was detected by the appearance of a zone of inhibition <16 mm in diameter on a MHA plate as part of antibiogram susceptibility test.<sup>(4)</sup>

### Results

In the last few years there have been a number of reports on the Coagulase –negative as significant

agents of infection.<sup>(10,11)</sup> During the study period 63 isolates of CoNS from clinical samples were collected. Among 63 isolates, 54 were isolated from blood, 5 isolation were from pus and only two from urine. All isolates were negative for bound and free coagulase.<sup>(5,12)</sup> In this study the most frequent isolate was *S. epidermidis* 22 (30.2 %) followed by *S.haemolyticus* 13(21%), *S.hominis* 11(17%), *S.sciuri* 6(10%), *S. cohnii* 4(6.3%), *S. arlettae* 2(3%), *S.warneri* 2(3%), *S.*

xylosus 1 (1.5%). Two *Staphylococcus saprophyticus* was isolated from urine sample. These data are in general agreement with other published reports. More than 41 species are recognized but only few are commonly incriminated in human infections. In most of the studies the majority of isolates were identified as *Staphylococcus epidermis* and *Staphylococcus*

*haemolyticus*. The percentage of *S. epidermidis* recovered was lower than in some reports and in this study there is concomitant rise in the percentage of *S. haemolyticus*. This study correlates with study of Shubha Singh, Gopa Banerjee et al where the common isolation was *S.epidermidis*.<sup>(13)</sup>

**Table: 2** showing resistance pattern of CoNS to different antibiotics

Species	P	Amp	G	E	Ceph	Clox	Ox	Ak	Van	Lin
<i>S.epidermidis</i> (22)	19 (86%)	18 (82%)	12 (55%)	18 (82%)	16 (73%)	14 (64%)	18 (82%)	8 (36%)	0	0
<i>S.haemolyticus</i> (13)	11 (85%)	10 (78%)	11 (85%)	10 (78%)	11 (85%)	12 (92%)	12 (92%)	1 (8%)	0	0
<i>S.homini</i> (11)	8 (73%)	8 (73%)	9 (82%)	8 (73%)	8 (73%)	7 (64%)	7 (64%)	1 (9%)	0	0
<i>S.sciuri</i> (6)	5 (83%)	5 (83%)	4 (67%)	3 (50%)	3 (50%)	5 (83%)	3 (50%)	0	0	0
<i>S..cohnii</i> (4)	3 (75%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	0	0	0	0	0
<i>S..arlettae</i> (2)	0	0	0	0	0	0	0	0	0	0
<i>S.warneri</i> (2)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0	0	0
<i>S.saprophyticus</i> (2)	0	0	0	0	0	0	0	0	0	0
<i>S.xylosus</i> (1)	0	0	0	0	0	0	0	0	0	0
Total isolation(63)	49 (78 %)	46 (73%)	38 (60%)	43 (68%)	42 (67%)	35 (56%)	40 (63%)	5 (8%)	0	0

P= penicillin A=ampicillin OX= oxacillin E=erythromycin  
Ceph=cephalexin Ak= Amikacin Lin=Linezolid Van= vancomycin

Coagulase-negative staphylococci are usually multi-drug resistant and glycopeptide antibiotics that have been considered as the drugs of choice, which vary according to species.<sup>(14)</sup> In this study antibiotic susceptibility testing showed maximum resistance to Penicillin (78%) and Ampicillin (73%). Methicillin resistance strains were (67%) and among this *S. epidermidis* was the common isolate. No resistance was found in two isolates of *S. saprophyticus* obtained from urine. There are studies showing more than 80 percent of Coagulase-negative staphylococcal isolates were resistant to methicillin and semi synthetic penicillin.<sup>(14,15)</sup> *S.epidermidis* (64%) and *S.haemolyticus* (92 %) are more MRCoNS.<sup>(16)</sup> There are many studies suggesting the relationship between methicillin-resistance and reduced susceptibility to glycopeptides in coagulase negative staphylococci. Antibiotic susceptibility

testing of the isolates showed maximum resistance to penicillin ampicillin followed by erythromycin. (68%) No resistance to vancomycin and linezolid was seen. In this study *S.epidermidis* showed maximum resistance to penicillin (86%) followed by ampicillin (82%)and erythromycin (82%) .

### Discussion

Staphylococci are members of the family Micrococcaceae. They are Gram positive, catalase positive and occur singly and in irregular grape like clusters. Staphylococci are broadly divided in to a group that produce coagulase and those that do not. Coagulase is an enzyme which causes clotting of the plasma which is easily demonstrable and produced exclusively by the pathogenic staphylococci, *Staphylococcus aureus*. This virulent factor is absent in coagulase negative staphylococci therefore the prefix "Coagulase

negative” is added. Hence CoNS represents a group of various species of staphylococci which can be differentiated using different biochemical reactions and more precisely by DNA hybridization.

