Manipal Journal of Medical Sciences

Volume 1 | Issue 1 Article 6

6-30-2016

The Changing Face of Coagulase-Negative Staphylococci: Diagnostic And Therapeutic Challenges

Shreya Singh
Senior Resident, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, dhawanb@gmail.com

Sujeesh Sebastian
Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi, dhawanb@gmail.com

Benu Dhawan
Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi, dhawanb@gmail.com

Review article

The Changing Face of Coagulase-Negative Staphylococci: Diagnostic And Therapeutic Challenges

Shreya Singh, Sujeesh Sebastian, Benu Dhawan*
Email: dhawanb@gmail.com

Abstract

Coagulase negative Staphylococci (CoNS) are now recognized as the etiological agents of an important range of infections in humans. The increasing importance of CoNS may be due in part to the growing appreciation of this group of organisms as opportunistic pathogens and to the increase in the use of medical devices in seriously ill and immunocompromised patients. The ability of biofilm formation seems to play an essential role in their virulence. CoNS are reservoirs of resistance genes. The similarity of SCCmec regions among *Staphylococcus aureus* and CoNS suggests horizontal transfer between species of Staphylococci. Hence the methicillin resistant CoNS which are also multidrug resistant act as a reservoir for drug resistance in hospitals. The increasing recognition of pathogenic potential of various species of CoNS and emergence of drug resistance amongst them denotes the need for better laboratory procedures to identify them along with continuous surveillance of their antimicrobial susceptibility pattern. Promptness in the detection of resistance pattern is of key importance to ensure appropriate antibiotic treatment in infected patients as well as control the spread of resistance in hospital environments. The usage of newer antimicrobial agents must be limited for the treatment of resistant and life threatening CoNS infections.

Key words: Coagulase negative Staphylococci, antimicrobial resistance, biofilm, Staphylococcal cassette chromosome

Introduction

Coagulase-negative Staphylococci (CoNS) are ubiquitous colonizers of skin and mucous membranes of human beings. In the past, when isolated from clinical specimens, CoNS were often discarded as contaminants. However, this has changed over the last decade with CoNS emerging as etiological agents of various hospital acquired infections. Advancement in medicine leading to the survival of elderly, multi-morbid and immunocompromised patients, and the increasing use of foreign medical devices has contributed to the pathogenic role of CoNS in health care settings.

A steady rise in resistance of CoNS isolates to multiple antibiotics is of concern, limiting the treatment options. The presence of mobile resistance genes and their horizontal transfer to drug sensitive *Staphylococcus* species necessitates the accurate diagnosis and treatment of CoNS infections.

Most CoNS infections are hospital acquired with the most important clinical entity being foreign body-related infections (FBRIs), also designated device-associated health care-associated infections (DA-HAIs). The CoNS species commonly encountered in clinical specimens include *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus* and *Staphylococcus lugdunensis*.

The present paper reviews current insights on taxonomy, clinical syndromes, pathogenic potential

Shreya Singh
Senior Resident, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh

Sujeesh Sebastian
PhD Scholar, Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi

Benu Dhawan
Professor, Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi

**Corresponding Author*

How to cite this article: Singh S, Sebastian S, Dhawan B. The changing face of coagulase-negative staphylococci: diagnostic and therapeutic challenges. *MJMS*. 2016; 1(1): 29-37

and epidemiology of medically important CoNS. In addition, the recent developments in diagnosis and treatment of CoNS infections are summarized.

Taxonomy

The Staphylococci belongs to the Phylum Firmicutes, order Bacillales, class Bacilli and family Staphylococcaceae. More than 47 species and 23 subspecies have been described by 2015.[1] Currently a simplified scheme divides Staphylococci into Coagulase positive Staphylococci (CoPS) and Coagulase negative Staphylococci or CoNS (Fig.1). A refined classification based on molecular data has been proposed which divides the genus into 6 species groups: Epidermidis–Aureus group, Hyicus-Intermedius group, Auricularis group, Saprophyticus group, Simulans group and Sciuri group. [2]

