International Journal of Research in Medical Sciences
*Golia S et al. Int J Res Med Sci. 2015 May;3(5):1242-1246*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: 10.5455/2320-6012.ijrms20150537

**Research Article**

# Speciation of clinically significant coagulase negative staphylococci and their antibiotic resistant patterns in a tertiary care hospital

**Saroj Golia, Deepali Bhimacharya Telsang*, Asha S. Kamath B, Devendrakumar Tiwari**

Department of Microbiology, Dr. B.R. Ambedkar Medical College, KG Halli, Bengaluru, Karnataka, India

**Received:** 19 March 2015
**Revised:** 20 March 2015
**Accepted:** 13 April 2015

***Correspondence:**
Dr. Deepali Bhimacharya Telsang,
E-mail: telsangd@yahoo.com



## ABSTRACT

**Background:** Coagulase negative Staphylococci (CONS) are normal human microbiota and sometimes cause infections, often associated with implanted devices, such as joint prosthesis, shunts and intravascular catheters, especially in very young, old and immunocompromised patients. These infections are difficult to treat because of the risk factors and the multiple drug resistant nature of the organisms. The study is undertaken to speculate CONS isolates from various clinical samples and to determine antibiotic susceptibility pattern of CONS by Kirby Bauer disc diffusion method.
**Methods:** A total of 134 clinically significant CONS isolated from pus, urine, blood, fluid, sputum, ear swabs, endotracheal tube, ophthalmic, semen and nail samples. These isolates initially identified by colony morphology, Gram staining, catalase test, slide coagulase test, tube coagulase test and mannitol fermentation. Speciation of CONS was done by novobiocin resistance test, urease activity, ornithine decarboxylase and aerobic acid production from mannose.
**Results:** S. epidermidis is the most frequent isolate 62 (46.3%) followed by S. saprophyticus 38(28.4%), S. haemolyticus 27(20.1%), S. lugdunensis 3(2.2%). S. warneri 3(2.2%), S. cohinii 1(0.7%). Antibiotic susceptibility testing of the isolates showed maximum resistance to penicillin 128 (95.5%) and ampicillin118 (88%) followed by erythromycin 96 (71.6%), cefoxitin 89 (66.4%), gentamicin 33(24.6%), piperacillin & tazobactam 31(23.8%), amoxicillin & clavulanic acid 25 (18.7%), linezolid 23 (17.2%), levofloxacin 9 (6.7%), vancomycin & teicoplanin 2 (1.5%), tigecycline 1 (0.7%).
**Conclusion:** S. epidermidis is the more common isolate identified and CONS are often resistant to multiple antibiotics (Penicillin, ampicillin) & glycopeptides have been considered as the drugs of choice for the management of infections caused by these organisms.

**Keywords:** Coagulase negative Staphylococci, Microbiota, Immunocompromised, Antibiotic susceptibility, Disk diffusion method, Ornithine decarboxylase

## INTRODUCTION

Coagulase negative Staphylococci (CONS) are normal human microbiota and sometimes cause infections, often associated with implanted devices, such as joint prosthesis, shunts and intravascular catheters, especially in very young, old and immunocompromised patients.[1] These infections are difficult to treat because of the risk factors and the multiple drug resistant nature of the

organisms.[2] Hence the study was undertaken to identify and speciate CONS and their antibiogram.

Coagulase negative Staphylococci embraces all species other than S. aureus. CONS they form clusters and colonies on solid media are similar but smaller than S. aureus. S. epidermidis, can able to colonize foreign bodies such as vascular catheters, or indwelling prosthesis S. saprophyticus is an important cause of UTI in younger, sexually active women. Patients acquire CONS infections in hospitals, carried by hospital staff and patients.[3]

### Objectives

1) To speciate CONS isolates from various clinical samples.
2) To determine antibiotic susceptibility pattern of CONS by Kirby Bauer disc diffusion method.

