Review Article

Coagulase Negative Staphylococci (CONS): A review

Abstract:

Coagulase-negative staphylococci (CoNS) has gain more importance as pathogenic organism ~~in recent~~in recent years as causative organism for infections in both human and animals. CONS ~~are specially~~are especially prevalent in immunocompromised patients~~-~~, critically ill patients and~~,~~ patients having invasive medical devices .

The incidence of CoNS varied across different geographic ~~locations in~~locations in humans and animals. Also, there is varying antibiotic resistance patterns observed in CoNS species, with high methicillin resistance and cross resistance against many antibiotics. *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus xylosus* are the most commonly reported species in various studies. Various virulence factors in CONS are responsible ~~for enhanced~~for enhanced pathogenicity. Because of advancement in diagnostic techniques, understanding of molecular mechanisms of CONS pathogenicity is possible. ~~Recent advances~~Recent advances in identification and typing methods, virulence screening ~~methods will~~methods will help to assess true pathogenic potential of CoNS species.

Keywords: CONS, speciation, Infection

Introduction

Coagulase-negative Staphylococci (~~CONS) classified~~CONS) classified as mere contaminants, are becoming clinically relevant ~~clinically~~ because of widespread of antibiotic resistance, biofilm formation and increased use of medical devices such as …. As there is marked species ~~diversity in~~diversity in CONS, there is need for increased laboratory capacity for effective speciation .

Coagulase-negative Staphylococci (CONS) are normal flora of human skin and mucous membranes, they have previously been considered nonpathogenic or contaminant having little clinical significance. [1].But now they have been considered as significant potential pathogen responsible for hospital acquired infection because ~~of widespread~~of widespread antibiotic resistance and increasing use of medical devices and occurs specially in immunocompromised patients and patients having indwelling devices. [1]

Because of bioflim formation on medical devices, maximum of hospital acquired infections are caused by CONS. Biofilm formation also increases the resistance to

Formatted: Highlight

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Highlight

Formatted: Highlight

antimicrobial agents and host defense mechanisms and because of that, it is very difficult to eradicate biofilm associated infections by conventional antibiotic treatment [1,2].

Milestones in CONS:

Table1. Milestones in CONS.

Year	Scientists	Milestones
1884	Rosenbach	First described CONS as Staphylococcus albus, an avirulent Staphylococcus [3].
1958	Smith and coworkers	First reported pathogenicity of CONS in patients with septicemia[3].
1965	Wilson and Stuart	Identified CONS in pure culture form [4].
1962	Pereira	UTIs were caused by certain group of CONS which is now known as S. saprophyticus [5].
1971	Pulverer and Pillich(Cologne, Germany)	Investigated pyogenic infections in Cologne, Germany and reported 10% infections were due to CONS and CONS were found in pure culture[6].
1971	Holt	Reported that CONS were responsible for colonization of ventriculoatrial shunts followed by septicemia[7].

Development in classification of Staphylococci have made clinicians more aware of various CONS species present in clinical specimens and as etiological agents. [8].

Table 2 shows various Staphylococcal species and subspecies.

Comment [U1]: Scientific names not properly written
All tables should be referred in text

Table2. staphylococcal species and subspecies (Lamers et al).[9]

Oxidase	**Negative**							
Novobiocin	**Susceptible**							
Coagulase	**Negative**		**Positive –variable-negative**				**Negative**	
Species group	**Hyicus-Intermedius**			**Epidermidis-Aureus**				
Cluster	Muscae	Hyicus	Intermediu	Aureus	Epidermi	Warneri	Haemolytic	Lugdunensis

group			s		dis		us	
Species	S.muscae S.microti S.rostri	S.hyicus S.agnetis S.chromogenes S.felis	S.intermedius S. delphini S.lutrae S.pseudintermedius S.schleiferi sp. Schleiferi sp. coagulans	S.aureus ssp. Aureus ssp. Anaerobius S.simiae	S. epidermidis S. capitis Sp. Capitis Sp. Urealyticus S.caprae S. saccharolyticus	S.warneri S.pasteuri	S.haemolyticus S.devriesei S.jettensis S.hominis Sp.hominis Sp.novobiosepticus S.petrasii Sp.croceilyticus Sp.petrasii	S.lugdunensis