More than 41 species are recognized but only few are commonly incriminated in human infections. In most of the studies the majority of isolates were identified as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Recently numerous reports have been implicated, a wide range of species as etiologic agents associated with confirmed infection process. They are *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, *S. capitis*, *S. hominis*, *S. lugdunensis*, *S. warneri*, *S. saccharolyticus* and *S. xylosus*. The different species of CoNS are found to have a specific site preference as normal commensal. Of these, *Staphylococcus epidermidis* is the most widely distributed all over the human body. They were recognized as opportunistic pathogen which could cause diseases only if the external barriers—skin, mucosa were breached. Other species are *S. haemolyticus*, *S. hominis* and *S. capitis*. The most important clinical entity associated with CoNS is foreign body related infections. *S. saprophyticus* is the second most frequent causative microorganism in uncomplicated lower urinary tract infection in young sexually active women.

### Prevention and Infection Control

Coagulase negative staphylococci are part of the normal nasal and cutaneous flora so they will never be nor should they be fully eradicated. The single most important factor that can reduce the incidence of infection due to CoNS is strict adherence to hand washing both prior to and after examining patients.

### Limitation of the Study

The most challenging problem in CoNS is the assessment of their clinical relevance. This study is limited by its small size. CoNS isolated are often deemed to be contaminants. So larger prospective, observational studies are required to define the

significance of CoNS isolate in critically ill patients.

### Conclusion

In general, isolation of CoNS has not been considered as significant unless repeated culture yields CoNS with identical antibiogram pattern. So in response to the pathogenic potential of these organisms, laboratory need an inexpensive, simple and reliable methods to identify species of Coagulase –negative Staphylococci. Recently, numerous commercial products for CoNS identification have become available as gram positive identification panels.

The present study suggest that if CoNS are repeatedly isolated from patients with infection, they should be taken seriously and susceptibility tests done on these isolates.

### References

1. Amoury, Bowman, F.O and Malm, J.R : Endocarditis associated with intracardiac prosthesis, Diagnosis, management and prophylaxis. *J. Thoracic and Cardiovasc. Surg*, 51:36-48, 1996.
2. Bauer, Kirby, W.M.M Sherris, J.C. and Tenckhoff, H. : Antibiotic susceptibility testing by a standardized single disc method. *Amer. J. Clin. Pathol.*, 45:493-496, 1966.
3. Abdulhadi Hassan Al-Mazroea ABP. Incidence and Clinical Significance of Coagulase Negative Staphylococci in Blood. *J. of Taibah university Medical Sciences* 2009;4(2): 137 -147.
4. Koneman EW, Allen SD Color atlas and Text book of diagnostic microbiology 5<sup>th</sup> edition, Philadelphia, Lippincott-Raven Publishers, 1997:547-549
5. Mackie and Mc Cartney. Practical medical Microbiology 14<sup>th</sup> edition. Churchill-Livingstone; Elsevier; 254-256-128p
6. Kloos WE, Schleifer KH: Simplified scheme for routine identification of staphylococcus species. *J Clin Microbiol* 1975;1:82-86

7. Bannerman TL. Staphylococcus, Micrococcus and other catalase positive cocci that grows aerobically Chapter 28 In :Manual of Clinical Microbiology, 8<sup>th</sup>ed; Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC, editors. ASM press: WashingtonDC;2003p.384
8. De paulis AN, Predari Sc Five simple scheme for the species level identification of clinically significant coagulase negative Staphylococci. J Clin Microbiol. 2003;41:1219-1224
9. Stephen Grojean, MT (ASCP), and Elliot L. Rank PhD Identifying Species of Coagulase-Negative Staphylococci using a Microtiter Plate panel. J.Laboratory Medicine.vol 16,No11, November 1985 p688 -692
10. Diekema, DJ Pfaller, Ma,Schmitz FJ. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United states, Canada, Latin America, Europe and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program,1997-1999.Clin Infect Dis.2001;32(2):114
11. Singh S Banerjee G Simple method for speciation of clinically significant coagulase negative Staphylococci and its antibiotic sensitivity/resistant pattern in NICU of a tertiary care centre. Biomedical research.2008;19(2)
12. Shubhra Singh, Gopa Banerjee et al .Prevalence of MecA Gene positive coagulase negative Staphylococci in NICU of a tertiary care hospital Boomedical Research.2009;20(2):94-98.
13. Silvia Natoli, Caria Fontana et al. Characterisation of coagulase-negative staphylococcal isolates from blood with reduced susceptibility to glycopeptides and their therapeutic options.J Antimicro Chemother 1992.29:459-466
14. Hamilton-Miller JM Iliffe A.1985. Antimicrobial resistance in coagulase-negative staphylococci. J .Med. Microbiol. 19:217-226.
15. Kloos WE, Bannerman TI.Update on clinical significance of coagulase negative staphylococci. Clin Microbiol Rev 1994;7:117-140.