## METHODS

The study was carried out in the department of microbiology, Dr. B.R.A.M.C, K.G. Halli, Bengaluru for a period of 9 months from November 2013 to July 2014. A total of 134 clinically significant CONS isolated from pus, urine, blood, fluid, sputum, ear swabs, endotracheal tube, ophthalmic, semen and nail samples. The Isolates were considered clinically significant when isolated in pure culture from infected site or body fluid or if the same strain was isolated twice. The isolates initially identified by colony morphology, Gram staining, catalase test, slide and tube coagulase test and mannitol fermentation.[4,5] Speciation of CONS was done by novobiocin resistance test, urease activity, ornithine decarboxylase and aerobic acid production from mannose[6-8] (Table 1).

**Table 1: Identification of CONS by simple scheme.**

| Species | Slide coagulate test | Tube coagulate test | Ornitine decarboxylase test | Urease activity | Mannose fermentation test | Novobiocin sensitivity (5 µg) |
|---|---|---|---|---|---|---|
| S. epidermidis | - | - | + | + | + | S |
| S. saprophyticus | - | - | - | + | - | R |
| S. haemolyticus | - | - | - | - | - | S |
| S. lugdunensis | + | - | + | + | + | S |
| S. warneri | - | - | - | + | - | S |
| S. cohnii | - | - | - | + | - | R |

S = Sensitive, R = Resistant, + = Positive, - = Negative

The antimicrobial susceptibility profiles of all isolates were done by Disc diffusion method using Mueller Hinton Agar (MHA) plates. Staphylococcal aureus ATCC 25293 was used as control.

The various antibiotic discs used are: Penicillin (10 µg), ampicillin (10 mg), erythromycin (15 mg), linezolid (30 mg), piperacillin/tazobactam (100/10 mg), cefoxitin (30 mg) amoxicillin & clavulanic acid (20/10 mg), gentamicin (10 mg), levofloxacin (5 mg), vancomycin (30 mg), teicoplanin (30 mg), tigecycline (15 mg).

## RESULTS

Among 134 isolates of CONS 63 (47%) were isolated from pus, 27 (20%) from the urine, 19 (14.2%) from blood, 9 (6.7%) from sputum, 5 (3.7%) from fluid, 3 (2.2%) each from ET tube and ear swabs, 2 (1.5%) each from semen and eye and 1 (0.7%) from nail samples. All isolates of CONS were negative for slide coagulase test & tube coagulase test except S. lugudensis which gave slide coagulase test positive. Identification of CONS showed S. epidermidis as most frequent isolate 62 (46.3%) followed by S. saprophyticus 38 (28.4%), S. haemolyticus 27 (20.1%), S. lugdunensis 3 (2.2%), S. warneri 3 (2.2%), S. cohinii 1 (0.7%).

Out of 62 S. epidermidis isolates 25 (40%) were isolated from pus followed by 12 (19.7%) from urine, 6 (9.7%) from blood, 5 (8%) from fluid and sputum, 3 (4.8%) from ear swab & 2 (3.2%) from ET tube, eye and 1(1.6%) nail and semen samples. Out of 38 S. saprophyticus, 18 (47.4%) were isolated from pus followed by 9 (23.7%) from urine, 6 (15.8%) from blood, 3 (7.9%) from sputum, 1 (2.6%) each from ET tube and semen samples. Out of 27 S. hemolyticus isolates 14 (51.9%) isolated from pus followed by 6 (22.2%) from blood and urine, 1 (3.7%) from sputum sample (Table 2).

Out of 134 CONS 52 (38.8%) cases >40 years of age group. The isolation was more in males 87 (64.9%) than females 47 (37.07%). Paediatrics accounted 20 (14.9%).

Antibiotic susceptibility testing of the isolates showed maximum resistance to penicillin 128 (95.5%) and ampicillin 118 (88%) followed by erythromycin 96 (71.6%), cefoxitin 89 (66.4%), gentamicin 33 (24.6%), piperacillin & tazobactam 31(23.8%), amoxicillin & clavulanic acid 25 (18.7%), linezolid 23 (17.2%), levofloxacin 9 (6.7%), vancomycin & teicoplanin 2 (1.5%), tigecycline 1 (0.7%) (Table 3).