Oxidase	**Negative**						**Positive**
Novobiocin	**Susceptible**		**Resistant**				
Coagulase	**Negative**						
Species group	**Auricularis**	**Simulans**	**Saprophyticus**				**Sciuri**
Cluster group	Auricularis	Simulans-Carnosus	Pettenkoferi-Massiliensis	Saprophyticus	Cohnii-Nepalensis	Arlettae-Kloosii	Sciuri
Species	S.auricularis	S.simulans S.carnosus sp. Carnosus sp utilis S.condimenti S.pisciferment ans	S.pettenkoferi S.massiliensis	S.saprophyticus sp.saprophyticus sp. Bovis S.equorum sp.eqorum sp.linens S.gallinarum S.succinus sp. Succinus sp. Casei S.xylosus	S.cohnii sp.cohnii sp.urealyticus S.nepalensis	S.arlettae S.kloosii	S. Sciuri sp. Sciuri sp.carnaticus sp.rodentium S.fleurettii S.lentus S.stepanovicii S.vitulinus

Is there a difference in the previous two tables?

Habitat:

CONS is a normal flora of skin and mucous membranes of humans and animals. [10,11].

Table 3 shows colonizing areas of different CONS species.

Table 3. Colonizing areas of different CONS species.

CONS species	Colonizing areas

S.epidermidis	axillae, inguinal and perineal areas, anterior nares, conjunctiva, and toe webs[12]
S.hominis S.haemolyticus	axillae and pubic region[12].
S. capitis	forehead and scalp following puberty [13].
S. lugdunensis	Pelvic and perineum regions, lower extremities, axillae [14].
S. saprophyticus subsp. saprophyticus	Rectum and genitourinary tract [12].
S. auricularis	Human external ear[15].

Transmission:

Maximum CONS infections are hospital-acquired or health-care related infections as they have the ability to survive in ~~ICU ,~~ICU, on medical devices and ~~medical~~ equipments for months [16,17,18]. Some clones are probably endemic in the hospital environment. [18 19]The mecA gene carriage in these clusters is usually very high, which suggests that antibiotic resistance is one of the major selective forces [20-23]

Emergence and spread of CONS- in hospitals is dependent on following factors:

- Duration of hospital stay (especially ICU stay),
- -Antibiotic treatment period
- antibiotic pressure in the environment
- hygiene standards[16].

Hand hygiene precautions is extremely important for preventing nosocomial colonization and infections.

Risk factors for CONS infections:

Risk factors for CONS infections includes medical conditions such as [24]

- immune suppression
- premature birth
- neutropenia
- dependence of renal dialysis
- malignancy
- cardiothoracic surgery
- long term hospitalization

Microbiological Profile of CONS:

Morphology:

CONS are gram-positive, nonmotile, non-spore-forming cocci. They are usually arranged in irregular (grape-like) clusters or singly, in short chains (three or four cells), in pairs or tetrads.

Classical approach for separation of CONS from coagulase positive Staphylococci:

Coagulase can contribute to pathogenicity by inhibiting the bactericidal activity of normal serum and by inhibiting phagocytosis through deposition of fibrin on the bacterial cell walls. In the laboratory, two types of coagulase tests are used such as slide test and tube test. Table 2 shows all the coagulase positive and coagulase negative Staphylococci species.

Grouping of CONS by novobiocin testing:

For CONS isolates which have been recovered from urinary tract specimens, novobiocin resistance is used to distinguish the intrinsically resistant S. saprophyticus subsp. saprophyticus from other clinically important CONS, using a 5 ug novobiocin disc on Mueller-Hinton agar[25].

