Indian Journal of Mednodent and Allied Sciences
Vol. 5, No. 2, June 2017, pp- 73-79

IndianJournals.com
A product of Diva Enterprises Pvt. Ltd.
DOI : 10.5958/2347-6206.2017.00012.7

Original Research

Biochemical Characterization and Antibiotic Susceptibility of Coagulase Negative Staphylococci Isolates from Exudates and Body Fluids

Nagaraju Vanaparthi[1]*, Ravishankarreddy Anukolu[2], Saileela Kondapaneni[3], Praful S. Patil[4] and Vijay Kumar A.N.[5]

ABSTRACT

Coagulase negative *Staphylococci* (CoNS) are increasingly being recognized as significant nosocomial pathogens, partly due to the growing appreciation of this group of organisms as opportunistic pathogens or due to increase in the use of transient or permanent medical devices in seriously ill and immunocompromised patients. **Aims and Objectives**: 1) Isolation of CoNS from exudates and body fluids. 2) Biochemical characterization of CoNS. 3) Antibiotic susceptibility pattern of CoNS. **Method**: 180CoNS isolated from various exudates and body fluids such as pus, wound swabs, endotracheal secretions, sputum, branchialaspitate, and central lining tube were collected. All the CoNS isolates were processed in the Microbiology Laboratory and identified by colony morphology, gram staining, catalase, slide, tube coagulase test, anaerobic acid from mannitol, and deoxyribonuclease. Bacitracin (0.04 U) and furazolidone (100 µg) susceptibilities were done to exclude *Micrococcus* and *Stomatococcus* spp. The following biochemical tests were done for the speciation of the CoNS: urease test, phosphatase test, polymyxin B disc test, novobiocin disk test, ornithine decorboxylase test, mannitol to acid, Voges–Proskauertest, mannose fermentation, trehalose fermentation and antibiotic susceptibility testing. **Result**: Out of 180 isolates, 78 are *Staphylococcus epidermidis (*43.3%), 63 are *Staphylococcus hemolyticus* (35%), 21 are *Staphylococcus hominis* (11.6%), and 18 are *Staphylococcus lugdunensis* (10.0%). Maximum number of CoNS were isolated from pus specimens (58.33%), followed by wound swabs (18.33%). A total of 164out of 180 strains were negative for both bound and free coagulase. A total of 60 strains were bound coagulase slow positive and free coagulase negative. *S. epidermidis* was the most frequent isolate and 68 *S. epidermidis* isolates were identified if ornithine decorboxylase was considered positive, while negative 10 *S. epidermidis* isolates required inclusion of trehalose and mannitol for speciation. Antibiotic susceptibility testing showed maximum resistance to penicillin (78.3) followed by chloramphenicol (41.6%). No resistance to vancomycin was seen. **Conclusion:** The study revealed *S. epidermidis* is the predominant CoNS from endotracheal secretions and also pus samples. *S. hemolyticus* was isolated from pus and central lining tubes, *S. hominis* and *S. lugdunensis* were isolated mainly from wound swabs. The present

[1]Assistant Professor, Department of Microbiology, MKCG Medical College & Hospital, Berhampur, District - Ganjam, Odisha. Pin: 760004
[2]Professor, Department of Microbiology, Kamineni Academy of Medical Sciences and Research Center Nalgonda Road, Near Andhra Bank, Suryodaya Colony, LB Nagar, Hyderabad, Telangana 500068
[3]Professor and Head, Department of Microbiology Katuri Medical College and Hospital, Katuri Nagar, Guntur, Andhra Pradesh
[4]Assistant Lecturer, Department of Microbiology S.M.B.T. Medical Sciences and Research Centre and Hospital, Nandi Hills, Dhamangaon Tal. Igatpuri, Dist. Nashik, Maharashtra State, India
[5]Assistant Professor, Department of Pharmacology Vikhe Patil Institute of Medical Sciences, Ahmednagar, Maharashtra
*Corresponding author email id: vnr2275@gmail.com

study suggests if coagulase –ve *Staphylococci* are repeatedly isolated from patients with infection they should be taken seriously and ABST done on these isolates for proper diagnosis and treatment especially in nosocomial infections.

