Significance of Staphylococcus Haemolyticus in Hospital Acquired Infections

Betty Daniel[1], Mehvish Saleem[1], Gousia Naseer[2], Alsaba Fida[3]

[1]*Postgraduate Department of Botany, St. Josephs College ,PG & Research Centre Bangalore, Bangalore, India*
[2]*Division of Clinical Microbiology, Trans world Muslim University Diagnostic Center, Jammu &Kashmir, India*
[3]*Department of Microbiology, Government Medical College Srinagar, Jammu &Kashmir, India*

Conflict of Interest: None declared

This article has been peer reviewed.

Article Submitted on: 17th December 2013

Article Accepted on: 28th March 2014

Funding Sources: None declared

Correspondence to: Dr Mehvish Saleem

Address: Division of Clinical Microbiology, Transworld Muslim Univeristy Diagnostic Center, Jammu and Kashmir, India

E-mail: mehvish.saleem@gmail.com

Cite this article: Daniel B, Saleem M, Naseer G, Fida A. Significance of staphylococcus haemolyticus in hospital acquired infections. J Pioneer Med Sci. 2014; 4(3):119-125

ABSTRACT

Staphylococcus haemolyticus is a commensal on skin in human beings and a member of coagulase negative staphylococci (CoNS). It is abundantly found in inguinal areas, perineum and axillae. S. haemolyticus is a well-known opportunistic pathogen and the most recurrently isolated among CoNS after S. epidermidis. It is the second most frequent cause of nosocomial infections associated with the insertion of medical devices. Staphylococcus haemolyticus also represents a multi-drug resistant phenotype recognized among CoNS. Its ability and pattern of forming bio-films that are different from other staphylococcus species makes it a complicated pathogen to treat. A multidisciplinary approach towards the awareness and control of infections due to CoNS will reduce the disease burden. This review describes the characteristics of S. haemolyticus along with mechanism of antimicrobial resistance, pathogenecity and clinical importance.

Keywords: Coagulase Negative Staphylococci; Staphylococcus Aureus; Glycopeptide Resistance; Bloodstream Infections (BSI), Bio-films

INTRODUCTION

S. haemolyticus is the second most frequently isolated CoNS in hospital acquired infections often associated with the insertion of medical devices [1]. S. haemolyticus is a gram positive, non-motile, non-spore forming, facultative anaerobic bacteria that grows on a wide range of substrates such as, glucose, maltose, sucrose and trehalose. It tests negative for coagulase, DNase, ornithinine decarboxylase, phosphatase, urease and oxidase [2]. In recent years, S. haemolyticus demonstrates the highest level of antimicrobial resistance among other coagulase negative staphylococci CoNS [3]. Glycopeptide resistance is a familiar phenomenon observed among CoNS species which limits the use of this therapy [4] Infections due to S. haemolyticus are severe and notoriously difficult to treat.

MORPHOLOGY OF STAPHYLOCOCCUS HEMOLYTICUS

S.hemolyticus is non-sporulating, non-motile, facultative gram-positive coccus usually seen in clusters. Sequenced genome of S.hemolyticus strain JCSC1435 genome shows a chromosome of 2,685,015 bp size with three prominent plasmids measuring 2,300 bp, 2,366 bp, and 8,180 bp in size. S.hemolyticus possesses a thick cell wall (60-80nm). Similar to other gram positive cocci, the cell wall is composed of peptidoglycan, teichoic acids, and proteins. L-lysine as the di-amino acid in position 3 of the peptide subunit and a glycine-rich interpeptide bridge present in the peptidoglycan is a characteristic feature of this microbe. The two predominant cross bridges found across peptidiglycan layer are COOH-Gly-Gly-Ser-Gly-Gly-NH2 and COOH-Ala-Gly-Ser-Gly-Gly-NH2[5]. Changes which may take place as a consequence of mutations in the cross bridging can play a role in the development of glycopeptide resistance.

MECHANISM OF ANTIBIOTIC RESISTANCE

Antibiotic resistance is defined as the ability of bacteria to resist the effects of an antibiotic to which they were once sensitive [6][7]. Antibiotic resistance is serious problem epitomized by the development of multidrug resistant variants of

different pathogens isolated in routine clinical laboratory tests. Well elucidated mechanisms of antibiotic resistance include the horizontal gene transfer, also referred to as lateral gene transfer, in which antibiotic resistant genes are transferred from one bacterial species to another via conjugation, transformation, transposons or plasmids [7].