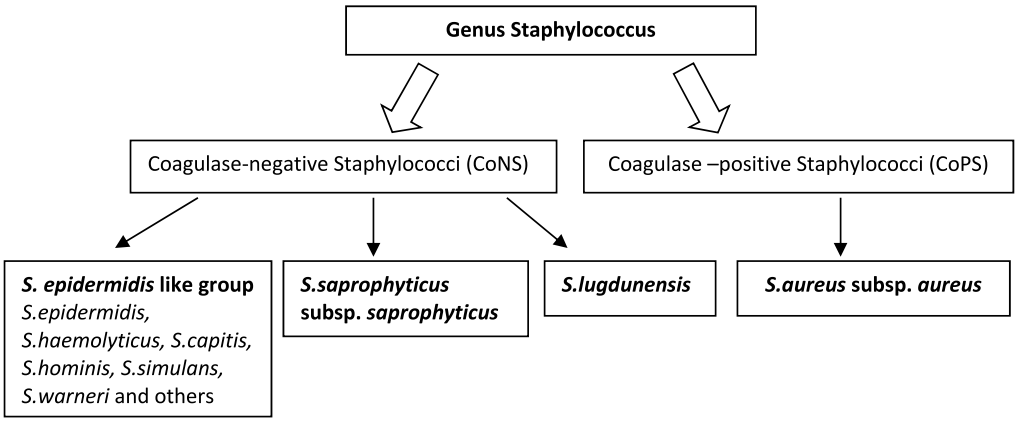

Fig 1: Classification of Human associated Staphylococcal species, based on the categorization of coagulase CoNS as part of Human Micro biota

Depending on the anatomic site, the population of CoNS in healthy human skin or mucous membranes may reach densities of 10^1 to 10^6 colony-forming units (CFU)/cm^2. [3]Recent evidence has shown that a *S. epidermidis* protease has the ability to inhibit biofilm formation by *Staphylococcus aureus*, suggesting that some strains of *S. epidermidis* may be important in the inhibiting S.*aureus* biofilm formation and nasal colonization.[4,5]

Differences in skin thickness, hair follicle and gland density define the distinct habitats of various CoNS species. Age-related changes in colonization may also occur. *S. epidermidis* is the most frequently recovered species. *S. haemolyticus* and *S. hominis* are isolated preferentially from axilla and pubic areas with high apocrine gland density. *S. capitis*is found surrounding sebaceous glands on the forehead and scalp following puberty. [6] *S. auricularis* is exclusively a part of external ear microbiota while *S. lugdunensis* is found particularly in the groin area, on the lower extremities and axilla.[7] *S. saprophyticus* colonizes the rectum or urogenital tracts of approximately 5 % to 10% women of childbearing age preferentially in summers.[8,9]

Pathogenesis

1. Biofilm formation: Development of a multilayered biofilm has been considered as the most critical step in the pathogenesis of FBRI's caused by CoNS. Large bacterial cell agglomerates encased in an extracellular amorphous material made of bacterial and host products constitute a biofilm.[10-13]They can be formed on abiotic surfaces (medical devices) or on biotic surfaces (host tissue). Varied physiologic states of bacteria found within a biofilm i.e. aerobic growth, anaerobic growth, dormantand dead cells provide tolerance to antibiotics. The formation of biofilm occurs in 4 stages- adherence, multiplication, maturation and dispersal.

Adherence: For attachment members of the genus Staphylococci produce :

- Proteinaceous adhesions - Noncovalently linked surface-associated proteins like AtlE an autolysin/ adhesin from *S.epidermidis* mediates initial adherence especially to polystyrene. It hydrolysis of cell wall peptidoglycan releasing eDNA, an important component of biofilms. Homologous autolysins Aae of S. epidermidis, Aas from *S.saprophyticus* and AtlC from *S.caprae* have also

been shown to bind to fibronectin and to abiotic surfaces. ClpP protease identified in *S.epidermidis* is involved in biofilm formation, stress adaptation and virulence. Another bifunctional surface molecule with both autolysin/ adhesion activity is GehD lipase found in *S. epidermidis* mediating attachment to collagen.

- Covalently linked surface proteins like biofilm – associated protein (Bap) described in *S.epidermidis*, *S. haemolyticus* and *S. cohnii* mediates attachment and the accumulation of biofilm. Staphylococcal surface proteins – SSP-1 and Staphylococcal surface proteins-SSP-2 contribute to the adherence.[14] Accumulation associated protein (Aap) supports adherence to corneocytes and has a role in skin colonization.
- Nonproteinaceous adhesins - Polysaccharide intercellular adhesin (PIA) (also known as poly-*N* –acetyl glucosamine (PNAG) is associated with initial adherence and slime production. Wall techoic acid (WTA) and Lipotechoic acid (LTA) determine the surface charge contributing to initial colonization on abiotic surfaces by acting as a bridge between fibrinectin coated biomaterial and the bacteria.