KEYWORDS: Coagulase negative staphylococci, Antibiotic susceptibility, Clinical isolates of exudates and body fluids, Biochemical chemical characterization, Bound coagulase, Free coagulase, Ornithine decarboxylase

INTRODUCTION

Coagulase negative *Staphylococci* (CoNS) are increasingly being recognized as significant nosocomial pathogens, partly due to the growing appreciation of this group of organisms as opportunistic pathogens or due to increase in the use of transient or permanent medical devices in seriously ill and immuno-compromised patients [1,2]. In a routine microbiology laboratory, *Staphylococcus aureus* is identified by a rapid screening test (coagulase test) and all non-*S. aureus* isolates are reported as CoNS [3,4]. CoNS are one of the most frequent causes of nosocomial infections and are reservoirs of multiple antimicrobial resistant determinants [5]. More than 30 species of CoNS are recognized but only a few are commonly incriminated in human infections [6]. The organisms are known pathogens in urinary tract infection, prosthetic valve endocarditis, patients with intravenous catheters and indwelling foreign devices, peritoneal dialysis, catheter associated peritonitis, and cerebrospinal fluid shunt infections in premature neonates [7,8]. They are also common opportunistic pathogens in patients who are immunocompromised [8]. However, there are a number of recent reports which state that CoNSare the most common pathogens of bloodstream infections [9,10]. Coagulase-negative *Staphylococci* are also associated with resistance to several commonly used antimicrobial agents such as the macrolides, lincosamides, quinolones, trimethoprim–sulfamethoxazole, and aminoglycosides. Multidrug resistant strains are common [11]. *Staphylococcus epidermidis* normal flora of human skin and mucous membranes distributed widely often in large numbers over body surface. Spread of patient's endogenous strain to normally sterile site, usually as a result of implantation of medical devices during hospitalization. Person to person spread in hospitals can lead to patients becoming colonized and potentially infected with antibiotic resistant strains. *Staphylococcus hemolyticus* and *Staphylococcus lugdunensis* are normal human flora similar to *S. epidermidis* but present in fewer numbers. *Staphylococcus saprophyticus* causes urinary tract infections in sexually active, young females, infections in sites outside urinary tract are not common. Virulence factors of production of exopolysacharide slime or biofilm enhances organism adhesion and provides mechanical barrier to antibiotics and host defence mechanisms. Ubiquitous member of normal flora makes this species the most commonly encountered in clinical specimens, usually as a contaminant and can be difficult to establish clinical significance [12].

AIMS AND OBJECTIVES

1. To isolate CoNS from exudates and body fluids.
2. To characterise CoNS biochemically.
3. Determine the antibiotic susceptibility pattern of CoNS.

MATERIALS AND METHODS

Place of study: Kamineni Institute of Medical Sciences, Narketpally, Nalgonda (Dist) Telangana (State).

Period of study: From June 2010 to June 2011.

Type of the study: Prospective analytical study.

Ethcialapproval : The present study was approved by the Institutional Ethics Committee of KIMS.

Inclusion criteria: 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 [1].

Sample size and sample collection: One hundred and eighty CoNS isolated from various exudates and body fluid samples such as pus, wound swabs, endotracheal secretions, sputum, branchialaspitate, and central lining tube were collected.

METHODOLOGY

All the CoNS isolates were processed in the Microbiology laboratory at KIMS and identified by colony morphology [12], gram staining [13], catalase [13], slide, tube coagulasetest [13] (i.e., bound coagulase and free coagulase) (read after 4, 24 h)and mannitolsait agar [12], deoxyribonuclease [14]. Bacitracin (0.04 U) and furazolidone [13] (100 μg) susceptibilities were done to exclude *Micrococcus* and *Stomatococcus* spp. [5,8].

The following biochemical tests were done for the speciation of the CoNS: urease test [13], phosphatase test [14], polymyxin B disc test [12], novobiocin disk test [13,14], ornithine decorboxylasetest [14], mannitol to acid [14], Voges–Proskauer test [13], mannose fermentation [14], trehalose fermentation [14]. The identification scheme was followed as per described by Goyal *et al.* [1], Forbes *et al.* [12], Winn *et al.* [13] antibiotic susceptibility testing [14].

Antibiotic Susceptibility Test

Antimicrobial susceptibility of all isolates were determined by Kirby–Bauer disk diffusion method on Mueller Hinton agar for the following antibiotics with their concentration given in parenthesis. Penicillin (10U), oxacillin (1μg), ciprofloxacin (5μg), gentamicin (10 μg), erythromycin (15 μg), chloramphenicol (30 μg), and vancomycin (30 μg) according to CLSI guidelines [15]. Antibiotics disks and media were obtained from HiMedia, Mumbai, India.