Inactivation or modification of antibiotics such as the production of β-lactmases like extended spectrum β-lactmases can lead to the deactivation of antibiotics such as penicillin G binding to the cell wall in penicillin-resistant bacteria. Mutations or alterations in the target sites by penicillin binding proteins (PBP) in MRSA strains is also a well-known phenomenon [7].

Increased activity of efflux pumps results in pumping out the drugs across the cell wall hence decreasing the drug permeability and concentration inside bacterial cells. Gram negative bacteria acquire resistant genes via plasmids. These genes produce proteins binding with DNA gyrase thus contributing to quinolone resistance [8]. Infections due to Staphylococcal species poorly respond to penicillin, methicillin, tetracycline and erythromycin. Intermediate resistance mechanisms to glycopeptides were reported in late 1990.Vancomycin resistant isolates of Staphylococcus were first identified in 2002 [9]. Antibiotic resistance has also lead to the emergence of "superbugs". A superbug is also referred to a multidrug resistant phenotype which carries several drug resistance genes.

The ability of S. haemolyticus to resist multiple antibiotics has been reviewed for a long time [10]. S. haemolyticus is naturally resistant to gentamicin, erythromycin and glycopeptides [11]. Methicillin resistance is conferred by the genes located on the bacterial chromosome. Resistance to erythromycin is acquired by plasmids. Gentamicin resistance is encoded on both chromosome and the plasmids [12]. Sequenced genome of (JCS1435) elucidates the mechanism of multi-drug resistance of S. haemolyticus [13]. MecA gene present on the staphylococcal cassette chromosome mec (SCCmec) is responsible for the methicillin resistance [14]. SCC mec type-V has also been found in S. haemolyticus [14].

It has been found that S. haemolyticus is also a potential (SCC) mec donor. Studies have supported the role of horizontal transfer of SCC mec type-V from methicillin resistant S. haemolyticus to methicillin susceptible S. aureus [14]. S. haemolyticus has a quick affinity to develop resistance to multiple antibiotics and shows the highest resistance to β-lactams [15]. Oxacillin resistance in S. haemolyticus strains is conferred due to either over production or over expression of penicillin binding proteins[16] . Complete genome sequencing of (JCSC1435) explained the genetic diversity of S. haemolyticus. There are 82 insertion sequences in its chromosome. This results in the frequent genomic rearrangements, phenotypic variations and acquisition of antibiotic resistance. The (SCC) mec type-V is the most widespread type of genetic aspect conferred to antibiotic resistance in S. haemolyticus [17]. Practically S. haemolyticus plays an efficient role as a recipient and a carrier of (SCC) mec elements [18].

GLYCOPEPTIDE RESISTANCE

Heterogeneous resistance among staphylococcus species is a well-known feature in methicillin resistant strains of staphylococci [19]. Experimental studies have verified that glycopeptide resistance can be expressed by the strains of Methicillin resistant Staphylococcus aureus (MRSA) and CoNS. The phenomenon of heterogeneous resistance is mainly associated with the failure of vancomycin therapy [20]. Resistance to glycopeptides such as teicoplanin is more common than vancomycin as represented in species of S. heamolyticus [21]. Strains of S. haemolyticus and S. epidermidis differ from their glycopeptides susceptible similar strains in several features such as ultrastructural morphology, glycopeptide binding capacity [22], membrane proteins , cell wall synthesis and susceptibility to antibiotics acting on cell wall. There has been an increase in the heterogeneous susceptibility to teicoplanin, observed more frequently in nosocomial isolates S. haemolyticus can be isolated from human blood cultures where it is suspected to be the cause of septicemia, peritonitis, otitis media and urinary tract infections. The appearance of glycopeptide resistance among CoNS is alarming due to the lack of effective alternatives. Since glycopeptides are the only available therapy for MRSA, it is advisable to remove the external medical devices causing infection in case of prolonged stay of patients in the hospital.[23]. Susceptibility to glycopeptides by MIC determination of isolated strains before and during prolonged treatment proves beneficial in guiding the treatment [23].

