Int J Pharm Bio Sci 2012 July; 3(3): (B) 787 - 799

Review Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

BIOFILMS, COAGULASE NEGATIVE STAPHYLOCOCCI AND THE SAGA OF CATHETER RELATED BLOODSTREAM INFECTIONS.

DR. CHANDAN KUMAR SHAW [1], DR. PRACHI SHAW.[2]

[1] Associate Professor, Pediatrics, SMVMCH, Pondicherry.
[2]Assistant Professor, Microbiology, SMVMCH, Pondicherry.

ABSTRACT

Catheter related bloodstream infections are a major problem in most tertiary care hospitals. Among the various organisms associated with nosocomial infections, coagulase negative staphylococci are responsible for majority of the catheter related infections. They are usually resistant to standard antibiotics necessitating prolonged hospital stay and amplifying the cost of treatment manifold which usually becomes a vicious cycle difficult to break and ultimately contributing to increased morbidity and mortality. Coagulase negative staphylococci are skin commensals but the strains producing biofilms manage to evade the host immune system. Biofilms consist of a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or each other, embedded in a matrix of extracellular polymeric substances, exhibiting an altered phenotype with respect to growth rate and gene transcription. This unnatural yet favourable ecological niche protects the organisms from host immune responses and antimicrobials. In the following account we present the characteristics of biofilms and the latter's relationship with catheter related blood stream infections particularly by coagulase negative staphylococcus and vice versa. Together, biofilms and coagulase negative staphylococci dominate the saga of catheter related sepsis and strict asepsis protocols related to catheter placement and maintenance and rational antibiotic policy are the only hope as other approaches to inhibition of biofilm formation are still experimental.

KEY WORDS: Biofilms, Coagulase negative staphylococci, *Staphylococcus epidermidis,* Catheter related blood stream infection,

DR. CHANDAN KUMAR SHAW
Associate Professor, Pediatrics, SMVMCH, Pondicherry

INTRODUCTION

The history of medical science comprises an age old battle between disease and cure. The result of this struggle has led to revolutionary discoveries by mankind in the field of medicine and many evolutionary changes in the organisms and agents causing disease states. The introduction of medical devices like "catheters" made of biopolymers like polysterene, polypropelene or latex is one such example of a revolution which has given us easy access to the various human body parts - be it the vein, artery, cerebrospinal space, heart or urinary bladder. But the very purpose of such devices such as in the treatment of infections has been defeated by some micro-organisms which produce a protective exopolymer layer, known as, the "biofilm" around themselves as they colonise these devices and acquire resistance to most antibiotics.[1,2] In other words some organisms have found a rather unnatural yet favourable ecological niche for themselves as a part of their survival instinct.

Various organisms colonise medical devices and are able to evade the immune system by producing biofilms. These organisms are usually resistant to standard antibiotics necessitating prolonged hospital stay and amplifying the cost of treatment manifold which usually becomes a vicious cycle difficult to break ultimately contributing to increased morbidity and mortality.[3,4] This article focuses on the nature and importance of "biofilm" with respect to medical device related infections and coagulase negative staphylococci (CNS) mainly, *S. epidermidis* as a nosocomial pathogen that utilizes biofilms as one of its main virulence factors.

Biofilms: A Historical Basis

Van Leeuwenhoek, using his simple microscopes, first observed microorganisms on tooth surfaces and can be credited with the discovery of microbial biofilms. However, a detailed examination of biofilms would await the electron microscope, which allowed high-resolution photomicroscopy. The initial research on biofilm was mainly centered around industries and waste water plants. Initial research by Jones et al[6] on biofilms on trickling filters in a wastewater treatment plant, showed them to be composed of a variety of organisms while in 1973, Characklis[7] noted that biofilms in industrial water systems were not only very tenacious but also highly resistant to disinfectants such as chlorine. Based on observations of dental plaque and sessile communities in mountain streams, Costerton et al.[8] in 1978 put forth a theory of biofilms that explained the mechanisms whereby microorganisms adhere to living and nonliving materials. Since that time, the studies of biofilms in industrial and ecologic settings and in environments more relevant for public health have basically paralleled each other.

Biofilm: Definition.

The definition of biofilm has evolved over the last 25 years mainly due to its structural and functional characterization. It has been defined as "very fine extracellular polymer fibrils that anchored bacteria to surfaces" (Marshall in 1976)[9] or "communities of attached bacteria in aquatic systems were found to be encased in a glycocalyx matrix" (Costerton et al.)[8] However, today a biofilm may be defined as "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription".[10] The latter helps us differentiate "nonbiofilm" populations, such as colonies of bacteria growing on the surface of agar and exhibit none of the inherent resistance characteristics of true biofilms.[10]

Structure of Biofilms.

Biofilms are composed primarily of microbial cells and EPS. EPS is primarily composed of polysaccharides which are usually neutral or polyanionic. Hussain et al[11] found that the slime of coagulase-negative bacteria consists of a teichoic acid mixed with small quantities of proteins. EPS is also highly hydrated because it can incorporate large amounts of water into its structure by hydrogen bonding.[12] Structure may also be influenced by the interaction of particles of nonmicrobial components from the host or environment such as erythrocytes and fibrin on native heart valves[13] and protect the organisms in these biofilms from the leukocytes of the host or precipitation of minerals such as calcium phosphate and magnesium ammonium phosphate[14] leading to encrustation of the catheter.