Novobiocin resistant species are S. saprophyticus subsp. Saprophyticus ,S. vitulinu S. xylosus

S. hominis subsp. Novobiosepticus,S. sciuri subsp. Sciuri,S. cohinii,S. cohinii subsp urealyticus.

CONS species and subspecies:

Currently at present, there are 32 recognized species and eight subspecies present in the genus Staphylococcus **(Table 2)** and about one-half of these are indigenous to humans.

EX. S. epidermidis S. capitis S. saccharolyticus S. warneri S. hominis S. lugdunensis S. auricularis S. cohnii S. saprophyticus S. xylosus S. caprae S. haemolyticus

Table 4 shows various CONS species causing human infections.

Table 6. CONS species causing human infections [25].

CONS species or subspecies	Site or source of infection (humans)	Clinical association on frequency	
		Device associated infections	Other infections
S.epidermidis	Skin (axillae, head, arms, legs) and mucous membranes of the nasopharynx	++++	Blood stream infections in neonates (++++)
S.auricularis	External auditory canal	-	Blood stream infections in preterm infant
S.capitis subspecies capitis	mainly scalp, arms,	+	Blood stream infections in neonates (+)
S. capitis subsp. Urealyticus	skin of (heads, ears and foreheads)	+	Blood stream infections in

			neonates (++)
S. caprae	Skin, anterior nares	+	Urinary tract infection(+)
S. cohnii subsp. Cohnii	Skin	++	Blood stream infections- in burn patient(+)
S. cohnii subsp. Urealyticus	Skin		Blood stream infections (+)
S.haemolyticus	Skin-, (legs and arms)	+++	Blood stream infections neonates(+++)
S. hominis subsp. hominis	Skin of axillae, arms, legs, pubic, inguinal regions)	++	Blood stream infections(+)
S.lugdunensis	Skin of lower abdomen and extremities)	++	wound infection (++)Native valve infectious endocarditis,(++)SSI (++)
S. saprophyticus subsp.saprophyticus	Skin	+	Urinary tract infections (++++) Blood stream infections (+)–, Native valve infectious endocarditis(+)
S. schleiferi subsp.schleiferi	Skin (preaxillary)	+	Blood stream infections (+) ,wound Infection(+)
S. sciuri subsp. carnaticus	Skin	-	Blood stream infections (?)
S. sciuri subsp. rodentium	Skin	-	Blood stream infections (?)
S. sciuri subsp. Sciuri	Skin	+	wound infection (?) Blood stream infections (?)
S. simulans	Skin (legs, arms, and heads of children)	+	-
S.warneri	Skin (mainly nares, head, legs, and arms)	++	Septic arthritis(+)

S. xylosus	Skin (rare)	+	-

Abbreviations:; **?**, questionable or unconfirmed; **+**, single cases; **++**, occasional detection; **+++**, frequent detection; **++++** most common origin.

Virulence factor in CONS:
CONS are seldom life-threatening except in immunocompromised patients as CONS do not produce aggressive virulence factors.[1]

Capsule:

Among CONS, capsule formation is frequent and they possess increased virulence compared to non-encapsulated variant strains. Slime may contain capsular polysaccharides, proteins and cell wall components. The capsule confers resistance to phagocytosis [26].

Slime: Glycocalyx is considered a slime layer when glycoprotein molecules are loosely attached with the cell wall. Slime material and biofilm formation has important role in colonization of uroepithelium and medical device- associated infections [27]. Slime has also been shown to inhibit the cell mediated immune response in vitro.

Biofilm:
Biofilm structures comprises mainly bacterial cells and an extracellular polymeric substance (EPS) provided by the polysaccharide intercellular adhesion (PIA) .PIA synthesis is associated with intercellular adhesion operon (ica ADBC) [28].