ADHERENCE TO MEDICAL DEVICES, FORMATION OF BIOFILM AND VIRULENCE

The knowledge about the nature of S. haemolyticus forming bio-films is limited [24] In S. haemolyticus bio-film formation is a two stage process involving initial attachment mediated by proteins on the cell surface (Fbe & Bhp) and lytic enzymes mainly autolysins (Atl E) [25]. Cell surface proteins (Fbe and Atl E) possess adhesion properties and bind to the host fibrinogen and vitronectin respectively [26]. Primary attachment and intracellular adhesion is due to the activity of (Bhp) [27]. The role of accumulation associated proteins (Aap) with operons like (ica) in S. haemolyticus are well described [28]. The ability to form bio-films is an important virulence factor in S. haemolyticus. Bio-films assist in the antibiotic resistance and persistent infections S. haemolyticus bio-films do not depend on polysaccharide independent adhesion (PIA) factors and lack the gene cluster encoding the production of polysaccharide independent adhesion [29]. This feature of forming bio-films easily differentiates S. haemolyticus from other CoNS. Evidence suggests that the extracellular DNA augments cell to surface and cell to cell adhesions during the initial process of bio-film formation .This role of DNA in the matrix of mature bio-films in S. haemolyticus is novel among CoNS [29].

The mechanism of molecular basis of the virulence of S. haemolyticus and device associated infections is largely unknown. Sequenced genome of (JCS1435) has assisted in the investigation of putative encoding virulence factors . Antibiotic resistance and genetic re-arrangements are mediated by insertion sequences found in S. haemolyticus.

Pathogenesis and progression of infection is governed by a number of virulence factors in S. haemolyticus [30]. These factors include adhesion factors autolysins (AtL E) that assist in the attachment to abiotic surfaces and bio-film production [31]. Attachment to the host matrix proteins is due to the extracellular matrix binding proteins (Embp) which play a vital role in fibronectin and collagen binding [32]. Accumulation associated proteins (AAP) produce exo-polysaccharides leading to cell to cell adhesion, haemagglutination and accumulation during infection. S. haemolyticus also secretes exoenzymes such as lipases and cysteine proteases [33]. These enzymes help in the persistence of the pathogen in fatty secretions and tissue damage during the infection process in the human host. Fatty acid modifying enzymes (FAME) detoxify the host produced bactericidal fatty acids. Regulatory virulence factors include accessory gene regulators (agr) which regulate the production of exo-zymes such as lipases and proteases. The entire process of virulence factors is controlled by staphylococcal accessory regulator (Sar) gene. Bio-film production is synchronized by Sig-B (Sigma factor B) and inflammatory processes are mediated by phenol soluble modulin (PSM). S. haemolyticus is also rich in producing siderophores for iron uptake by staphlyoferrin A and B. Cell wall components such as peptidoglycan and lipoteichoic acids stimulate the inflammatory processes .

Capsular polysaccharide is an important virulence factor in S.hemolyticus [34]. Some strains of S. haemolyticus are capable of producing capsular polysaccharides. A capsular operon in (JCS1435) is located within the "OriC environ". The (Capsh) locus has an operon containing (13ORF's) in a 14652bp region. First seven genes of the capsular polysaccharide Capsh (capAsh, capGsh) are homologous to the S. aureus. CapH and CapM are unique in S. haemolyticus. Growth of capsular polysaccharides is enhanced by the culture medium and the stages of growth in the medium. Since S. heamolyticus uses a wide variety of carbohydrates for metabolism, therefore culture in tryptic soy broth with 1% glucose favors the production of capsular polysaccharides. Capsular polysaccharide provides excellent resistance to the complement mediated polymorphonuclear (PMN) phagocytosis.

PATHOGENESIS AND CLINICAL IMPORTANCE

Enterotoxin Production: Some strains of S. haemolyticus abundantly produce a series of enterotoxins and hemolysins [35] classified into SE(A), SE(B) and SE(C). 31.3% of S. haemolyticus strains produce at least one type of enterotoxin [36].Staphylococcal enterotoxins are exoproteins that if ingested by humans can give rise to acute gastroenteritis and emesis. Enterotoxins of S.hemolyticus have been discussed here as important virulence determinants homologous to other staphylococcal species. To our knowledge staphylococcal enterotoxins have no clinical significance with the insertion of medical devices.