Accumulation and maturation: After primary attachment to biotic or abiotic surfaces, bacteria multiply and accumulate in multilayered cell aggregates. Polysaccharide adhesins producedby the icaADBC operon- IcaA has glycosyl transferase activity and directing the synthesis of β-1, 6-linked GlcNAc oligosaccharides. IcaD encodes a chaperone directing the folding and membrane insertion of IcaA linking IcaA with IcaC. IcaC is involved in elongation and externalization of the growing polysaccharide. [15]IcaB has polysaccharide deacetylase activity crucial for PIA activity in biofilm formation.Proteinaceous adhesins such as Bap and Aap also play a role in intercellular adhesion.

Detachment: Following the maturation of biofilms, bacteria disintegrate and disperse via the bloodstream. This may be mediated by different mechanisms, such as a variety of extracellular enzymes such as protease, sugar hydrolases and nucleases or the phenol-soluble modulins (PSMs) which have surfactant like action.

Clinical Significance of CoNS

Overall, *S.epidermidis* is the most common species in CoNS infections, followed by *S.haemolyticus*, *S.hominis* and *S.capitis*. *S.lugdunensis* also being increasingly reported as the common pathogenic species. Although CoNS cause a wide variety of clinical infections, most of them are related to foreign bodies and prosthetic medical devices.

Blood Stream Infections (BSI): CoNS are the most common cause of nosocomial BSI, responsible for 30% to 40% of these infections.[16] Most CoNS BSI's result from infected catheters, commonly from CoNS colonizing patient's skin, migrating via the surface of the catheter to gain access to the blood stream.

CoNS of the *S. epidermidis* group are the most frequent agents of central venous catheter (CVC)- and umbilical catheter associated BSIs in neonatal ICUs [17]and in neutropenic patients, accounting for approximately 20 - 40% of cases. [18-21] Immunosuppressed patients, neonates, oncology patients particularly those with severe neutropenia, are at high risk of CoNS BSI.

Endocarditis: CoNS are major pathogens infecting prosthetic vascular grafts, prosthetic heart valves, cardiac devices, and coronary stents. They frequently cause prosthetic valve infective endocarditis (PVIE), being responsible for 37 to 47% of early cases and about 25% of late cases, followed by *S.aureus*. [22] The typical course of IE due to CoNS is chronic and features are subtle however, International Collaboration on Endocarditis merged database has shown that heart failure was encountered significantly more frequently with CoNS (54%) than with either *S.aureus* (33%) or viridans group Streptococci. [23]

Central nervous system Shunt Infections: CoNS are the most common bacteria isolated form cerebrospinal fluid shunt infections (48% to 67% isolates) with *S.epidermidis* as the predominant species.[24]They are introduced most likely during shunt system implantation or subsequent revisions and manipulations. Clinical manifestations can be nonspecific and may include fever and /or signs of shunt malfunction. Removal of the shunt is usually necessary.

Surgical site infections: CoNS mainly cause superficial incisional infections. The major risk factors include long duration of the surgical procedure, host factors and experience of the surgeon and surgical staff.

Urinary Tract Infection: *S.saprophyticus* subsp. *Saprophyticus* is a frequent agent implicated in uncomplicated lower UTI in young, sexually active women but there are reports of its association with UTIs in young girls and males. Complications include acute pyelonephritis and nephrolithiasis, urethritis, epididymitis and prostatitis.

Prosthetic Joint Infections: Infection is generally initiated at the time of the arthroplasty and owing to their relatively avirulent nature, may be quite indolent in presentation. CoNS prosthetic joint infections (PJI's) are usually caused by *S.epidermidis* with a few cases caused by *S.lugdunensis* or other CoNS species.[25] Risk factors include previous joint surgery, long duration of surgery, another infection at the time of surgery and rheumatoid arthritis.