Biofilm provides

- protective environment to microorganisms
- quorum sensing (the exchange of genetic material between cells and intercellular communication)[29]
- the micro-organisms becomes more resistant ~~to antibiotics~~to antibiotics and to host defense mechanisms.

.**Cytolytic toxins:**

Delta-toxin (PSM ~~is produced~~is produced by S. epidermidis . It forms pores in the cell membrane which leads to erythrocytes and other mammalian cells lysis. [25].

Production of Lantibiotics:
antibiotic-like peptides produced by commensal staphylococci are called lantibiotics and belongs to the class of cationic antimicrobial peptides (CAMPs) and are active against gram-positive bacteria. Lantibiotics production has role in bacterial interference on skin and mucous membranes. Type A lantibiotics induce pores in the cytoplasmic membrane. Lantibiotics produced by S.epidermidis are epidermin , Pep5, epilancin K7, epidermicin

NI01, and epicidin 280 . Other species such as S. gallinarum (gallidermin), S. hominis (hominicin), and S. warneri (nukacin ISK-~~1)also~~1) also show lantibiotic production.[25].

Siderophore:

Microorganisms produce low molecular weight (<1000D) chelating compounds called siderophore in their iron especially in free form. Siderophores are helpful to overcome host's non-specific defense mechanisms and thus helpful in survival within the host, [30].

Meiwes et al [31] has detected two iron binding compounds, staphyloferrin A and B which were highly hydrophilic and anionic.

Extracellular Enzymes:

CONS produces variety of enzymes and extracellular proteins such as proteases, lipases, phospholipases, esterase's, protein A, and fatty acid modifying enzymes. Protease are responsible for proteolytic inactivation of ~~antibodies ~~,antibodies, platelet microbicidal proteins, and destruction of tissue protein which leads to increased invasiveness. S. epidermidis has two lipase ~~genes involved~~genes involved in skin colonization [32].

Exopolymers:

Polysaccharide intercellular adhesin (PIA) and poly gamma-glutamate (PGA)s are produced by S. epidermidis.

Functions of ~~PGA :~~PGA:

- protecting ~~against neutrophill~~against neutrophill phagocytosis and antimicrobial peptides.
- important for survival in biofilm and as a commensal on the skin,
- during high salt concentrations it promotes growth by increase osmotolerance.

PIA has similar functions as PGA and also protects against complement deposition and immunoglobulins[33].

Table 5 shows various virulence factors of S. epidermidis.

Table 5. Important virulence factors of S. epidermidis [33].

Virulence factor	Gene	Function
Intercellular aggregation		
PIA (PNAG)	icaA,icaD,icaB, and icaC	Polysaccharide intercellular adhesion
Aap Bhp	Aap ,Bhp	Protein intercellular adhesion
Teichoic acids	Multiple biosynthetic genes	Components of the biofilm matrix
Protective exopolymers		
PIA	icaA,icaD,icaB, and icaC	Protects from IgG, AMPs, phagocytosis

Virulence factor	Gene	Function
PGA	capA,capB,capC and capD	Protects from AMPs and phagocytosis
Resistance to AMPs		
SepA protease	sepA	Involved in AMP degradation
Aps system	apsR, apsS, and apsX	senses AMPs and regulates AMP resistance mechanism
Toxins		
PSMs	psma,psmd,psme, hld	Pro-inflammatory cytolysins
Exoenzymes		
Glutamylendopeptidase GluSE and serine proteases SspA and Esp	sspA	Degrades fibrinogen and complement factor C5
Cysteine proteases SspB and Ecp	sspB	Possibly responsible for tissue damage
Other factors		
Staphyloferrins A and B	Sfna locus	Siderophores (iron acquisition)
SitA, SitB and SitC	sitA, sitB and sitC	Involved in iron uptake

Figure 1 shows scheme for identification of human CONS

Figure 1. Dichotomous key for identification of common human CONS[8]