Implanted Medical Device Infection: Infections due to CoNS are commonly associated with implanted medical devices. Their ability to form bio-films promotes progression of infection often

leading to persistent infection [37]. Furthermore, S.hemolyticus is a significant cause of bacteremia associated with vascular catheters .S.hemolyticus can colonize central venous catheters. Common infections include valve endocarditis, septicemia, peritonitis, and urinary tract infections [38]. Other complications include chronic infections of the surgical wounds, and osteomyelitis; while in immunocompromised patients infections of the soft tissue are prominent [39]. S. haemolyticus also colonizes medical devices such as prosthetic valves, CSF shunts, orthopedic prosthesis, intravascular and urinary catheters induced during surgical interventions [39]. The overall mortality associated with the infections due to CoNS is 9% in neonates [40]. Isolation of S.hemolyticus by cultures becomes necessary in patients presenting with the clinical picture of sepsis specially when patients have above described clinical risk factors.

Infections related to vascular catheters are a growing concern in intensive care units in patients with long periods of hospitalization as S. haemolyticus can colonize central venous catheters leading to severe medical complications. It easily migrates from skin, with external surface of the device. Severity of these infections depends on catheters, frequency of carriage and virulence factors of the strain involved. Some studies have strongly recommended the removal of external medical devices such as catheters in case of localized infections [41].

Suggested therapy includes glycopeptides antibiotics (vancomycin and teicoplanin) . Glycopeptides can be supplemented with β-lactams as a strong antibiotic treatment for S. haemolyticus infections [42]. However, other studies have mentioned concentration dependent antagonistic effects of glycopeptides when combined with β-lactams as observed in some strains of S.aureus. Thus, the use of combinational therapy in infections due to CoNS is poorly understood.

Interaction with Immune System: Macrophages act as the primary line of innate defense against bacterial infections [43]. This is attributed to cell mediated killing which represents a major defense mechanism against host's nonspecific immunity. Many pathogens have developed specific mechanisms to evade the antimicrobial immune response of the cells in order to avoid the immune defense of the host. S. haemolyticus triggers caspase dependent apopto-is of the macrophages during pathogenesis and causes a number of alterations in their structure [43]. Evidence suggests that S. haemolyticus produces abundant cytotoxins that kill macrophages. This could be an important mechanism for the successful establishment of the infection. Cytotoxity is also evoked by hemolysins and pore forming toxins. It is interesting to note that S. haemolyticus also induces mitochondrial dysfunction in phagocytes by regulating the apoptotic cell death This is achieved through the permeabilization of the outer membrane produced by the perturbation of the mitochondrial transmembrane potential [43].

APPROACHES FOR PREVENTION AND CONTROL OF S.HAEMOLYTICUS INFECTIONS

Infections due to CoNS have shown a progressive increase in the antibiotic resistance. In an ICU setting, infection control measures such as hand washing and isolation precautions in patients with device associated infection remain doubtful especially in clinical settings where infection control practices are questionable [44].

Prophylactic measures may include the use of topical anti staphylococcal agents which may eliminate the nasal colonization in high risk ICU patients such as neonates [45][46]. It is also recommended to clean skin sores and abrasions with topical antimicrobial agents. Wounds should be covered until fully healed. Transmission of the infection can also be prevented by proper disposal of wound dressings, use of hand sanitizers in dressing areas, rest rooms and waiting areas of the hospital [47]. Proper sterilization by autoclaving is mandatory in preventing infections through equipment. Antibiotic coated catheters should be used for patients expected to have the device implanted more than 7 days[48]. Infection control measures should be practiced by everyone involved in direct as well as indirect patient care. In a hospital setting with critically ill patients nursing staff should be comprehensive in observing practices associated with the insertion, care and maintenance of implanted medical devices such as venous catheters which often lead to bloodstream infections. Use of barrier precautions such as gowns, gloves and masks should be made mandatory while attending patients. Venous catheters should be impregnated with antiseptics such as chlorohexidine and silver sulfadiazine compounds to reduce the risk of

catheter related bacteremia in intensive care patients [48]. Development of anti-staphylococcal vaccines is currently being investigated and is an area of immense research interest.