"Substratum Effects" such as physical characteristics that is - the roughness[14] or smoothness[15] of a surface, influence bacterial adhesion to only a minor extent. The physicochemical properties of the surface exert a strong influence on the rate and extent of attachment. Most investigators have found that microorganisms attach more rapidly to hydrophobic, nonpolar surfaces such as teflon and other plastics than to hydrophilic materials such as glass or metals which is more relevant to medical practice.[16,17,18] "Conditioning films" such as the acquired pellicle, on tooth enamel comprising albumin, lysozyme, glycoproteins, phosphoproteins, lipids, and gingival crevice fluid also determines the attachment and growth of a biofilm.[19] Higher linear velocities of the surrounding fluid results in rapid association with the surface, until velocities become high enough to exert substantial shear forces on the attaching cells, resulting in detachment of these cells[20,21] – a phenomenon related to device related infection. Other characteristics of the aqueous medium[22] and the hydrophobicity of the cell surface such as fimbriae[23] containing a high proportion of hydrophobic amino acid residues may play a role in attachment.

Biofilms and Disease*:*

The suggested mechanisms by which biofilm-associated organisms elicit disease in the human host include the following: (i) detachment of cells or cell aggregates from indwelling medical device biofilms, resulting in bloodstream or urinary tract infections, (ii) production of endotoxins, (iii) resistance to the host immune system, and (iv) provision of a niche for the generation of resistant organisms (through resistance plasmid exchange). Biofilms also provide an ideal niche for the exchange of plasmids (gene transfer) providing a mechanism for selection, and promoting the spread of bacterial resistance to antimicrobial agents.[24,25] Ghigo[26] showed that the F conjugative pilus (encoded by the *tra* operon of the F plasmid) acts as an adhesion factor for both cell-surface and cell-cell interactions, resulting in a three-dimensional biofilm of *Escherichia coli*. Cell-to-cell signaling or "quorum sensing" has recently been demonstrated to play a role in cell attachment and detachment from biofilms. "RNAIII-inhibiting peptide" (RIP), a heptapeptide, known to be produced by *S. aureus* and *S. epidermidis* inhibits cell adhesion and biofilm formation by inhibition of the phosphorylation of a protein called "target of RNAIII activating protein" (TRAP) by inhibiting the activity of the gene locus *agr*.[27] Davies et al.[28] showed that two different cell-to-cell signaling systems in *P. aeruginosa*, *lasR-lasI* and *rhlR-rhlI,* were involved in biofilm formation. At sufficient population densities, these signals reach concentrations required for activation of genes involved in biofilm differentiation.

Biofilms and Resistance to the Host Immune System

Interference with the phagocytic activity has been classically incriminated as one of the modes by which organisms within biofilms acquire resistance to host immune system.[29,30] Shiau and Wu[31] found that extracellular slime produced by *S. epidermidis* interfered with macrophage phagocytic activity.

Biofilms and Resistance to Antimicrobial Agents

The extracellular polymeric substances constituting this matrix present a diffusion barrier for antimicrobials by influencing either the rate of transport of the molecule to the biofilm interior or the reaction of the antimicrobial material with the matrix material. Suci et al.[32] demonstrated a marked delay in penetration of ciprofloxacin into *Pseudomonas aeruginosa* biofilms. DuGuid et al.[33] concluded that the organization of cells within biofilms could in part explain the resistance of *Staphylococcus epidermidis* to tobramycin.

Another proposed mechanism for biofilm resistance to antimicrobial agents is that biofilm-associated cells grow significantly more slowly than planktonic cells and, as a result, take up antimicrobial agents more slowly. Anwar et al.[34] found that older (10-day-old) chemostat-grown *P. aeruginosa* biofilms were significantly more resistant to tobramycin and piperacillin than younger (2-day-old) biofilms.

Organisms producing biofilms:

Both gram positive as well as gram negative organisms may form biofilms, however CNS have been most commonly reported to be associated with biofilms. Anisio Storti (2005 Brazil)[35] reported biofilm formation by *Staphylococcus intermedius*, *Staphylococcus saprophyticus*, *Acinetobacter baumanii*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Moro et al.[36] observed biofilm formation by CNS, with highest frequency amongst CRBI.

Biofilms on Medical Devices

Biofilms may be forms on a variety of medical devices like - central venous catheters, prosthetic heart valves, urinary (Foley) catheters, contact lenses, intrauterine devices, and dental unit water lines; however this article outlines its importance with respect to catheter related infections, more so, associated with CNS.

Maki [37] noted that central venous catheters (CVCs) pose a greater risk of device-related infection than does any other indwelling medical device, with infection rates of 3 to 5%. Biofilms have been shown by scanning electron microscopy and transmission electron microscopy to be universally present on CVCs and may be associated with either the outside of the catheter or the inner lumen.[2] Organisms that colonize the CVC originate either from the skin insertion site, migrating along the external surface of the device, or from the hub, due to manipulation by health care workers, migrating along the inner lumen.[38] Because the device is in direct contact with the bloodstream, the surface becomes coated with platelets, plasma, and tissue proteins such as albumin, fibrinogen, fibronectin, and laminin.[38] These materials act as “conditioning films”; *S. aureus* adheres to proteins such as fibronectin, fibrinogen, and laminin, and *S. epidermidis* adheres only to fibronectin.[38] Raad et al.[38] also showed that catheters in place for less than 10 days tended to have more extensive biofilm formation on the external surface of the catheter; for longer-term catheters (up to 30 days), biofilms were more extensive on the internal lumen.