RESULTS

Distribution of CoNS (Table 1). Out of total 180 CoNS, 18 (10%) were isolated from endotrachealsecretions,

Table 1: Distribution of CoNS isolates among various specimens

Specimen	No of Isolates (%)
Endotracheal secretions	18 (10)
Wound swabs	33 (18.33)
Pus	105 (58.33)
Bronchial aspirate	6 (3.33)
Sputum	15 (8.33)
Central lining tube	3 (1.66)

33 (18.33) were isolated from wound swabs, 105 (58.33) were isolated from pus, 6 (3.33) were isolated from bronchial aspirate, 15 (8.33) were isolated from sputum, and 3 (1.66) were isolated from central lining tube.

Most of the CoNS isolates were from pus, sputum, wound swabs, and endotracheal secretions except for *S. hemolyticus* which was isolated from all the specimens. Pus and wound swab specimens yielded all strains of CoNS (Figure 1), while central lining tube yielded only *S. hemolyticus*, *S. epidermidis* was not isolated from sputum and bronchial aspirate. Out of 180 isolates, 78 are *S. epidermidis (*43.3%), 63 are *S. hemolyticus* (35%), 21 are *Staphylococcus hominis* (11.6%), and 18 are *S. lugdunensis* (10.0%). Maximum number of CoNS were isolated from pus specimens (58.33%), followed by wound swabs (18.33%) (Table 2).

One hundred and sixty four of 180 strains were negative for both bound coagulase and free coagulase. Sixteen strains were bound coagulase slow positive and free coagulase negative. The scheme could identify 95% of CoNS isolated from clinical samples. *S. epidermidis* was the most frequent isolate and 68 *S. epidermidis* isolates were identified if ornithine decorboxylase was considered positive, while ornithine decorboxylase negative 10 *S. epidermidis* isolates required inclusion of trehalose and mannitol for speciation (Figure 2) (Tables 3 and 4).

Antibiotic sensitivity was performed for 180 strains. Antibiotic susceptibility testing showed maximum

Table 2: Different strains of CoNS isolates from various clinical samples

Species	No. (%)	Pus	Sputum	Wound swabs	Endotracheal secretions	Bronchial aspirate	Central lining tube
S. epidermidis	78 (43.3)	54(30)	–	12(6.67)	12(6.67)	–	–
S. hemolyticus	63(35)	36(20)	12(6.67)	6(3.33)	3(1.6)	3(1.6)	3(1.6)
S. hominis	21(11.6)	9(5)	–	6(3.33)	3(1.6)	3(1.6)	–
S. lugdunensis	18(10)	6(3.33)	3(1.6)	9(5)	–	–	–
Total	180(100)	105(58.33)	15(8.33)	33(18.33)	18(10)	6(3.33)	3(1.6)

Table 3: Biochemical characters of CoNS strains

Test or characteristic	*S. epidermidis*	*S. hemolyticus*	*S. hominis*	*S. lugdunensis*
Bound coagulase–	78	63	21	2
Free coagulase+	0	0	0	0
Mannose+	78	0	0	18
Novobiocin sensitive	78	63	21	18
Polymixin-B Resistant	78	0	0	0
Urease+	78	0	21	18
Phosphatase+	78	0	0	0
Ornithine Decorboxylase–	10	63	21	0

Table 4: Additional tests for speciation of CoNS

Test or Characteristic	*S. epidermidis*	*S. hemolyticus*	*S. hominis*	*S. lugdunensis*
Bound coagulase slow+	0	0	0	16
Ornithine Decorboxylase+	68	0	0	18
Mannitol to acid	0	55	21	0
Trehalose+	0	63	21	18

resistance to penicillin (78.3) followed by chloramphenicol (41.6%), oxacillin (33.3%), ciprofloxacin (31.6%), gentamicin (28.3%), and erythromycin (25%). No resistance to vancomycin was seen (Table 5).

Table 5: Antibiotic sensitivity pattern# of CoNS; *n*=180

Antibiotic	Sensitive#	Intermediate	Resistant
Penicillin	39(21.6)	0	141(78.3)
Oxacillin	120(66.7)	0	60(33.3)
Ciprofloxacin	96(53.3)	27(15)	57(31.6)
Gentamicin	120(66.7)	9(5)	51(28.3)
Erythromycin	120(66.7)	45(25)	15(8.33)
Chloramphenicol	105(58.3)	0	75(41.6)
Vancomycin	180(00)	0	0

#Sensitivity pattern considered as mentioned in CLSI guidelines [17].