CONCLUSION

Implanted medical device associated infections due to *S. haemolyticus* pose a significant economic burden on the country's health care, therefore prevention and management of such infections is of paramount importance. Efforts in the past have focused on basic control measures and use of traditional antimicrobial therapy, however, infections due to medical devices continue to be a challenge. Since bio-film formation is a critical aspect of infection, novel therapies should focus on the prevention of infection using antibiofilm agents which may inhibit the microbial attachment. Recent approaches include (QS) perturbation, immunotherapy, disruption and disintegration of the protective extra polymer matrix. Use of synergistic combination of novel anti biofilm strategies with more traditional bactericidal approaches can be utilized to prevent and control further infections. Other effective materials include antiseptic impregnated dressings with chlorohexidine. Sliver alginate coated dressings have been previously tested in neonatal ICUs and results have shown a significant decline in peripheral inserted central catheters (PICC) infections. Antimicrobial catheters coated externally with chlorohexidine have proven to reduce the risk of catheter related blood stream infections(CR-BSI). Catheters impregnated both internally and externally with a combination of chlorohexidine /sulfadiazine are now being introduced.

It is mandatory for hospitals and health care settings to have a comprehensive infection control team comprising of infectious disease experts, paramedical staff and microbiologists. Guidelines should be provided to healthcare workers for proper insertion of catheters, hand hygiene with correct protocol of hand washing ,insertion of catheters , use of hand rubs, sanitizers and antiseptics such as alcohol before and after handling the patients, inspection of the insertion site daily as well as change of catheter as soon as possible when soiled or infected. Although the recommended guidelines mentioned above are simple , these are often neglected, particularly in developing countries. Newly identified antimicrobials with approved. These drugs include ceftobiprole, dalbavancin, daptomycin, linozolide, tigecycline and telavancin. Newer designs of antistaphylococcal mechanisms can decrease the infection only in patients who spend a short span of time in intensive care units .Although new drugs recommended for antimicrobial therapy may prove beneficial, infections due to CoNS will remain a significant problem in patients with longer duration of hospital stay. Therefore, the implementation of strict surveillance guidelines in hospitals specially in developing world will significantly decrease the burden of BSI due to CoNS and would not only improve patient care but also decrease the economic burden on health care.

REFERENCES

1. Barros EM, Ceotto H, Bastos MC, Dos Santos KR, Giambiagi-Demarval M. Staphylococcus haemolyticus as an important hospital pathogen and carrier of methicillin resistance genes. *J Clin Microbiol.* 2012;50(1):166-168.
2. Paul De Vos, George Garrity, Dorothy Jones, Noel R. Krieg, Wolfgang Ludwig, Fred A. Rainey, Karl-Heinz Schleifer and William B. Whitman, ed et al. *Bergey's Manual of Systematic Bacteriology*. 3 The *Firmicutes* (2nd ed.) Springer-Verlag.
3. Biavasco F, Giovanetti E, Montanari MP, Lupidi R, Varaldo PE et al. Development of in-vitro resistance to glycopeptide antibiotics: assessment in staphylococci of different species. *J Antimicrob Chemother* 1991; 27 (1): 71-79.
4. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 2000; 19(6):403-417.
5. Billot-Klein D, Gutmann L, Bryant D, Bell D, Van Heijenoort J, Grewal J, et al. Peptidoglycan synthesis and structure in Staphylococcus haemolyticus expressing increasing levels of resistance to glycopeptide antibiotics. *J Bacteriol.* 1996;178(15):4696-4703.
6. Centers for Disease Control and Prevention, "Antibiotic Resistance Questions & Answers". Get Smart: Know When Antibiotics Work 30 June 2009. Retrieved 20 March 2013.
7. wikipedia.org/wiki/Antibiotic_resistance#cite_note-pmid2115217-2
8. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis.* 2006;6(10):629-40.
9. Bozdogan B1, Esel D, Whitener C, Browne FA, Appelbaum PC. Antibacterial susceptibility of a vancomycin-resistant Staphylococcus aureus strain isolated at the HersheyMedical Center. *J Antimicrob Chemother.* 2003;52(5):864-868.
10. Froggatt JW, Johnston JL, Galetto DW, Archer GL. Antimicrobial resistance in nosocomial isolates of Staphylococcus haemolyticus. *Antimicrob Agents Chemother*. 1989;33(4):460-466.
11. Raponi G, Ghezzi MC, Gherardi G, Dicuonzo G, Caputo D, Venditti M, et al. Antimicrobial susceptibility, biochemical and genetic pr ofiles of Staphylococcushaemolyticus strains