Coagulase negative staphylococci: A historical Perspective.

In the past, CNS were considered as harmless skin commensal and dismissed as culture contaminants. The fact that they could be pathogenic was not accepted overnight! The earliest report In 1958, Smith and coworkers noted the potential pathogenicity of CNS by collecting data from patients with septicemia[39] Several years later, Pulverer and Halswick reported on 128 cases of CNS endocarditis[40] while data from 2,276 ventriculoatrial or peritoneal shunt operations and estimated that 8% of the patients acquired shunt infections, with 58% of the cases probably caused by CNS.[41] In 1971, Pulverer and Pillich investigated the incidence of CNS pyogenic infections in Cologne, Germany, presenting

data for the years 1960, 1969, and 1970. CNS were found in about 10% of all pyogenic lesions observed in hospital patients, and in about 50% of these cases, CNS were believed to be present in pure culture.[42] In light of recent advances in staphylococcal systematics and epidemiological typing methods, conclusions concerning the etiology of CNS infections reported prior to the 1980s should be made with some caution. However, during the last decade, considerable progress in the classification of staphylococci and in the development of methods for identifying them at the genus, species, subspecies, and strain levels has been made which have not only made clinicians more aware of the variety of CNS present in clinical specimens, but also enhanced the credibility of CNS as etiologic agents.[43]

Coagulase negative staphylococci and Disease:

S. epidermidis and other CNS are the most frequently reported pathogens in nosocomial blood stream infections.[43] According to the Center for Disease Control and Prevention's National Nosocomial infection surveillance system, *S. epidermidis* is responsible for 33.5% of nosocomial blood stream infections.[44] Unfortunately, nosocomial bacteremia due to *S. epidermidis* is a rapidly increasingly problem.[45,46,47] A study has demonstrated that the isolation of CNS was attributed to the colonization of the implanted catheter since the same microorganism had been isolated from the blood of patients during the preceding weeks, some of them with multiple positive cultures.[48] *S. epidermidis* is the most prominent cause of CRBI. Migration of skin organisms at the insertion site into the cutaneous catheter tract with colonization of the catheter tip is the most common route of infection for peripherally inserted, short-term catheters.[49,50] Contamination of the catheter hub contributes substantially to intraluminal colonization of long-term catheters and implicated as an additional entry point leading to catheter related sepsis justifying local use of antibiotics in preventive control measures.[49]Rarely, catheter might become hematogenous seeding from another focus of infection.[49,50] *S. epidermidis*, are the predominant cause of nosocomial prosthetic valve endocarditis (PVE), can be acquired in the theatre (or shortly thereafter) at the time of the original valve replacement and presents within weeks or more often diagnosed within 60 days after surgery (early onset). The vast majority of CNS causing PVE, when speciated, were *S. epidermidis*. In contrast, when infection involves native valves, only 50% of isolates were *S. epidermidis*.[51] Prosthetic infection can also be acquired from an infected intravascular device. Community acquired endocarditis, which may involve native (usually) or prosthetic valves, is increasingly recognized. The commonest pathogen is *S. epidermidis*, but there are increasing reports of other species, particularly *S. lugdunesis*, which seems to be especially virulent. Some cases of endocarditis following implantation of a prosthetic valve were recently shown to be attributable to polyclonal *S. epidermidis* populations.[52] Therefore, the detection in samples from the same patient of *S. epidermidis* strains with different antibiograms does not necessarily indicate contamination of the samples during collection. Late onset nosocomial neonatal septicemia by CNS, the most common organism accounting for more than 50% cases, show multiple antibiotic resistance including resistance to methicillin.[53,54] There is a clear co-relation between very low birth weight and the risk of a nosocomial infection with CNS.[55] The intensive use of antibiotics in an NICU setting with highly susceptible patients causes selection of multiresistant clones of CNS, which subsequently becomes endemic.[56] *S. epidermidis* distinct clones have become endemic in NICUs as long as a decade and nosocomial transmission plays an important role in *S. epidermidis* bacteremia.[57] Quantitative biofilm production is significantly greater in strains isolated from either the blood

or skin of neonates with *S. epidermidis* bacteremia.[58] Garland JS et al (2008)[59] in a prospective nested cohort (82 neonates) study at a level III NICU performed cultures of peripheral and catheter-drawn blood samples, and quantitative cultures of catheter hub samples if blood stream infection (BSI) was suspected clinically along with semiquantitative cultures of the catheter tip and the catheter hub and the skin at the insertion site when the catheter was removed. Nosocomial BSI was identified in 23 neonates. 15 of these infections, 14 of which were caused by CNS, were considered definite or probable catheter-related BSIs. Catheter-related BSI was intraluminally acquired in 10 (67%) of 15 patients, extraluminally acquired in 3 (20%), and indeterminate in 2 (13%). Thus they concluded that most catheter-related BSIs in neonates with peripherally inserted central venous catheters are caused by CNS and derive from intraluminal contamination.