Vascular Graft Infections: Prosthetic vascular infection incidence ranges from 1% to 6%, with Infra-inguinal grafts from the groin having the highest rates of infection. CoNS are a common cause of these infections, which may occur within 30 days of surgery, but are common months or years after implantation with mortality rate of 17%.[25]

Endophthalmitis: Following penetrating eye injury and after vitrectomy or cataract surgery CoNS frequently cause infections, with rates as high as 15% -73%. *S.epidermidis* is the most common species isolated with the patient's own flora being the primary source. [25, 26]

CoNS Prevalence & Species distribution: **Indian Scenario**

Various Indian studies from 1993 onwards have shown a rising trend in CoNS infections, from 2.6 % to 39.5%.[27-38] Among the different species isolated, *S.epidermidis* was the predominant followed by S.*haemolyticus*, *S.saprophyticus*, *S.hominis*, *S.capitis and S.lugdunensis*. Methicillin resistance in these studies varied from 18.44% to 67.7%. (Table.1)

Laboratory Detection and identification

The most challenging issue in CoNS diagnosis to assess whether the isolated CoNS species is a contaminant, part of normal flora or a true pathogen. An evaluation of their clinical importance requires close cooperation between clinicians and microbiologists.

Isolation

Routinely, for isolation of Staphylococci from clinical specimens, Columbia or tryptic soy broth containing 5% sheep blood is used. Growth usually occurs within 18 to 24 hours but in case of Small colony variants (SCV) 48 to 72 hrs incubation is recommended.

Procedures for detection of Foreign body related infections

1. Catheter related- blood stream infections (CRBSIs).[39]

With catheter removal

a. Semi-quantitative Maki roll-plate culture technique: the distal segment of the central venous catheter be cut and rolled across the surface of a Columbia blood agar plate at least four times. After overnight incubation, a colony count of >/=15 CFU/plate may indicate catheter colonization.[40]
b. Sonication and/ or vortexing :detachment of biofilms from different implants can be improved by this method.

Without catheter removal

a. Time to positivity: On examination of paired quantitative blood cultures drawn simultaneously from the catheter and a peripheral vein, Central line associated blood stream infection (CLABSI) is probable if the catheter sample becomes positive first and the time difference between both samples samples is 2 h.
b. Paired Quantitative culture: A difference in count of catheter and peripherally collected blood sample by > 5-10 indicates CLABSI.
c. Endoluminal brush: It can be used for in situ diagnosis of CRBSI and hence no line sacrifice is needed.

Table 1. Prevalence, species distribution and Methicillin resistance of CoNS in Indian studies

Study (Year)	**Shrikhande** et al [27] **1996**	**Mohan** et al [28] **2002**	**Jain** et al [29] 2004	**Manikandan** et al [30] **2005**	**Goyal** et al [31] **2006**	**Singhal** et al [32] **2006**	**Singh** et al [33] **2009**	**Sharma** et al [34] **2010**	**Surekha** et al [35] **2011**	**Usha** et al [36] **2013**	**Priya** et al [37] **2014**	**Dhawan** et al [38] **2014**
Samples	Various Samples*	Various samples	Blood and skin	Corneal ulcer	Various samples	Various samples	Various samples	Various samples	Various samples	Various samples	Ocular samples	Various samples
No. of Isolates	103	192	100	35	102	83	100	300	96	102	100	128
S.epidermidis (%)	17.5	82.3	24	57.1	41	33.7	40	54	44.8	32	43	17.9
S.haemolyticus (%)			34	2.8	14.7	13.3	12	40	19.7	18	10	35.9
S.saprophyticus (%)	6.7	15.6	8	2.8	16.6		20	2.3	27.1	8		1.5
S.capitis (%)	1.9		1		1.9	9.6	4			6	16	8.5
S.cohnii (%)	1.9		6					10	1	3		
S.hominis (%)	8.7		4	22.8	14.7	2.4	6			10	5	
S.lugdunensis (%)				5.7	4.9	13.3	6		2.1	12		
S. intermedius (%)	1.9											0.8
S. xylosus (%)						5.6				4	8	
S.schleiferi subsp. Coagulans (%)												14.8
S.schleiferi subsp. Schleiferi (%)					1.9	12.1	2					10.3
S.warneri (%)			3			1.2	6	2.67		3		9.3
S.simulans (%)			1	5.7								
S.sciuri (%)			2									
S.caprae (%)										4		
Methicillin Resistance(%)	18.5	20	66			62.7	54	52	67.7			34.4

*various samples: Blood, bone, sterile body fluids, pus, urine and tissue

2. Implant associated infections: sonication prior to culture increases chances of isolation, particularly in patients who have been on antibiotics 2 weeks prior to implant surgery.[41]
3. Ocular examination and gram stain followed by culture of sample obtained by vitrectomy can be used for diagnosis of endophthalmitis.