**Table 2: Frequency of clinically significant CONS in different clinical samples.**

| Species | Pus | Urine | Blood | Fluid | Sputum | Ear | ET tube | Ophthalmic | Semen | Nail |
|---|---|---|---|---|---|---|---|---|---|---|
| S. epidermidis (62) 46.3% | 25 (40%) | 12 (19.4%) | 6 (9.7%) | 5 (8%) | 5 (8%) | 3 (4.8%) | 2 (3.2%) | 2 (3.2%) | 1 (1.6%) | 1 (1.6%) |
| S. saprophyticus (38) 28.4% | 18 (47.4%) | 9 (23.7%) | 6 (15.8%) | - | 3 (7.9%) | - | 1 (2.6%) | - | 1 (2.6%) | - |
| S. haemolyticus (27) 20% | 14 (51.9%) | 6 (22.2%) | 6 (22.2%) | - | 1 (3.7%) | - | - | - | - | - |
| S. lugdunensis (3) 2.2% | 3 (100%) | - | - | - | - | - | - | - | - | - |
| S. warneri (3) 2.2% | 3 (100%) | - | - | - | - | - | - | - | - | - |
| S. cohnii (1) 0.7% | - | - | 1 (100%) | - | - | - | - | - | - | - |
| **Total (134)** | 63 (47%) | 27 (20%) | 19 (14.2%) | 5 (3.7%) | 9 (6.7%) | 3 (2.2%) | 3 (2.2%) | 2 (1.5%) | 2 (1.5%) | 1 (0.7%) |

**Table 3: Showing resistance patterns of CONS to different antibiotics.**

| | P | AMP | E | CX | PIT | LZ | GEN | AMC | LE | VA | TEI | TG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S. epidermidis (62) | 59 (95%) | 54 (87%) | 35 (36%) | 40 (64.5%) | 9 (14.5%) | 6 (9.7%) | 14 (22.5%) | 13 (21%) | 5 (8%) | 2 (3.2%) | 2 (3.2%) | 1 (1.6%) |
| S. saprophyticus (38) | 37 (97%) | 34 (89.5%) | 33 (86.8%) | 23 (60.5%) | 5 (13%) | 17 (44.7%) | 10 (26.3%) | 6 (15.8%) | 4 (10.5%) | - - | - - | - - |
| S. haemolyticus (27) | 25 (92.6%) | 24 (88.8%) | 24 (88.8%) | 24 (88.8%) | 17 (63%) | - - | 7 (26%) | 4 (14.8%) | - - | - - | - - | - - |
| S. lugudensis (3) | 3 (100%) | 2 (66.7%) | 3 (100%) | 1 (33.3%) | - - | - - | 2 (66.7%) | 2 (66.7%) | - - | - - | - - | - - |
| S. warneri (3) | 3 (100%) | 3 (100%) | - - | 1 (33.3%) | - - | - - | - - | - - | - - | - - | - - | - - |
| S. cohnii (1) | 1 (100%) | 1 (100%) | 1 (100%) | - - | - - | - - | - - | - - | - - | - - | - - | - - |
| **Total (134)** | 128 (95.5%) | 118 (88%) | 96 (71.6%) | 89 (66.4%) | 31 (23.1%) | 23 (17.2%) | 33 (24.6%) | 25 (18.7%) | 9 (6.7%) | 2 (1.5%0 | 2 (1.5%) | 1 (0.7%) |

P-Penicillin, AMP-Ampicillin, E-Erythromycin, CX-Cefoxitin, PIT-Piperacillin & Tazobactam, LZ-Linezolide, GEN-Gentamycin, AMC-Amoxicillin & Clavulanic acid, LE-Levofloxacin, VA-Vancomycin, TEI-Teicoplanin, TG-Tigecycline

S. epidermidis showed a significant percentage of isolates resistant to penicillin 59 (95%), followed by ampicillin 54 (87%), cefoxitin 40 (64.5%) erythromycin 35 (56%).