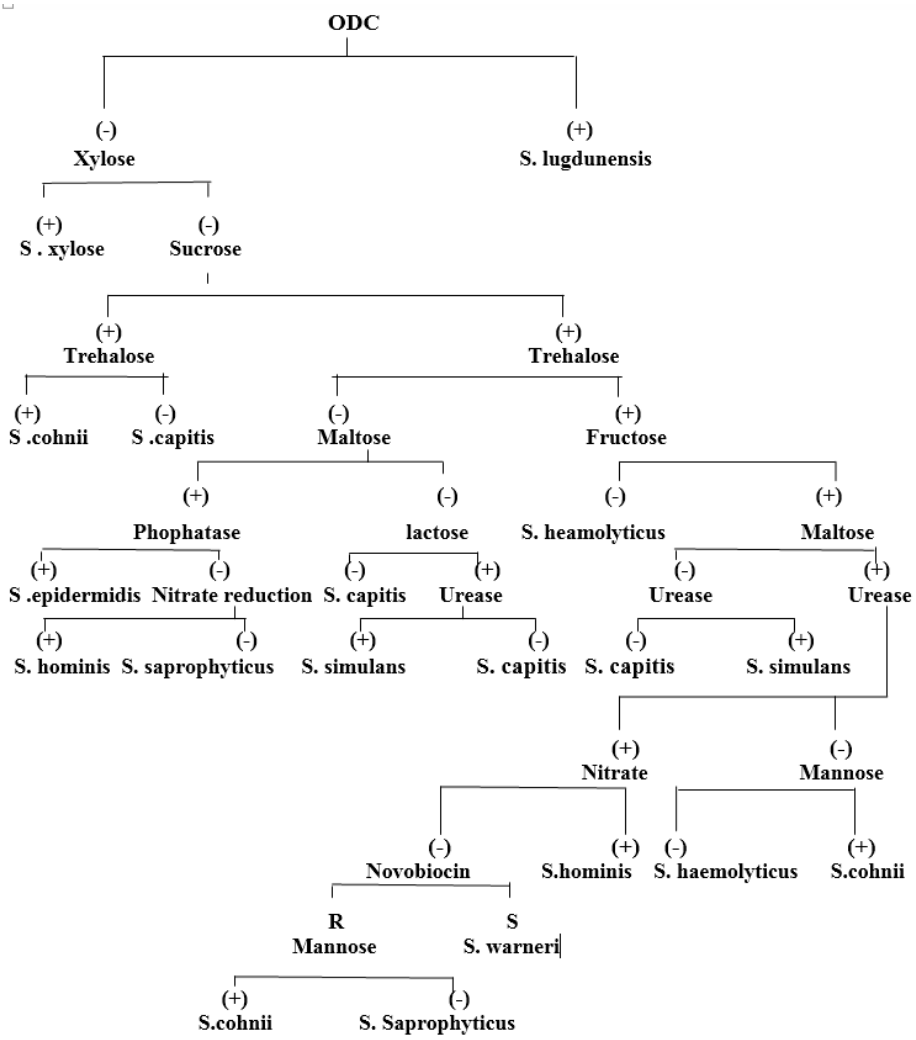
ODC
(-)
Xylose
(+)
S. lugdunensis
(+)
S . xylose
(-)
Sucrose
(+)
Trehalose
(+)
Trehalose
(+)
S .cohnii
(-)
S .capitis
(-)
Maltose
(+)
Fructose
(+)
(-)
(-)
(+)
Phophatase
lactose
S. heamolyticus
Maltose
(+)
(-)
(-)
(+)
(-)
(+)
S .epidermidis
Nitrate reduction
S. capitis
Urease
Urease
Urease
(+)
(-)
(+)
(-)
(-)
(+)
S. hominis
S. saprophyticus
S. simulans
S. capitis
S. capitis
S. simulans
(+)
(-)
Nitrate
Mannose
(-)
(+)
(-)
(+)
Novobiocin
S.hominis
S. haemolyticus
S.cohnii
R
S
Mannose
S. warneri
(+)
(-)
S.cohnii
S. Saprophyticus