isolated fromthe bloodstream of patients hospitalized in critical care units. *J Chemother*. 2005;17(3):264-269.

12 Berglund C, Söderquist B. The origin of a methicillin-resistant Staphylococcus aureus isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette chromosome mec between methicillin-resistant Staphylococcus haemolyticus and Staphylococcus aureus. *Clin Microbiol Infect.* 2008;14(11):1048-1056.

13 Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-genome sequencing of staphylococcus haemolyticus uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. *J Bacteriol.* 2005;187(21):7292-7308.

14 International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCC mec elements. *Antimicrob. Agents Chemother* 2009; 53(12): 4961-4967.

15 Vignaroli C, Biavasco F, Varaldo PE. Interactions between Glycopeptides and β-Lactams against Isogenic Pairs of Teicoplanin Susceptible and Resistant Strains of Staphylococcus haemolyticus. *Antimicrobial Agents and Chemotherapy* 2006; 50 (7): 2577-2582.

16 Domaracki BE, Evans A, Preston KE, Fraimow H, Venezia RA. Increased oxacillin activity associated with glycopeptides in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 1998; 17(3):143-150.

17 Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev* 1997; 10 (4):781-791.

18 Caierao J, Musskopf M, Superti S, Roesch E, Dias CG, d'Azevedo PA, et al. Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS). *J Med Microbiol* 2004; 53(12): 1195-1199.

19 Chiew YF, Charles M, Johnstone MC, Thompson KM, Parnell KD, Penno EC. Detection of vancomycin heteroresistant Staphylococcus haemolyticus and vancomycin intermediate resistant Staphylococcus epidermidis by means of vancomycin screening agar. *Pathology* 2007; 39(3): 39375-39377.

20 Blans M, Troelstra A. Glycopeptide resistance in Staphylococcus haemolyticus during treatment with teicoplanin. Infect Control Hosp Epidemiol. 2001;22(5):263-264.

21 Bertin M, Muller A, Bertrand X, Cornette C, Thouverez M, Talon D. Relationship between glycopeptide use and decreased susceptibility to teicoplanin in isolates of coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis.* 2004;23(5):375-379.

22 Biavasco F, Pugnaloni A, Lupidi R, Biagini G, Varaldo PE et al. An electron microscopic study of clinical and laboratory-derived strains of teicoplanin-resistant Staphylococcus haemolyticus. *Microb Drug Resist* 1996; 2 (2):239-243.

23 Viale P, Stefani S. Vascular catheter-associated infections: a microbiological and therapeutic update. *J Chemother.* 2006 ;18(3):235-249.

24 Fredheim EG, Klingenberg C, Rohde H, Frankenberger S, Gaustad P, Flaegstad T, Sollid JE. Biofilm formation by Staphylococcus haemolyticus. *J Clin Microbiol.* 2009; 47(4):1172-1180.

25 Heilmann C, Hussain M, Peters G, Gotz F. Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. *Mol Microbiol* 1997; 24 (5):1013-1024.

26 Davis SL1, Gurusiddappa S, McCrea KW, Perkins S, Höök M. SdrG, a fibrinogen-binding bacterial adhesin ofthe microbial surface compo nents recognizing adhesivematrix molecules subfamily f rom Staphylococcus epidermidis, targets the thrombin cl eavage site in the Bbeta chain. *J Biol Chem.* 2001;276(30):27799-277805

27 Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, et al. The intercellular adhesin involved in biofilm accumulation of Staphylococcus epidermidis is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *J Bacteriol.* 1996;178(1):175-183.