Coagulase negative staphylococci and Drug Resistance:

Over the last decades, there has been an enormous increase and emergence of CNS strains particularly *S. epidermidis*, *S. haemolyticus* and *S. hominis*, resistant to the antibiotic methicillin, especially in nosocomial settings.[60,61] Detection of resistance to oxacillin in staphylococci is important to guide the therapy and prevent the patient from being unnecessarily treated with vancomycin, which is an antimicrobial agent that presents therapeutic complications, high costs and may lead to the selection of resistant mutants.[62] A Finnish study (1995) reported the percentage of *S. epidermidis* isolates resistant to the 20 tested antibiotics was oxacillin (58%), penicillin (82%), amoxicillin/clavulanic acid (34%), cephalothin (4%), cefuroxime (31%), cefotaxime (20%), imipenem (46%), gentamycin (46%), tobramycin (57%), netilmicin (16%), ciprofloxacin (23%), ofloxacin (21%), erythromycin (36%), fusidic acid (27%), clindamycin (34%), chloramphenicol (19%), rifampin (4%), vancomycin (0%), co-trimaxazole 62%, trimethoprim (53%).[63]

Coagulase negative staphylococci and Biofilm:

S. epidermidis is the most commonly isolated and well characterised CNS associated with CRBI and biofilm formation.[45,64] Minto E C et al (1999 São Paulo, Brazil) studied a total of 126 coagulase-negative staphylococci strains (CNS) isolated from blood samples and from the intravenous catheters and cerebrospinal fluid of 103 patients. *Staphylococcus epidermidis* (68.2%), *S. haemolyticus* (11.1%) and *S. hominis* (3.2%) were the most frequent species. CNS were the agents of infection in 10.7% of the patients and the agents of intravenous catheter colonization in 18.4% of the cases.[48] Recently, the genetic control of the slime production has begun to be elucidated, first in the *S. epidermidis* and then in *Staphylococcus aureus*. Synthesis of the capsular polysaccharide is mediated by the ica operon (intercellular adhesion gene cluster).[65] The adherence process is mediated by polysaccharide intercellular adhesin, which is synthesized by products of the chromosomal ica gene locus, which comprises intercellular adhesion genes (ica A, ica D, ica B, and ica C) organized, in an operon.[66,67,68] Arciola CR et al (2001)[65] studied the presence of icaA and icaD in a collection of 91 staphylococcal (68 *S. epidermidis* and 23 *S. aureus*) strains from intravenous catheter-associated infections along with slime-forming ability on Congo red agar plates; 49% of *S. epidermidis* strains from catheters and, surprisingly, 61% of *S. aureus* strains were icaA and icaD positive and slime forming. Of the 151 isolates of CNS analyzed by Muller E et al (1993)[69] from all clinical infections examined except peritonitis, capsular polysaccharide/adhesin (PS/A) positive isolates bound significantly ($P < 0.001$) more colony-forming units after 15 min to 1.5-cm segments of silicone-elastomer catheter than did PS/A negative isolates. Thus, PS/A expression is common among clinical isolates

of coagulase-negative staphylococci, accounting for most slime-positive and a proportion of slime-negative isolates. Knobloch J.K.M et al (2001)[70] studied that *S. epidermidis* is a common pathogen in medical devices-associated infections and reported that in 11 clinical *S. epidermidis* strains, a restriction fragment length polymorphism of the Sig B operon was detected which was independent of the presence of the ica ADBC locus and a biofilm positive phenotype. Yufeng Yao et al.[71] in their analysis of gene expression in *S. epidermidis* biofilms provided insights into the pathophysiology of *S. epidermidis* biofilms and the role of Phenol-Soluble Modulins (PSMs) in formation of biofilms. They observed decreased production of the proinflammatory PSMs, increased production of specific protective factors and low activity of the quorum-sensing system *agr*, (for *a*ccessory *g*ene *r*egulator) which controls expression of several aggressive virulence factors, including the PSMs. *S. epidermidis* possesses a well-characterized global regulator, particularly cell density–dependent (quorum-sensing) regulatory system known as *agr*, which controls expression of several virulence determinants, including biofilm factors.[72,73] Earlier observations by them[73] demonstrated that the expression of genes in the *agr* operon and of RNAIII, the regulatory molecule of the *agr* system, was significantly lower in biofilms. Other authors have noted transcriptional regulator SarZ as a novel important determinant of biofilm formation and biofilm-associated infection, on the basis of the significant impact of SarZ on the transcription of the biosynthetic operon for biofilm exopolysaccharide.[74] In addition, sarZ influenced the expression of a series of virulence genes, including genes that influence the expression of lipases and proteases, resistance to an important human antimicrobial peptide, and hemolysis.[74] Physiological changes in *S. epidermidis* biofilms thus protect the bacteria by two mechanisms. First, they lower the sensitivity toward harmful molecules, such as antibiotics, antibacterial peptides, and cytokines. Second, they cause a shift to a nonaggressive state, reducing inflammation and the attraction of immune cells to the site of infection. Thus, “immune evasion” by *S. epidermidis* biofilms appears to be based on multiple physiological changes, which underlines the importance of immune-evasion mechanisms during epidermal colonization and biofilm-associated infection by *S. epidermidis.*