Table 6 shows Various biochemical characteristics of CONS

Formatted: Highlight

Table ~~7.Biochemical~~7. Biochemical characteristics of coagulase negative Staphylococci [34]

Formatted: Highlight

Species	Coagulase test									Carbohydrate fermentation test							
	Slide	Tube	NV	Pol-B	PYR	Nit	VP	Ure	ODC	Glu	Mal	Su	La	Man	Mo	Xy	Tre
S. epidermidis	_	_	S	R	_	+	+	+	V	+	+	+	V	_	+	_	_
S. saprophyticus subsp saprophyticus	_	_	R	S	_	_	+	+	_	+	+	+	V	v	_	_	+
S. haemolyticus	_	_	S	S	+	_	+	_	_	+	+	+	V	V	-	-	+
S. hominis subsp hominis	_	_	S	S	_	V	V	+	_	+	+	+	V	-	-	-	V
S. hominis subsp novobiosepticus	_	_	R	NA	_	V	V	+	_	+	+	+	V	-	-	-	-
S. lugdunensis	+	_	S	S/R	+	+	+	V	+	+	+	+	+	-	+	-	+
S. schleiferi subsp schleiferi	+	V	S	S	+	+	+	_	_	+	-	-	-	-	+	-	V
S. schleiferi subsp coagulans	V	+	S	NA	NA	+	+	+	NA	+	-	v	V	V	+	-	-
S. warneri	_	_	S	S	_	V	+	+	_	+	+	+	v	V	-	-	+
S. xylosus	_	_	R	S	V	V	V	+	_	+	+	+	v	+	+	+	+
S.intermedius			S	S	+	+	-	+	-	+	v	+	V	V	+	-	+
S.hyicus	-	V	S	R	-	+	-	V	-	+	-	+	+	-	+	-	+
S.cohnii subsp. Cohnii	-	-	R	S	-	-	V	-	-	+	V	-	-	V	V	-	+

Abbreviations: NV-Novobiocin,Pol-B- Polymyxin-B, Nit- Nitrate reduction test, Ure-Urease Production test, ODC- Ornithine Decarboxylase test, Glu-Glucose, Mal-Maltose, Su-Sucrose, La- Lactose, Man-Mannitol, Mo-Mannose, Xy-Xylose, Tre-Trehalose. V-Variable, R-Resistant, S-Susceptible, + ~~Positive,~~ Positive, - Negative

Molecular methods:

Genotypic ~~methods have~~methods have higher discriminatory power and are less laborious.[35,36].

Disadvantages:

1. Costly, expensive

2. Time consuming

Commercial identification systems:

With ~~these commercial~~these commercial kits , identification of human CONS species can be possible with accuracy of 70->90%.For organism identification these kits use adaptations of standard bacteriologic identification tests , chromogenic enzyme substrate tests and modified carbohydrate fermentation tests.

Different systems available for identification of CONS are [34]

1. API Staph
2. BD Phoenix system
3. BD Phoenix ID-13 system
4. VITEK 2 ID-GP system
5. ID 32 STAPH system
6. Rapidec STAPH
7. API Staph- IDENT
8. MICROSCAN RAPID POS COMBO PANEL
9. STAF- SISTEM 18-R
10. STAPH-ZYM
11. MICROBIAL IDENTIFICATION SYSTEM

As there is addition of more discriminating tests and availability of growing data bases, the reliability of these commercial systems will continue to increase [34].