S. saprophyticus showed resistance to penicillin 37 (97%) followed by ampicillin 34 (89.5%), erythromycin 33 (86.8%), cefoxitin 23 (60.5%) and S. haemolyticus showed resistance to penicillin 25 (92.6%), ampicillin, erythromycin, cefoxitin 24 (88.8%).

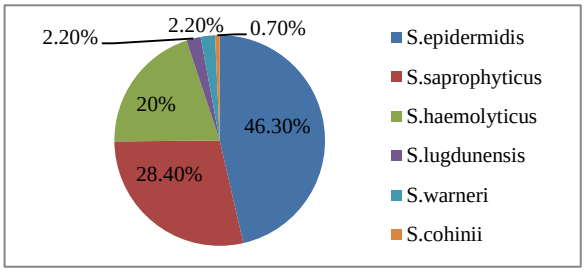


**Figure 1: Resistant pattern of CONS to different antibiotics.**

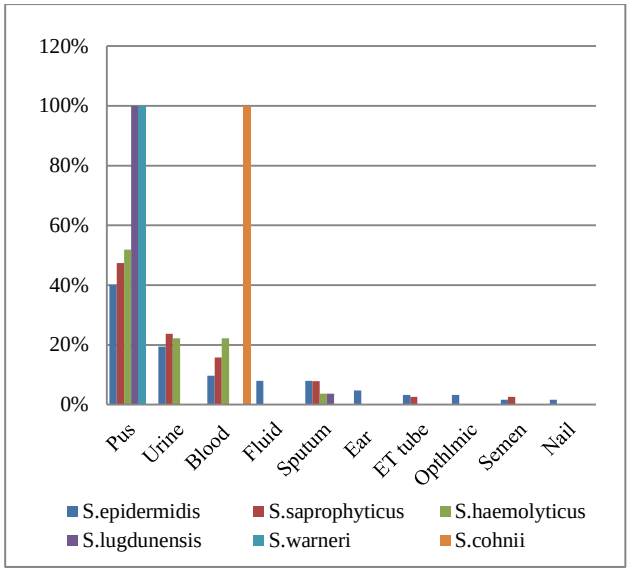


**Figure 2: Frequency distribution of various clinical samples in CONS.**

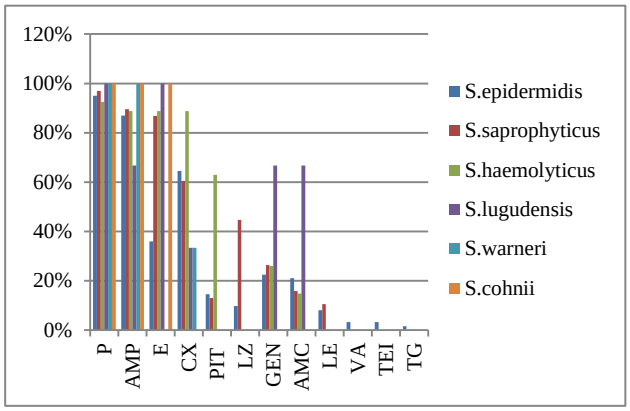


**Figure 3: Resistant pattern of CONS to different antibiotics.**

P-Penicillin, AMP-Ampicillin, E-Erythromycin, CX-Cefoxitin, PIT-Piperacillin & Tazobactam, LZ-Linezolide, GEN-Gentamycin, AMC-Amoxicillin & Clavulanic acid, LE-Levofloxacin, VA-Vancomycin, TEI-Teicoplanin, TG-Tigecycline

## DISCUSSION

Coagulase Negative Staphylococci form a part of normal flora. Moreover, if CONS isolated along with another organism, its pathogenic potential may be totally neglected. Hence it is necessary to speciate CONS and understand the pathogenic potential of individual CONS.[9] Coagulase-negative Staphylococci species formerly known as contaminants bacteria, but are now as important possible pathogens with the augment in number of sternly incapacitated patients.[10]