Francisco Draz-Mitone et al (1987), studied l7 patients with ventriculoperitoneal shunts infected with coagulase negative staphylococci. Out of 19 episodes 2 episodes of ventriculitis were by slime producing organisms. Pirkko Kotilainen (1990)[45] in his retrospective analysis of 64 CNS strains from 62 adult septicemias reported 34 (53%) adherent slime producers. In comparison, only 142 (29%) of 489 single blood culture isolates were adherent slime producers. ($P < 0.001$). The epidemiologic findings revealed that slime-producing coagulase-negative staphylococci were common in the hospital environment and suggested that epidemic spread of such strains was influenced by antimicrobial therapy. M. G. Ammendolia (1999)[75] noted in their study that out of 115 isolates of *S. epidermidis*, 43 (37.4%) and 16 (13.9%) from i.v. catheter and blood respectively were biofilm producers. Expression of the slime-associated antigen appeared to be species specific and confined to the *Staphylococcus epidermidis* sensu stricto isolates; its strong association with the ability of these strains to produce thicker biofilms indicated slime-associated antigen as a possible virulence marker for *S. epidermidis*. Total 100 invasive, 50 colonizing and 50 commensal CNS isolates were studied by Amita Jain et al (2009). Of 100 invasive isolates 74% (74/100) were biofilm positive while only 68% (34/50) colonizing and 32% (16/50) commensal isolates were biofilm positive. The difference in biofilm production by commensal, colonizing and invasive strains was statistically significant ($p < 0.0001$).[76]

The scanning electron microscope remains the gold standard for identification of biofilms, however, biofilms on CVCs have routinely been detected by a semiquantitative procedure termed the roll-plate technique, in which the distal tip of the catheter is removed aseptically and rolled over the surface of a nonselective medium; a colony count of > 15 colony forming units (CFUs) being regarded as significant.[37,77,78] However, this technique will not detect organisms on the inner lumen of the catheter and is unable to detect more than 1,000 CFU per tip. Other techniques include sonication plus vortexing (Raad et al.)[79], acridine orange staining.(Zufferey et al.)[80] Congo red agar (DJ Freeman et al)[81] method Regardless of the technique used to quantify biofilms, any attempt to relate the occurrence of biofilms with infection should take into consideration the method of blood sampling. Duplicate blood samples should ideally be drawn peripherally (from a vein rather than through the CVC) to ascertain that the organisms in the blood sample have not originated from the device biofilms during sampling.[38]

CONCLUSION

Biofilm formation thus remains the most important mechanism by which the otherwise low virulent commensals like CNS and even other resistant clones of nosocomial pathogens wreath havoc in CRBIs world-wide. Awareness of the latter may help all hospitals to formulate infection control programmes and antibiotic policies. Elucidation of the structure and the genetic mechanisms today provide a beacon light for future strategies to combat the very production of biofilms. The relevance of the age old adage “prevention is better than cure” cannot be overstressed when referring to nosocomial infections It would be appropriate to conclude with a few strategies to prevent CRBIs associated with “biofilm formation” by various organisms. The Association for Vascular Access (AVA) has initiated a program called “SAVE” THAT LINE! Campaign[82] which stands for: Scrupulous hand hygiene before and after contact with all vascular access devices and prior to insertion, Aspetic technique during catheter insertion and care, Vigorous friction to hubs - Vigorous friction with alcohol wherever you make or break a connection to give medications, flush, change tubing or access injection port or add on device and Ensure Patency - flush all lumens with adequate amount of saline or heparinized saline to maintain patency per institution policy. Identification of epitopes in the *S. aureus* fibronectin-binding protein for the generation of adhesion-blocking antibodies[83] may aid in preventing future infections. Prevention of microbial growth on the surface of future intravascular catheters may be mediated by inhibitors of the acyl homoserine lactone-based chemical messengers involved in cell-to-cell signaling that control bacterial gene expression.[84]

REFERENCES

1. Mermel LA, McCormick RD, Springman SR, Maki DG. The pathogenesis and epidemiology of catheter-related infection with pulmonary artery Swan-Ganz catheters: a prospective study utilizing molecular subtyping. *Am J Med* 1991;91(suppl):197S-205S.
2. Raad II, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. *J Infect Dis* 1993;168:400-407.
3. Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients: excess length of stay,