Identification

Direct examination: CoNS are Gram-positive, nonmotile, non-spore forming usually arranged singly, in pairs, tetrads, in irregular (grape –like) clusters, or in short chains of three or four cells.

Colony characteristics: On Sheep blood agar colonies are usually nonpigmented, smooth, entire margins, glistening, and opaque. Some CoNS species display a hazy or distinct zone of beta-haemolysis around the colonies. SCVs of CoNS have pinpoint colonies reaching only 10% of the wide-type colony size.

Biochemical characterization: Kloos and Schleifer published a scheme for the biochemical identification of CoNS in 1975.[42] In this scheme, coagulase test and a variety of biochemical tests are used for differentiating the CoNS species. Although the tube coagulase test is still used, the usefulness of detecting clumping factor by the slide agglutination test is obsolete due to low sensitivity and specificity.[1] These biochemical tests are time consuming and labor-intensive, but a little extra effort can easily help in the speciation of CoNS, especially in laboratories where automated or molecular techniques are not available.

Amplification-based assays: Commonly used universal target genes for CoNS includes ribosomal genes (16S and 23SrRNA), glyceraldehyde-3-phosphate dehydrogenase- gene (*gap*), RNA polymerase beta subunit gene (*rpoB*) and gyrase gene (*gyrA*).

Spectroscopic and spectrometric methods for CoNS identification: Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is rapid, high

throughput and most studies have demonstrated specificities of >97% for CoNS speciation.[43,44]

Antimicrobial resistance

During the 1970s it was seen that methicillin resistance was more prevalent in CoNS than in MRSA, an observation that continues to be true today. Nearly 60% of CoNS show methicillin resistance (MRCoNS). *S. haemolyticus* strains show highest resistance followed by *S. epidermidis*, *S. lugdunensis* and *S. hominis* strains. [45, 47]

Indian studies have demonstrated a resistance of 20 - 66% and increasing trends of MRCoNS in neonatal septicaemia were observed in South India over a period of 3 years with prevalence MRCoNS rising from 41.57% to 57.36%. Coresistance to different classes of antibiotic is higher in MRCoNS than MSCoNS, particularly ciprofloxacin, norfloxacin, gentamicin, nitrofurantoin, erythromycin and Amikacin. [28, 29, 34, 48, 49]

In Staphylococci, methicillin resistance is mainly due the expression of the *mec*A gene, which specifies penicillin binding protein 2a (PBP2a), a transpeptidase with low affinity for β-lactams.. The detection of the *mec*A gene in Staphylococci is the gold standard for detecting methicillin resistance. Amongst the various phenotypic methods used, cefoxitin disc diffusion test is considered as a surrogate marker of methicillin resistance.

SCC*mec* typing

The *mec*A gene is a part of the mobile genetic element Staphylococcal cassette chromosome *mec* (SCC*mec*), which acts as vehicle for horizontal transfer of antibiotic resistance genes from methicillin-resistant CoNS (MR-CoNS) to organisms like *Staphylococcus aureus*. [50] Hospital-acquired infection associated MR-CoNS serve as a reservoir of genetically diverse SCC*mec* types, each being associated with a different antibiotic resistance pattern. Thus, molecular characterization by SCC*mec* typing of MR-CoNS is an essential epidemiological tool for studying the evolution of these genetic elements and providing useful information regarding the antibiotic resistance pattern in Staphylococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) and MR-CoNS strains can be described in terms of the SCC*mec* composition. Till date, 11 types and several subtypes of SCC*mec* have been reported. In CoNS the most prevalent types are SCC*mec* types III, IV, and V, either alone or in various combinations. [1] Limited data on diversity of SCC*mec* types in CoNS is available from India. Few studies have shown the predominance of type I with it constituting approximately 50% of all MR-CoNS.[38, 51]

S. haemolyticus is the first CoNS species in which vancomycin and teicoplanin resistance was identified. [52] Despite extensive, vancomycin use the majority of CoNS isolates have been shown to still be susceptible. [53, 54] Recent reports however, of CoNS with linezolid resistance and decreased susceptibility to vancomycin have alarmed the medical community. [55]

3.8 Treatment and management

For CoNS, scant data is available regarding treatment guidelines and most recommendation are based on limited studies and some case reports for the treatment of *S. aureus* infections. Thus, a careful risk-benefit assessment is mandatory if combination therapy is applied.Therapeutic options for the treatment of CoNS are limited because the vast majority of clinically recovered isolates are methicillin resistant. Thus, most infections by CoNS of the *S. epidermidis* group require treatment with a glycopeptide.