REFERENCES:

Comment [U2]: The format does not comply with the journal guideline

1. Otto, M. (2004). Virulence factors of the Coagulase negative staphylococci. Front. Biosci.9: 841-863.
2. Rupp, M.E. and Archer, G.L.(1994). Coagulase negative Staphylococci: pathogens associated with medical progress.Clin. Infect. Dis.19:231-245.
3. Rosenbach FJ. 1884. Micro-Organismen bei den Wund-Infections-Krankheiten des Menschen. J F Bergmann, Wiesbaden, Germany.
4. Wilson, T. S., and R. D. Stuart. 1965. Staphylococcus albus in wound infection and in septicemia. Can. Med. Assoc. J. 93:8-16.
5. Pereira, A. T. 1962. Coagulase-negative strains of Staphylococcus possessing antigen 51 as agents of urinary infection. J. Clin. Pathol. 15:252-259.
6. Pulverer, G., and J. Pillich. 1971. Pathogenic significance of coagulase-negative staphylococci, p. 91-96. In M. Finland, W. Marget, and K. Bartmann (ed.), Bacterial infections: changes in their causative agents; trends and possible basis. Springer- Verlag, New York.
7. Holt, R. J. 1971. The colonization of ventriculoatrial shunts by coagulase-negative staphylococci, p. 81-87. In M. Finland, W. Marget, and K. Bartmann (ed.), Bacterial infections: changes in their causative agents, trends and possible basis. Springer- Verlag, Stuttgart, Germany.

8. Götz F., Bannerman T., Schleifer KH. (2006) The Genera *Staphylococcus*and*Macrococcus*. In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. (eds) The Prokaryotes. Springer, New York, NY.
9. Lamers RP, Muthukrishnan G, Castoe TA, Tafur S, Cole AM, Parkinson CL. 2012. Phylogenetic relationships among Staphylococcus species and refinement of cluster groups based on multilocus data. BMC Evol. Biol. 12:171.
10. Grice, E. A., H. H. Kong, S. Conlan, et al. (2009). Topographical and temporal diversity of the human skin microbiome. Science 324(5931): 1190-1192.
11. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation in human body habitats across space and time. Science 326:1694–1697.
12. Schleifer KH, Kloos WE. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of Staphylococcusepidermidis and Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, andStaphylococcus xylosus. Int. J. Syst. Bacteriol. 25:50–61.
13. Kloos WE, Schleifer KH. 1975. Isolation and characterization of staphylococci from human skin. II. Description of four new species: Staphylococcuswarneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcussimulans. Int. J. Syst. Bacteriol. 25:62–79.
14. Bieber L, Kahlmeter G. 2010. Staphylococcus lugdunensis in several niches of the normal skin flora. Clin. Microbiol. Infect. 16:385–388.
15. Kloos WE, Schleifer KH. 1983. Staphylococcus auricularis sp. nov.: an inhabitant of the human external ear. Int. J. Syst. Bacteriol. 33:9–14.
16. Widerström, M., Monsen, T., Karlsson C., Wiström, J., 2006. Molecular epidemiology of methicillin-resistant coagulase-negative staphylococci in a Swedish county hospital: evidence of intra and interhospital clonal spread. J. Hosp. Infect. 64, 177-183.
17. Kloos, W. E. and Bennerman, T.L. (1994). Update on clinical significance of coagulase negative staphylococci. Clin. Microbiol. Rev. 7:117-40.
18. Neely, A.N., Maley, M.P., 2000. Survival of Enterococci and Staphylococci on hospital fabrics and plastic. J. Clin. Microbiol. 38, 724–726.
19. Agvald-Ohman, C., Lund, B., Edlund, C., 2004. Multiresistant coagulase-negativestaphylococci disseminate frequently between intubated patients in a multidisciplinary intensive care unit. Crit. Care. 8, R42-R47.
20. Krediet, T.G., Mascini, E.M., van Rooij, E., Vlooswijk, J., Paauw, A., Gerards, L.J., Fleer, A., 2004. Molecular epidemiology of coagulase-negative staphylococci causing sepsis in a neonatal intensive care unit over an 11-year period. J. Clin. Microbiol. 42, 992-995.
21. Kozitskaya, S., Olson, M.E., Fey, P.D., Witte, W., Ohlsen, K., Ziebuhr, W., 2005. Clonal analysis of Staphylococcus epidermidis isolates carrying or lacking

biofilm-mediating genes by multilocus sequence typing. J. Clin. Microbiol. 43, 4751–475.