extra costs, and attributable mortality. *JAMA* 1994;271:1598-1601.
4. Jarvis WR, Edwards JR, Culver DH, et al. Nosocomial infection rates in adult and pediatric intensive care units in the United States: National Nosocomial Infections Surveillance System. *Am J Med* 1991;91 (suppl):185S-191S.
5. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis* [serial online] 2002 Sep [cited 2009 October 29]. Available from: URL:http://www.cdc.gov/ncidod/EID/vol8no9/02-0063.htm
6. Jones HC, Roth IL, Saunders WM III. Electron microscopic study of a slime layer. *J Bacteriol* 1969;99:316–25.
7. Characklis WG. Attached microbial growths-II. Frictional resistance due to microbial slimes. *Water Res* 1973;7:1249–58.
8. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. *Sci Am* 1978;238:86–95.
9. Marshall KC. Interfaces in microbial ecology, Harvard University Press, Cambridge, 1976 Mass. p. 44-47.
10. Donlan RM, Costerton JW. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. *Clin Microbiol Rev* 2002;15(2):167-93.
11. Hussain M, Wilcox MH, White PJ. The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. *FEMS Microbiol Rev* 1993;104:191–208.
12. Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 2001;147:3–9.
13. Characklis WG, McFeters GA, Marshall KC. Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; 1990. p. 341–94.
14. Durack DT. Experimental bacterial endocarditis. IV Structure and evolution of very early lesions. *J Pathol* 1975;115:81–9.
15. Costerton, JW, Lewandowski Z, Caldwell DE, Korber DR and Lappin-Scott H.M. Microbial biofilms. *Annu Rev Microbiol* 1995;49:711-745.
16. Fletcher M, Loeb GI. Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. *Appl Environ Microbiol* 1979;37:67–72.
17. Pringle JH, Fletcher M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. *Appl Environ Microbiol* 1983;45:811–17.
18. Bendinger B, Rijnaarts HHM, Altendorf K, Zehnder AJB. Physicochemical cell surface and adhesive properties of coryneform bacteria related to the presence and chain length of mycolic acids. *Appl Environ Microbiol* 1993;59:3973–77.
19. Marsh PD. Dental plaque. In: Lappin-Scott HM, Costerton JW, editors. Microbial biofilms. Cambridge: Cambridge University Press; 1995. p. 282–300.
20. Characklis WG. Microbial fouling. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; 1990. p. 523–84.
21. Rijnaarts HH, Norde W, Bouwer EJ, Lyklema J, Zehnder. Bacterial adhesion under static and dynamic conditions. *Appl Environ Microbiol* 1993;59:3255–65.
22. Fletcher M. The applications of interference reflection microscopy to the study of bacterial adhesion to solid surfaces. In: Houghton DR, Smith RN, Eggins HOW, editors. Biodeterioration 7. London: Elsevier Applied Science; 1988. p. 31–5.
23. Rosenberg M, Kjelleberg S. Hydrophobic interactions in bacterial adhesion. *Adv Microb Ecol* 1986;9:353–93.
24. Ehlers LJ, Bouwer EJ. RP4 plasmid transfer among species of *Pseudomonas*

in a biofilm reactor.*Water Sci Technol* 1999;7:163–171.
25. Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Appl Environ Microbiol* 1999;65:3710–13.
26. Ghigo J-M. Natural conjugative plasmids induce bacterial biofilm development. *Nature* 2001;412:442–5.
27. Balaban N, Giacometti A, Cirioni O, Gov Y, Ghiselli R, Mocchegiani F et al. Use of the Quorum-Sensing Inhibitor RNAIIIInhibiting Peptide to Prevent Biofilm Formation In Vivo by Drug-Resistant *Staphylococcus epidermidis*. *J Infect Dis* 2003;187:625–30.
28. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998;280:295–8.
29. Meluleni G J, Grout M, Evans DJ, and Pier GB. Mucoid *Pseudomonas aeruginosa* growing in a biofilm in vitro are killed by opsonic antibodies to the mucoid exopolysaccharide capsule but not by antibodies produced during chronic lung infection in cystic fibrosis patients. *J Immunol* 1995;155:2029-2038.
30. Yasuda H, Ajiki Y, Aoyama J, and Yokota T. Interaction between human polymorphonuclear leucocytes and bacteria released from in-vitro bacterial biofilm models. *J. Med. Microbiol*. 1994;41:359-367.
31. Shiau AL., and Wu CL. The inhibitory effect of *Staphylococcus epidermidis* slime on the phagocytosis of murine peritoneal macrophages is interferon-independent. *Microbiol Immunol* 1998;42:33-40.
32. Suci, P. A., M. W. Mittelman, F. P. Yu, and G. G. Geesey. Investigation of ciprofloxacin penetration into *Pseudomonas aeruginosa* biofilms. *Antimicrob. Agents Chemother* 1994;38:2125-2133.
33. DuGuid, I. G., E. Evans, M. R. W. Brown, and P. Gilbert. Effect of biofilm culture on the susceptibility of *Staphylococcus epidermidis* to tobramycin. *J Antimicrob Chemother* 1992;30:803-810.
34. Anwar H, Strap JL, Chen K, and Costerton JW. Dynamic interactions of biofilms of mucoid *Pseudomonas aeruginosa* with tobramycin and piperacillin. *Antimicrob. Agents Chemother* 1992;36(6):1208-14.
35. Storti A, Pizzolitto AC; Pizzolitto EL. Detection of mixed microbial biofilms on central venous catheters removed from intensive care unit patients. *Braz. J. Microbiol.* 2005;36:275-280.
36. Moro ML, Viganó EF, Lepri AC. Risk factors for central venous catheter-related infections in surgical and intensive care units. *Infect Control Hosp Epidemiol* 1994;15: 253-264.
37. Maki DK, Stolz SM, Wheeler S, Mermel LA. Prevention of central venous catheter–related bloodstream infection by use of an antiseptic-impregnated catheter: a randomized, controlled trial. *Ann Intern Med* 1997;127:257-266.
38. Raad I. Intravascular-catheter-related infections. *Lancet* 1998;351:893-898.
39. Smith IM, Beals PD, Kingsbury KR, and Hasenclever NF. Observations on Staphylococcus albus septicemia in mice and men. *Arch Intern Med* 1958;102:375-388.
40. Pulverer G and Halswick R. Coagulase-negative Staphylokken (*Staphylococcus albus*) als Krankheitserreger. *Dtsch Med Wochenschr* 1967;92:1141-5.
41. Pulverer G. and Pillich J. Pathogenic significance of coagulase-negative staphylococci. In Finland M., Marget W., and Bartmann K. (eds.). Bacterial infections: changes in their causative agents; trends and possible basis. New York: Springer- Verlag; 1971. p. 91-96.