Replacement of vancomycin by β-lactamase resistant penicillins, cotrimoxazole or cephalosporins (first or second generation) is advised for methicillin-susceptible strains. New antibiotic agents, such as daptomycin, cephalosporins with MRSA activity, Arbekacin, streptogramins, and linezolid are new antibiotics being evaluated as alternatives to glycopeptides.

Whenever an FBRI is suspected, decisions of whether to remove the colonized foreign body and/or to initiate empirical antimicrobial treatment to salvage the device have to be made.This is still a matter of debate because of the lack of controlled study data. Polyclonal and monoclonal antibodies have been developed against the cell-wall components

of Staphylococcus and may have role in immune prophylaxis and treatment of CoNS infection.

To conclude, CoNS will likely remain a major cause of infections, evolve further resistance mechanisms and require development of newer antimicrobials.

References

1. Karsten Becker, Christine Heilmann and Georg Peters. Coagulase-Negative Staphylococci. Clin Microbiol Rev 2014; 27(4):870.
2. Lamers RP, Muthukrishnan G, Castoe TA, Tafur S, Cole AM, Parkinson CL. Phylogenetic relationships among Staphylococcus species and refinement of cluster groups based on multilocus data. BMC Evol Biol 2012;12:171.
3. Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas and Bennett's Principles and practice of infectious diseases. 8 th edition 2015;2273
4. Suginoto S, Iwamoto T, Takada K, et al. Staphylococcus Esp degrades specific proteins associated with Staphylococcus aureus biofilm formation and host pathogen interaction.J Bacteriol 2013;195:1645-55.
5. Iwase T, Uehara Y, Shinji H, et al. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature 2010;20:346-9.
6. Kloos WE and Schleifer KH. 1975. Isolation and characterization of staphylococci from human skin. Description of four new species: Staphylococcuswarneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcussimulans. Int. J. Syst. Bacteriol. 25:62-79.
7. Bieber L, Kahlmeter G. Staphylococcus lugdunensis in several niches of the normal skin flora. Clin Microbiol Infect 2010;16:385-8.
8. Nys S, van Merode T, Bartelds AI, Stobberingh EE. Urinary tract infections in general practice patients: diagnostic tests versus bacteriological culture. J Antimicrob Chemother 2006;57:955-8.
9. Rupp ME, Soper DE, Archer GL. Colonization of the female genital tract with Staphylococcus saprophyticus. J Clin Microbiol 1992;30:2975-2979.
10. Hussain M, Wilcox MH, White PJ. The slime of coagulasenegative staphylococci: biochemistry and relation to adherence. FEMS Microbiol Rev 1993;10:191-207.
11. Sadovskaya I, Vinogradov E,Flahaut S, Kogan G, Jabbouri S. Extracellular carbohydrate-containingpolymersof amodelbiofilmproducing strain, Staphylococcus epidermidis RP62A. Infect Immun2005;73:3007-17.
12. Schommer NN, Christner M, Hentschke M, Ruckdeschel K, Aepfelbacher M, Rohde H. Staphylococcus epidermidis uses distinct mechanisms of biofilm formation to interfere with phagocytosis and activation of mouse macrophage-like cells 774A.1. Infect Immun2011;79: 2267-76.
13. Izano EA, Amarante MA, Kher WB, Kaplan JB. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Appl Environ Microbiol 2008;74:470-6.
14. Veenstra GJ, Cremers FF, van Dijk H, Fleer A. Ultrastructural organization and regulation of a biomaterial adhesin of Staphylococcus epidermidis. J Bacteriol1996; 178:537-41.
15. Gerke C, Kraft A, Sussmuth R, Schweitzer O, Götz F.Characterization of the Nacetylglucosaminyltransferase activity involved in the biosynthesis of the Staphylococcus epidermidis polysaccharide intercellular adhesin. J BiolChem 1998; 273:18586-93.
16. Rupp ME. Nosocomial bloodstream infections. In: Mayhall CG, editor. Hospital epidemiology and infection control, 3rd Edition, vol. 1. Philadelphia: Lippincott Williams and Wilkins; 2004: 253-66.
17. Yalaz M, Altun-Köroglu O, Ulusoy B, Yildiz B, Akisu M, Vardar F, O¨ zinel MA, Kültürsay N. Evaluation of device-associated infections in a neonatal intensive care unit. Turk JPediatr 2012;54:128-35.
18. Horasan ES, Ersoz G, Tombak A, Tiftik N, Kaya A. Bloodstream infections and mortality-related factors in febrile neutropenic cancer patients. Med SciMonit 2011;17:CR304-CR309.