22. Hira, V., Sluijter, M., Estevão, S., Horst-Kreft, D., Ott, A., de Groot, R., Hermans, P.W., Kornelisse, R.F., 2007. Clinical and molecular epidemiologic characteristics of coagulase-negative staphylococcal bloodstream infections in intensive care neonates. Pediatr. Infect. Dis. J. 26, 607-612.
23. Nouwen, J.L., van Belkum, A., de Marie, S., Sluijs, J., Wielenga, J.J., Kluytmans, J.A.,Verbrugh, H.A., 1998. Clonal expansion of Staphylococcus epidermidis strains causing Hickman catheter-related infections in a hemato-oncologic department. J. Clin. Microbiol. 36, 2696-2702.
24. Bozkurt H, Kurtoglu MG, Bayram Y, Keşli R, Berktaş M. Correlation of slime production investigated via three different methods in coagulase-negative staphylococci with crystal violet reaction and antimicrobial resistance. The Journal of International Medical Research.2009;37:121-128.
25. Becker K, Heilmann C, Peters G. Coagulase-Negative Staphylococci. Clinical Microbiology Reviews. 2014; 27(4):870-926.
26. Hancock, C, (1989). Encapsulation of Coagulase negative staphylococci.Zentralbi, Bakteriol.272(1);11-18.
27. Bayston, R and Rodgers, J.(1990).Production of extracellular slime by Staphylococcus epidermidis during stationary phase of growth.J.Clin. Pathol.43:866-870.
28. Heilmann,C.,Thumm,G.,Chhatwal,G,S.,Hartleib,J.,Uekotter,A.,Peters G.(2003)Identification and Characterization of a noval autolysin(Aac) with adhesive proportion from Staphylococcus epidermidis. Microbiology.149:2769-2778.
29. Rodney,M.D and Costerton, J.W(2002). Biofilms:survival mechanism of clinicallyrelevant microorganisms. Clin. Microbiol.Rev.15(2):167-193.
30. Bullen,J.J. and Griffiths,E.(1999). Iron binding proteins and host defense in Iron and infections, In: Bullen. J.J and Griffiths,E.(eds).Molecular,Physiologicaland Clinical Aspects, John Wiley and sons,Chichester, UK.Pp-327-368.
31. Meiwes,J.,Fiedler,H.P.,Haag,H.,Zahner,H.,Koneschny,R.,Jung,G.(1990).Isolation and characterization of staphyloferrin a, a compound with siderophore activity from Staphylococcus hyicus.FEMS,Microbiol,Lett.67,201-206.
32. Simons,J.W.,Vankampen,M.D.,Riel, S.,Gotz,F.,Egmond, M.R.,Verheij, H.M. (1998). Cloning,purification and characterization of the lipase from Staphylococcus epidermidis, comparison of the substrate selectivity with those of other microbial lipases. Eur. J. Biochem. 253; 675-683.
33. Otto, M. (2009). Staphylococcus epidermidis-the accidental pathogen. Nat.Rev. Microbiol.7(8) ;555-567.
34. Washington CW Jr, Stephen DA, William MJ et al. Koneman"s color Atlas and Textbook of diagnostic Microbiology, in Gram positive cocci.Ch 12; 6th ed, Lippincott Williams and Williams, USA, 2006; p623-71.

35. Heikens, E., Fleer, A., Paauw, A., Florijn, A.C., Fluit, A., 2005. Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol. 43, 2286-2290.
36. Layer, F., Ghebremedhin, B., Moder, K., König, W., König, B., 2006. Comparative study using various methods for identification of Staphylococcus species in clinical specimens. J. Clin. Microbiol. 44, 2824-2830.