42. Pulverer, G. On the pathogenicity of coagulase-negative staphylococci. In Jeljaszewics J.(ed.), The Staphylococci: Proceedings of Vth International Symposium on Staphylococci and Staphylococcal Infections. Stuttgart, Germany: Gustav Fischer Verlag; 1985. p. 1-9.
43. Kloos WE, Bannerman TL. Update on clinical significance of coagulase - negative staphylococci. *Clin Microbiol Rev* 1994;7:117-40.
44. U.S. Department o f Health & Human Services, Public Health Service. National nosocomial infections surveillance (NNIS) report, data summary from October 1986-April 1997. *Am J Infect Control* 1997; 25:477-87.
45. Kotilainen P. Association of coagulase negative staphylococcal slime production & adherence with the development & outcome of adult septicemias. *J Clin Microbiol* 1990;28:2779-85.
46. Rupp M E, Archer G L. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis*1994;19:231-45.
47. Banerjee S N, Emori TG, Culver DH, Gaynes R P, Jarvis WR, Horan T et al. The National Nosocomial Infections Surveillance Systm.. Secular trends in nosocomial primary blood stream infections in the United States, 1980-1989. *Am J Med* 1991;91(Supp l. 3B) :3B-86S3B8 9S.
48. Minto EC, Barelli C, Martinez R, da Costa Darini A. Identification & medical importance of coagulase negative staphylococci species. *Sao Paulo Med J* 1999;117(4):175-8.
49. O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG et al. Guidelines for the Prevention of Intravascular Catheter-Related Infections. *Pediatrics* 2002;110(5):e51.
50. Salzman MB, Isenberg HD, Shapiro JF, Lipsitz PJ, Rubin LG. A prospective study of the catheter hub as the portal of entry for micro organisms causing catheter-related sepsis in neonates. *J Infect Dis* 1993; 167:487-90
51. Whitener C, Caputo GM, Weite Kamp MR, Karchmer AW. Endocarditis due to CNS: Microbiologic, epidemiologic and clinical considerations. *Infect Dis Clin North Am*. 1993; 7:81.
52. van Wijngaerden E, Peetermans WE, van Lierde S, van Eldere J. Polyclonal Staphylococcus Endocarditis. *Clin Infect Dis* 1997;25:69 -71.
53. Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, et al. Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1996;129:63-71.
54. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatr* 2002;110:285-91.
55. Khadilkar V, Tudehope D, Fraser S. A prospective study of nosocomial infection in a neonatal intensive care unit. *J Pediatr Child Health* 199 5;31:387 -91.
56. Vermont C L, Hartwig N G, Fleer A , Peter de Man, Verbrugh H, John vanden Anker, et al. Persistence of Clones of Coagulase-Negative Staphylococci among Premature Neonates in Neonatal Intensive Care Units: Two-Center Study of Bacterial Genotyping & Patient Risk Factors: *J Clin Microbiol* 1998; 36(9): 2485-90.
57. Huebner J, Pier GB, Maslow JN, Muller E, Shiro H, Parent M, et al. Endemic nosocomial transmission of *S. epidermidis* bacteremia isolates in a neonatal intensive care unit over 10 years: *J Infect Dis* 199 4 ;169(3):526 -31
58. de Silva GDI, Kantzanou M, Justice A, Massey RC., Wilkinson AR, Day NPJ et al. The ica Operon & Biofilm Production in

Coagulase-Negative Staphylococci Associated with Carriage & Disease in a Neonatal Intensive Care Unit. *J Clin Microbiol* 2002;40(2):382-8.