28 Toledo-Arana A, Merino N, Vergara-Irigaray M, Débarbouillé M, Penadés JR, Lasa I. Staphylococcus aureus develops an alternative, ica-independent biofilm in the absence of the arlRS two-component system. *J Bacteriol.* 2005;187(15):5318-5329.

29 Frank KL, Patel R. Poly-N-acetylglucosamine is not a major component of the extracellular matrix in biofilms formed by icaADBC-positive Staphylococcus lugdunensis isolates. *Infect Immun*. 2007;75(10):4728-4742.

30 Klingenberg C, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, et al. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. *Clin Microbiol Infect.* 2007;13(11):1100-1111.

31 Heilmann C, Hussain M, Peters G, Götz F. Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. *Mol Microbiol.* 1997;24(5):1013-1024.

32 Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés JR. Bap, a Staphylococcus aureus surface protein involved in biofilm formation. *J Bacteriol.* 2001;183(9):2888-2896.

33 Hussain M, Herrmann M, von Eiff C, Perdreau-Remington F, Peters G. A 140-kilodalton extracellular protein is essential for the accumulation of Staphylococcus epidermidis strains on surfaces. *Infect Immun.* 1997;65(2):519-524.

34 Flahaut S, Vinogradov E, Kelley KA, Brennan S, Hiramatsu K, Lee JC.Structural and biological characterization of a capsular polysaccharide produced by Staphylococcus haemolyticus. *J Bacteriol.* 2008;190(5):1649-1657

35 Valle J, Gomez-Lucia E, Piriz S, Goyache J, Orden JA, Vadillo S. Enterotoxin production by staphylococci isolated from healthy goats. *Appl Environ Microbiol.* 1990;56(5):1323-6.

36 Vasconcelos NG1, Pereira VC, Araújo Júnior JP, da Cunha Mde L. Molecular detection of enterotoxins E,G,H and I in Staphylococcus aureus and coagulase-negative staphylococci isolated from clinical samples of newborns in Brazil. *J Appl Microbiol*. 2011;111(3):749-762

37 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999;284(5418):1318-1322.

38 Fischetti, A.; Novick, R. P.; Ferretti, J. J.; Portnoy, D. A.; Rood, J. I.; Lina, G., Etienne, J., and Vandenesch, F. (2000). "Biology and pathogenicity of staphylococci other than Staphylococcus aureus and Staphylococcus epidermidis". Gram-positive pathogens. Washington, D.C.: ASM Press. pp. 450–462.

39 Rosenthal VD, Maki DG, Salomao R, Moreno CA, Mehta Y, Higuera F, et al. Device-associated nosocomial infections in 55 intensive care units of 8 developing countries. *Ann Intern Med.* 2006;145(8):582-591.

40 Klingenberg C1, Rønnestad A, Anderson AS, Abrahamsen TG, Zorman J, Villaruz A, et al. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. *Clin Microbiol Infect*. 2007;13(11):1100-1111.

41 de Allori MC, Jure MA, Romero C, de Castillo ME. Antimicrobial resistance and production of biofilms in clinical isolates of coagulase-negative Staphylococcus strains. *Biol Pharm Bull.* 2006;29(8):1592-1596.

42 Climo MW, Patron RL, Archer GL. Combinations of vancomycin and β-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. *Antimicrob Agents Chemother* 1999; 43 (7):1747-1753.

43 Sansonetti PJ, Di Santo JP. Debugging how bacteria manipulate the immune response. *Immunity.* 2007;26(2):149-161.

44 Patient population at an increased risk of infection by coagulase negative staphylococci. www.inkling.com

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 *Therapeutics and Clinical Risk Management* 2007:3(6) 1143–1152

46 Hill ML, Baldwin L, Slaughter JC, Walsh WF, Weitkamp JH: A silver-alginate-coated dressing to reduce peripherally inserted central catheter (PICC) infections in NICU patients: a pilot randomized controlled trial. *J Perinatol* 2010, 30 (7):469-473

47 Guide to the elimination of catheter related bloodstream infections 2009. www.apic.org

48 Timsit JF, Schwebel C, Bouadma L, Geffroy A, Garrouste-Orgeas M, Pease S, Herault MC, et al. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA* 2009, 301 (12):1231-1241