19. González-Barca E, Fernández-Sevilla A, Carratalá J, Grañena A, Gudiol F.Prospective study of 288 episodes of bacteremia in neutropenic cancer patients in a single institution. Eur J Clin Microbiol Infect Dis 1996;15:291-6.
20. Cherif H, Kronvall G, Björkholm M, Kalin M.Bacteraemia in hospitalised patients with malignant blood disorders: a retrospective study of causative agents and their resistance profiles during a 14-year period without antibacterial prophylaxis. Hematol. J 2003;4:420-6.
21. Rubio M, Palau L, Vivas JR, del Potro E, Diaz-Mediavilla J, Alvarez A, Martinez R, Picazo JJ. Predominance of gram-positive microorganisms as a cause of septicemia in patients with hematological malignancies. Infect Control Hosp Epidemiol 1994;15:101-4.
22. Alonso-Valle H, Fariñas-Alvarez C, García-Palomo JD, Bernal JM, Martín-Duran R, Gutiérrez Díez JF, Revuelta JM and Fariñas MC. Clinical course and predictors of death in prosthetic valve endocarditisover a 20-year period. J ThoracCardiovasc Surg 2010; 139: 887-93.
23. Lalani T, Kanafani ZA, Chu VH, Moore L, Corey GR, Pappas P, Woods CW, Cabell CH, Hoen B, Selton-Suty C, Doco-Lecompte T, Chirouze C, Raoult D, Miro JM, Mestres CA, Olaison L, Eykyn S, Abrutyn E, Fowler VG, Jr. 2006. Prosthetic valve endocarditis due to coagulase-negative staphylococci: findings from the International Collaboration on Endocarditis Merged Database. Eur. J. Clin. Microbiol. Infect. Dis. 25:365-8.
24. Roos KL and WM Scheld. Central nervous system infections. The Staphylococciin Human Disease, ed. KB Crossley, GL Archer, New York: Churchill Livingston. 1997; 1:413-39.
25. Kathie RL, Paul DF and Rupp EM. Coagulase-Negative Staphylococcal Infections. Infect Dis Clin NAm 2009; 23: 73-98.
26. Karacal H, Kymes SM and Apte RS. Retrospective analysis of etiopathogenesis of all cases of endophthalmitis at a large tertiary referral center. Int Ophthalmol2007; 27: 251-9.
27. Shrikhande S, Thakar YS, Pathak AA, Saoji AM. Species distribution of clinical isolates of Staphylococci. Indian J Pathol Microbiol 1996;39:207-10.
28. 28.Mohan U, Jindal N and Aggarwal. P. Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococci isolated from various clinical specimens. Ind Journ of Med Micro.2002; 20: 45-46.
29. Jain A, Agarwal J and Bansal S. Prevalence of methicillin-resistant, coagulasenegative staphylococci in neonatal intensive care units: findings from a tertiary care hospital in Ind J of Med Micro. 2004; 53: 941-4.
30. Manikandan P, Bhaskar M, Revathy R, Jhon RK, Narendran K, Narendran V. Speciation of coagulase negative Staphylococcus causing bacterial keratitis. Indian J Ophthalmol 2005;53:59-60.
31. Goyal R, Singh NP, Kumar A, Kaur I, Singh M, Sunita N, Mathur M. Simple and economical method for speciation and resistotyping of clinically significant coagulase negative staphylococci. Indian J Med Micro. 2006;24:201-4.
32. Singhal R et al. Species distribution & antimicrobial susceptibility of Coagulase negative staphylococci in a tertiary care hospital. Indian J Med Res. 2006; 123: 569-70.
33. Singh S, Banerjee G et al. Prevalence of mec A gene positive Staphylococci in NICU of a tertiary care hospital. Biomedical Research.2009;20:94-98.
34. Sharma V, Jindal N and Devi P. Prevalence of methicillin resistant coagulase negative staphylococci in a tertiary care hospital. Iran. J. Microbiol. 2010; 2: 185-88.
35. Surekha YA , Mariraj J , Sathyanarayan MS , Nagabhushan and Rashmi. Speciation of clinically significant Coagulase Negative Staphylococci and their antibiotic resistant patterns in a tertiary care hospital.Int J Biol Med Res. 2011; 2: 735-39.
36. Usha MG, Shwetha DC and Vishwanath G. Speciation of coagulase negative Staphylococcal isolates from clinically significant specimens and their antibiogram.Ind J of Path and Micro. 2013; 56: 258-60.