DISCUSSION

CoNS are generally considered normal inhabitants of skin and nares and are capable of causing only opportunistic infections, so many clinical laboratories do not identify clinical isolates of CoNS to the species level. As CoNS is increasingly being implicated as

Figure 1: Coagulase negative *Staphylococci* on blood agar plate

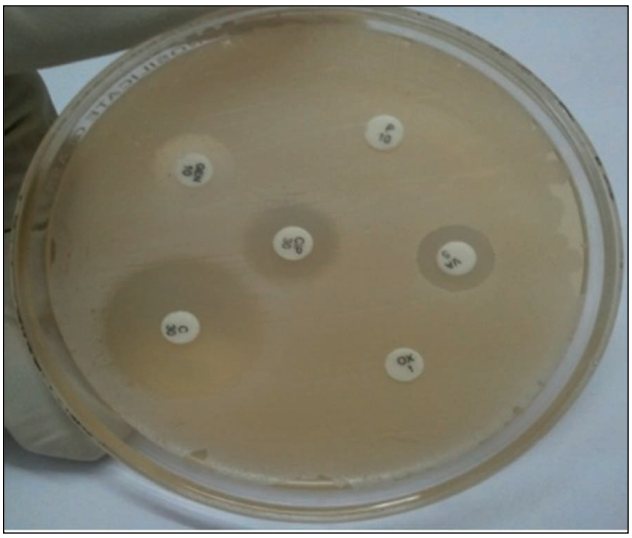

Figure 3: Antibiotic sensitivity test

Figure 2: Mannitol salt agar test, phosphatasetest

significant nosocomial pathogen, several reviewers have emphasized the need for species identification, which is possible only by a simple, easily adaptable, inexpensive method. The species identification is important in monitoring the reservoir and distribution of CoNS involved in nosocomial infections and determining the etiological agent.

The present scheme adapted by us conveniently identified the most frequently encountered clinical isolates in our hospital as *S. epidermidis* (43.3%), *S. hemolyticus* (35%), *S. hominis* (11.6%), and *S. lugdunensis* (10.0%).

Table 6: Comparison of CoNS isolates from different samples in different studies

Different studies	Total No. of isolates	Pus	Sputum	Wound swabs	Endotracheal secretions	Bronchial aspirates	Central lining tube
Present stud	180	105(58.33)	15(8.33)	33(18.33)	18(10)	6(3.33)	3(1.66)
Goyal *et al.*[1]	120	–	–	39(38.2)	–	–	–
Mohan *et al.*[6]	192	17.8%	–	–	–	–	–
Singh *et al.*[16]	150	36(24)	–	–	–	–	–
Ieven *et al.*[4]	444			48(10.81)	–	–	12.16
Gaikwad *et al.*[17]	352	76.92	–	–	–	–	–

Table 7: Comparison of different isolates of CoNS in different studies

	Total No. of isolates	*S. epidermidis*	*S. hemolyticus*	*S. hominis*	*S. lugdunensis*
Present study	180	78(43.3)	63(35)	21(11.6)	18(10)
Goyal *et al.*[1]	102	41%	14.7%	14.7%	–
Mohan *et al.*[6]	192	158(82.29)	3(1.56)	–	–
Singh *et al.*[16]	150	60(40)	18(12)	9(6)	9(6)
Ieven *et al.*[4]	444	304(69.1)	53(12)	39(8.9)	6(1.4)
Kawamura *et al.*[18]	1230	385(31.3)	150(12.2)	49(4.)	16(1.3)
Gaikwad *et al.*[17]	352	44.25%	–	–	–

Table 8: Antibiotic resistance % of CoNS isolates in different studies

	Total No. of isolates	P	OX	CF	G	E	C	VA
Present study	180	141(78.3)	60(33.3)	57 (31.6)	51(28.3)	15(25)	75(41.6)	0(0)
Goyal *et al.*[1]	120	–	–	29%	20%	23%		
Mohan *et al.*[6]	192	90.6%	–	51%	46.3%	–	40%	0(0)
Singh *et al.*[16]	150	120(80%)	57(38%)	81(54%)	42(28%)	81(54%)	48(32%)	0(0)

In our study, maximum number of CoNS isolates were from pus samples (58.33%). This is in comparison with the reports of Mohan *et al.* and Gaikwad *et al.* who also reported more number of isolates from pus samples. Other authors such as Singh *et al.* reported urine as common source of CoNS isolates (Table 6).

In the present study, *S. epidermidis* was most frequently isolated. This result is comparable with studies of Goyal *et al.*, Mohan *et al.*, and Gaikwad *et al*. The prevalence of next common isolate, *S. hemolyticus* in the present study is similar to reports of Goyal *et al.*, Singh *et al.*, Ieven *et al.*, and Kawamura *et al*. The isolation rate of *S. lugdunensis*was more in our study as compared to the studies of Singh *et al.*, Ieven *et al.* and Kawamura *et al.* (Table 7).