In our study S. epidermidis was the most frequent isolate 62 (46.3%) followed by S. saprophyticus 38 (28.4%), S. hemolyticus 27 (20.1%), S. lugudensis & S. warneri 3 (2.2%), S. cohinii 1 (0.7%) which correlated with study like Asangi et al. which showed S. epidermidis as the most frequent isolate (43, 44.8%), followed by S. saprophyticus (26, 27.1%), S. haemolyticus (19, 19.7%), S. lugdunensis (2, 2.1 %), S. warneri (2, 2.1%), S. cohnii (1, 1%).[11] Our study also correlated with study like Shubhra Singh and Gopa Banerjee et al., where they identified S. epidermidis (40%) as the most frequently encountered clinical isolates in their hospital followed by S. saprophyticus (14%), S. haemolyticus (12%).[9] Our study also correlated with study conducted by M. G. Usha et al. that among the 100 isolates, epidermidis was the most common species isolated, seen in (32%), followed by hemolyticus (18%), S. cohni and S. warneri (3% each).[12]

In our study out of 134 samples 19 (14.2%) from blood cultures and 5 (3.7%) from fluid which correlated with R. Goyal et al study that out of 102 strains of CONS 15 (14.7%) from blood cultures and 2 (1.9%) each from synovial fluid and ascitic fluid.[13] In our study we got 27(20%) urine samples, which correlates to study by Sadhvi Parashar that 185, 36 (19.46%) from urine.[14]

In our study resistance to penicillin 95.5%, ampicillin 88% cefoxitin 66.4% which correlated to study by Asangi et al. that penicillin 94.7%, Ampicillin 88.5%, cefoxitin 67.7%.[11] In our study 88%Ampicillin resistant CONS, gentamicin 24.6% and vancomycin 1.5% which correlates with study by M. G. Usha et al., that majority of the CONS species were resistant to ampicillin and amoxyclav (89%) each, followed by gentamicin (25%) & none of the isolates showed resistance to vancomycin.[13] Our study also correlated with R. Goyal et al. which revealed no resistance to vancomycin with 89% resistant to ampicillin and gentamicin (20%).[14] In our study, antibiotic susceptibility testing showed maximum resistance to penicillin 128 (95.5%) and ampicillin 88%, this study correlates with a study by Shubhra Singh, Gopa Banerjee et al. were the antibiotic susceptibility testing showed maximum resistance to penicillin & ampicillin 80%.[9] Our study also correlated to the study done by U. Mohan, N. Jndal, P. Aggarwal that penicillin (90.6%) and all the isolates were sensitive to vancomycin.[15] Our study also correlated to Ahmad Farajzadeh Sheikh and Manijeh Mehdinejad et al. which showed ampicillin (AM) 88.05%.[10]

In our study out of 134 CONS 52 (38.8%) cases >40 years of age group. The isolation was more in males 87 (64.9%) than females 47 (37.07%). Paediatrics accounted 20 (14.9%). Our study correlated to Asangi et al. that out of 96 CONS, 38(39.5%) cases in >40 years of age group. The isolations were more in males (63, 65.6%) than females (33, 34.4%).[11]

CONS primarily S. epidermidis and S. haemolyticus are often resistant to multiple antibiotics and glycopeptides have been considered the drugs of choice for the management of infections caused by these organisms.[16]

## CONCLUSION

Recently CONS have emerged as a potential pathogen. CONS have become the 3rd cause of nosocomial bloodstream infections as a result of the combination of increased use of intravascular devices and an increase number of hospitalised immunocompromised patients. S. epidermidis & S. saprophyticus are the more common isolates identified & CONS are often resistant to multiple antibiotics (Penicillin, ampicillin) & glycopeptides have been considered as the drugs of choice for the management of infections caused by these organisms.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: Not required, as clinical samples were processed that received in central laboratory Dr. B.R.A.M.C. KG Halli, Bengaluru*