37. Priya R, Mythili A et al. Virulence, speciation and antibiotic susceptibility og ocular coagulase negative Staphylococci (CoNS). Journal of Clin and Diag Res. 2014; 8:33-37.
38. Dhawan B et al. Staphylococcal Cassette Chromosome mec(SCCmec) Typing of Clinical Isolates of Coagulase-Negative Staphylococci (CoNS) from a Tertiary Care Hospital in New Delhi. Ind J Med Res. 2014 (in press).
39. Betty A Forbes, Daniel F Sahm, Alice S Weissfeld. Bailey & Scott's Diagnostic Microbiology. 13 th Edition.2014
40. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. N Engl J Med 1977;296:1305-9.
41. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, Mandrekar JN, Cockerill FR, Steckelberg JM, Greenleaf JF, Patel R. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007.357:654-63.
42. Kloos WE, Schleifer KH. Simplified scheme for routine identification of human Staphylococcus species. J Clin Microbiol1975;1:82-8.
43. Carbonnelle E, Beretti JL, Cottyn S, Quesne G, Berche P, NassifX,Ferroni A. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization- time of flight mass spectrometry. J Clin Microbiol2007;45:2156-61.
44. Dubois D, Leyssene D, Chacornac JP, Kostrzewa M, Schmit PO, TalonR, Bonnet R, Delmas J. Identification of a variety of Staphylococcus species by matrix-assisted laser desorption ionization-time of flight massspectrometry. J Clin Microbiol 2010;48:941-5.
45. Joseph F John and Alexander M Harvin. History and evolution of antibiotic resistance in coagulase-negative staphylococci: Susceptibility profiles of new anti-staphylococcal agents .Ther and Clin Risk Manag 2007; 3 :1143-52.
46. Sahm DF, Marsilio MK and Piazza G. Antimicrobial resistance in key bloodstream bacterial isolates: electronic surveillance with the surveillance network database-USA. Clin Infect Dis. 1999; 29: 259-63.
47. Pereira VC, Lourdes M and Cunha RS.Coagulase-negative staphylococci strains resistant to oxacillin isolated from neonatal blood cultures. Mem Inst Osw Cruz. 2013; 108: 939-42.
48. Deepa S, Kumari AB, Venkatesha D. Increasing Trends of Methicillin Resistant Coagulase Negative Staphylococcus in Neonatal Septicaemia - A Study in a Tertiary Care Hospital, Mysore, South India. On J Health Allied Scs. 2010; 9: 11.
49. Jain A, Agarwal A, Verma RK, Awasthi S and Singh KP. Intravenous device associated blood stream staphylococcal infection in paediatric patients. Indian J Med Res. 2011; 134: 193-199.
50. Hanssen AM and Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immuno Med Micro. 2006; 46: 8-20.
51. Saravanan et al.: Prevalence of SCCmec types among Methicillin resistant Coagulase negative Staphylococci isolated from HIV patients in Chennai, South India. BMC Infectious Diseases 2014 14(Suppl 3):P51.
52. Schwalbe RS, Stapleton JT and Gilligan PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. N Engl J Med.1987; 316: 927-31.
53. Sader HS and Jones RN.Antimicrobial activity of daptomycin in comparison to glycopeptides and other antimicrobials when tested against numerous species of coagulase-negative Staphylococcus. Diagn Microbiol Infect Dis. 2012; 73:212-4.
54. Natoli S et al. Characterization of coagulase-negative staphylococcal isolates from blood with reduced susceptibility to glycopeptides and therapeutic options. BMC Infect Dis. 2009; 9:83.
55. Gu B, Kelesidis T, Tsiodras S, Hindler J and Humphries RM. The emerging problem of linezolid-resistant Staphylococcus. J Antimicrob Chemother. 2013; 68: 4-11.