Antibiotic sensitivity testing of CoNS showed multidrug resistance to routinely used antibiotics (Figure 3), and variability in sensitivity and resistance patterns similar to the studies of Mohan *et al.*, Goyal *et al.*, and Singh *et al.* (Table 8).

CONCLUSION

The study revealed *S. epidermidis* is the predominant CoNS from endotracheal secretions and also pus samples. *S. hemolyticus* was isolated from pus and central lining tubes, *S. hominis* and *S. lugdunensis* were isolated mainly from wound swabs. Many CoNS isolates are true contaminants and do not require identification to the species level.

In our study, CoNS showed maximum sensitivity to erythromycin, and maximum resistance to penicillin.

The present study suggests that if CoNS are repeatedly isolated from patients with infection they should be taken seriously and antibiotic susceptibility tests done on these isolates for proper diagnosis and treatment especially in nosocomial infections.

RECOMMENDATIONS

It is important to monitor the CoNS infection in ICU patients, patients with central lining tubes, and patients with post-operative wounds. However, with an

economical, user-friendly methodology, rapid species identification of virtually all clinically significant CoNS becomes possible, leading ultimately to improved, clinically relevant predictions concerning CoNS isolates.

REFERENCES

[1] Goyal R, Singh NP, Kumar A, Kaur I, Singh M, Sunita N, *et al.* Simple and economical method for speciation and resistotyping of clinically significant coagulase negative staphylococci, Indian J Med Microbiol 2006;24:201–4.

[2] Kloos WE, Bannerman TL. Update on clinical significance of coagulase negative Staphylococci, Clin Microbiol Rev 1994;7:117–40.

[3] Goel MM, Singh AV, Mathur SK, Singh M, Singhal S, Chaturvedi UC. Resistant coagulase negative Staphylococci from clinical samples, Indian J Med Res 1991;93:350–2.

[4] Ieven M, Verhoeven J, Pattyn SR, Goossens H. Rapid and economical method for species identification of clinically significant coagulase negative Staphylococci, J Clin Microbiol 1995;33:1060–3.

[5] Archer GL, Climo MW. Antimicrobial susceptibility of coagulase negative Staphylococci, Antimicrob Agents Chemother 1994;38:2231–7.

[6] Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococci isolated from various clinical specimens, Indian J Med Microbiol 2002;20:45–6.

[7] Bodonaik NC, Moonah S. Coagulase negative staphylococci from blood culture contaminants or pathogens, West Indian Med J 2006;55(3):174–82.

[8] Archer GL, Climo MW. *Staphylococcus epidermidis* and other coagulase negative staphylococci. In: Mandel GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia, PA: Elsevier Churchill Livingston; 2005. pp. 2352–62.

[9] Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: a three-year analysis, Clin Infect Dis 1999;29:239–44.

[10] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study, Clin Infect Dis 2004;39:309–17.

[11] Betriu C, Redondo M, Boloix A, Gomez M, Culebras E, Picazo JJ. Comparative activity of linezolid and other new agents against Methicillin-resistant *Staphylococcus aureus* and teicoplanin intermediate coagulase negative *Staphylococci*, J Antimicrob Chemother 2001;48:911–3.

[12] *Staphylococcus*, *Micrococcus*, and similar organisms. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scotts Diagnostic Microbiology. 12th ed. Amsterdam, The Netherlands: Elsevier; 2007. pp. 254–64

[13] Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, *et al.* Koneman' scolor atlas and textbook of diagnostic microbiology, 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006. pp. 1443–71.

[14] Collee JA, Marmion BP, Fraser AG. Simmons Antony Mackie & McCartney practical medical microbiology, 14th ed. India: Churchill Livingstone; 2006. pp. 245–58.

[15] Performance standards for antimicrobial disc susceptibility tests; Approved Standard-Eleventh Edition Clinical and Laboratory Standards Institute; International organization of Standardization Technical Committee 212 approved and draft standards, 2012:32(1):1–76. Available from: http://clsi.org/standards/micro/M02-A11.

[16] Singh S, Banerjee G, Agarwal SK, Kumar M, Singh RK. Simple method for speciation of clinically significant CoNS and its antibiotic sensitivity/resistant pattern in NICU of tertiary care centre, Biomed Res 2008; 19 (2): 97-101.

[17] Gaikwad SS, Deodhar LP. Study of coagulase-negative Staphylococci in clinical infection, J Postgrad Med 1983;29:162–4.

[18] Kawamura Y, Hou XG, Sultana F, Hirose K, Miyake M, Shu S-E, *et al.* Distribution of *Staphylococcus* species among human clinical specimens and emended description of *Staphylococcus caprae*. J Clin Microbiol 1998;36(7):2038–42.