## REFERENCES

1. Geo. F. Brooks, Karen C. Carroll, Stephen A. Morse, Timothy A. Mietzner. Coagulase negative Staphylococci. In: Geo F. Brooks, Karen C. Carroll, Stephen A. Morse, Timothy A. Mietzner, eds. Jawetz, Melink & Adelberg's Medical Microbiology. 26th ed. New York: McGraw Hill Education; 2013: 199-205.
2. Roth RR, James WD. Microbial ecology of the skin. Annu Rev Microbiol. 1988;42:441-64.
3. College JG, Fraser AG, Marmion BP, Simmons A. CONS. In: College JG, Fraser AG, Marmion BP, Simmons A, eds. Mackie & Mccartney Practical Microbiology. 19th ed. USA: Elsevier; 2012: 250-252.
4. Shubhra Singh, Gopa Banerjee, S. K. Agarwal, Anuradha Rajput, Piyush Tripathi, Mala Kumar, et al. Prevalence of MecA Gene positive coagulase negative Staphylococci in NICU of a tertiary care hospital. Biomed Res. 2009;20(2):94-8.
5. Koneman EW, Allen SD. Identification of bacteria. In: Koneman EW, Allen SD, eds. Colour Atlas and Text Book of Diagnostic Microbiology. 5th ed. Philadelphia: Lippincott-Roven Publishers; 1997: 547-549.
6. Bannerman TI. Staphylococcus, Micrococcus and other catalase positive coccid that grows aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, eds. Manual of Clinical Microbiology. 8th ed. Washington DC: ASM Press; 2003: 384.
7. De Pauli AN, Predari SC. Five test simple scheme for the species level identification of clinically significant coagulase negative staphylococci. J Clin Microbiol. 2003;41:1219-24.
8. Baird D. Staphylococcus; cluster forming Gram-positive cocci. In: Colle JG, Fraser AG, Marimom BP, Simmons A, eds. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Living Stone; 1996:245.
9. Singh S, Banerjee G. Simple method for speciation of clinically significant coagulase negative Staphylococci and its antibiotic sensitive/resistant pattern in NICU of tertiary care centre. Biomed Res. 2008;19(2):97-101.
10. Ahmad Farajzadeh Sheikh, Manijeh Mehdinejad. Identification and determination of coagulase-negative Staphylococci species and antimicrobial susceptibility pattern of isolates from clinical specimens. Afr J Microbiol Res. 2012 Feb;6(8):1669-74.
11. Surekha Y. Asangi, Mariraj J, Sathyanarayan MS, Nagabhushan, Rashm. Speciation of clinically significant Coagulase negative Staphylococci and their antibiotic resistant patterns in a tertiary care hospital. Int J Biol Med Res. 2011;2(3):735-9.
12. Usha MG, Shwetha DC, Vishwanath G. Speciation of coagulase negative Staphylococcal isolates from clinically significant specimens and their antibiogram. Indian J Pathol Microbiol. 2013;56(3):258-60.
13. Goyal R, Singh NP, Kumar A, Kaur I, Singh M, Sunita N, et al. Simple and economical method for speciation and resist typing of clinically significant coagulase negative staphylococci. Indian J Med Microbiol. 2006;24:201-4.
14. Sadhvi Parashar. Significance of Coagulase Negative Staphylococci with Special Reference to Species Differentiation and Antibiogram Sadhvi Parashar.
15. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from various clinical specimens. Indian Med Gazette. 2014;CXLVII(7):255-8.
16. Silvia Natoli, Carla Fontana, Marco Favaro, Alberto Bergamini, Gian Piero Testore, Silvia Minelli, et al. Characterization of coagulase negative staphylococcal isolates from blood with reduced susceptibility to glycopeptides and therapeutic options. J Antimicrob Chemother. 1992;29:459-66.

DOI: 10.5455/2320-6012.ijrms20150537
**Cite this article as:** Golia S, Telsang DB, Kamath B AS, Tiwari D. Speciation of clinically significant coagulase negative staphylococci and their antibiotic resistant patterns in a tertiary care hospital. Int J Res Med Sci 2015;3:1242-6.