59. Garland JS, Alex CP, Sevallius JM, Murphy DM, Good MJ, Volberding AM, Hofer LL, Gordon BJ, Maki DG. Cohort study of the pathogenesis and molecular epidemiology of catheter-related bloodstream infection in neonates with peripherally inserted central venous catheters. *Infect Control Hosp Epidemiol* 2008;29(3):243-9.
60. Jaffe R, Lane J.D, Albury S.V, Niemeyer D.M. Rapid extraction from & direct identification in clinical samples of methicillin-resistant staphylococci using the PCR. *J Clin Microbiol* 2000;38:3407-12.
61. Brakstad OG, Maeland JA. Mechanisms of methicillin resistance in staphylococci. *APMIS* 1997;105: 264-76.
62. Marshall SA, Pfaller MA, Jones RN. Ability of the modified vitek card to detect coagulase-negative staphylococci with mecA & oxacillin resistant phenotypes. *J Clin Microbiol* 1999;37:2122-23.
63. Hyvarinen J, Huovinen P, Jarvinen H, Kotilainen P, Finnish Study Group for Antimicrobial Resistance (FiRe) (mukana Katila M-L). Multiresistance in Staphylococcus spp. blood culture isolates in Finland with special reference to t he distribution of the mecA gene among the *Staphylococcus epidermidis* isolates. *APMIS* 1995:103:885-9 1.
64. Mack D. Molecular mechanism of *S. epidermidis* biofilm formation. *J Hosp Infect*1999;43. Suppl:S113-25.
65. Arciola CR, Baldassarri L B, Montanaro L. Presence of ica A & ica D genes & slime production in a collection of staphylococcal strains from catheter-associated infections. *J Clin Microbiol* 2001;39(6):2151-56.
66. Heilmann, C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F. Molecular basis of intercellular adhesion in the biofilm-forming *S. epidermidis*. *Mol Microbiol* 1996;20:1083 -91.
67. Gerke C, Krafts A, Smuth R, O. Schweitzer, F. Gotz. Characterization of the N- acetylglucosaminyl transferase activity involved in the biosynthesis of the *S. epidermidis* polysaccharide intercellular adhesin. *J Biol Chem* 1998;273:18586-94
68. Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H, et al. The intercellular adhesin involved in biofilm accumulation of *S. epidermidis* is a linear 1,6linked glucosaminoglycan: purification & structural analysis. *J Bacteriol* 1996;178:175-83.
69. Muller E, Takeda S, Shiro H, Goldmann D, Pier GB. Occurrence of capsular polysaccharide/adhesin among clinical isolates of coagulase-negative staphylococci. *J Infect Dis* 1993 Nov;168(5):1211-8.
70. Knobloch JKM, Bartscht K, Sabottke A, Rohde H, Fevcht HH, Mack D. Biofilm Formation by *Staphylococcus epidermidis* Depends on Functional RsbU, an Activator of the *sigB* Operon: Differential Activation Mechanisms Due to Ethanol and Salt Stress. *J Bacteriol* 2001;183(8):2624- 33.
71. Yao Y, Sturdevant DE, Otto M. Genomewide Analysis of Gene Expression in *Staphylococcus epidermidis* Biofilms: Insights into the Pathophysiology of *S. epidermidis* Biofilms and the Role of Phenol-Soluble Modulins in Formation of Biofilms. *J Infect Dis* 2005;191:289–98.
72. Vuong C, Gotz F, Otto M. Construction and characterization of an agr deletion mutant of *Staphylococcus epidermidis*. *Infect Immun* 2000; 68:1048–53.
73. Vuong C, Gerke C, Somerville GA, Fischer ER, Otto M. Quorum-sensing control of biofilm factors in *Staphylococcus epidermidis*. *J Infect Dis* 2003;188:706–18.

74. Wang L, Li M, Dong D, Bach THL, Sturdevant DE, Vuong C et al. SarZ Is a Key Regulator of Biofilm Formation and Virulence in *Staphylococcus epidermidis*. *J Infect Dis* 2008;197:1254–62.
75. Ammendolia MG, Di Rosa R, Montanaro L, Arciola CR, Baldassarri L. Slime Production and Expression of the Slime-Associated Antigen by Staphylococcal Clinical Isolates. *J Clin Microbiol* 1999;37(10):3235–8.
76. Jain A, Agarwal A. Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. *J Microbiol Method* 2009;76:88–92.
77. Aufwerber E, Ringertz S, and Ransjo U. Routine semiquantitative cultures and central venous catheter-related bacteremia. *APMIS*1991; 99:627-630.
78. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of Biofilm Formation among the Clinical Isolates of Staphylococci: An Evaluation of Three Different Screening Methods. *Ind J Med Microbiol* 2006;24(1):25-9.
79. Raad II, Sabbagh MF, Rand KH, Sherertz RJ. Quantitative tip culture methods and the diagnosis of central venous catheter-related infections. *Diagn Microbiol Infect Dis* 1992;15:13-20.
80. Zufferey J, Rime R, Francioli P, Bille J. Simple method for rapid diagnosis of catheter-associated infection by direct acridine orange staining of catheter tips. *J Clin Microbiol* 1988;26(2):175-7.
81. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative Staphylococci. *J Clin Pathol* 1989;42:872-4.
82. Association for Vascular Accses [home page on the internet]. Herriman: The Association. c2009. SAVE That Line! Campaign. [one screen] Available from: http://www.avainfo.org/website/article.asp?id=40777.htm
83. Huesca M, Sun Q, Peralta R, Sauder DN, McGavin MJ. Synthetic peptide immunogens elicit polyclonal and monoclonal antibodies specific for linear epitopes in the D motifs of Staphylococcus aureus fibronectin-binding protein, which are composed of amino acids that are essential for fibronectin binding. *Infect Immun* 2000;68:1156-63.
84. Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP. Acyl homoserine-lactone quorum-sensing signal generation. *Proc Natl Acad Sci* 1999;96:4360-5.