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Detection of biofilm formation of a collection of fifty strains of *Staphylococcus aureus* isolated in Algeria at the University Hospital of Tlemcen

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The burden of disease caused by *Staphylococcus aureus* continues to grow; this organism has the ability to form biofilm and it is also a frequent cause of medical device and implant-related infections. The objective of this study was to evaluate the biofilm-forming ability of a collection of clinical isolates of *S. aureus*. In a total of 240 *Staphylococcus* spp. isolated from catheters, retrieved at five services (neonatology, internal medicine, pneumology, pediatric and neurology), only 50 (20.83%) strains were identified by conventional microbiological methods as *S. aureus* species; these strains were screened by microtiter plate assay for detection of biofilm formation. Of the 50 clinical isolates, 16 (32%) were non adherent, 20(40%) weakly, 10 (20%) moderately and 4(8%) strongly adherent. The quantitative method of microtiter plate can be involved as a simple, rapid, inexpensive and reproducible assay to assess biofilm formation which is further an important feature of pathogenecity of *S. aureus* in the clinical setting.

Key words: Microbial biofilm, *Staphylococcus aureus*, catheter, microtiter plate assay.

INTRODUCTION

Staphylococci are most often associated with chronic infections of implanted medical devices (Dunne, 2002; Raad, 2000). Such infections are predominately caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. The first one is known as an ubiquitous bacteria. It also has an inherent ability to form biofilms on biotic and abiotic surfaces (McCann et al., 2008; Begun et al., 2007). The biofilms protect the cells not only from host immune response but also from antimicrobial agents (Donlan et al., 2002). Indeed, biofilm formation is a major concern in nosocomial infections because it protects microorganisms from opsonophagocytosis and antibiotics, leading to chronic infection and sepsis (Martí et al., 2010). These qualities have converged to make *S. aureus* a significant burden on our current health care system (Hobby et al., 2012). One of the patient populations most vulnerable to *Staphylococcus aureus* infection are those with implanted medical devices such as central venous catheters, cardiac valves and pacemakers, artificial joints and various orthopedic devices (Hobby et al., 2012). Therefore, once biofilm-associated *S. aureus* infections occur, they are difficult to be treated by conventional procedures (Trampuz and Widmer, 2006).

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In fact, the biofilm formation involves the production of a polysaccharide intracellular adhesion (PIA) (Ziebuhr et al., 2001; Mack et al., 1996) which is the formal name of slime. This polysaccharide depends on the expression of the intercellular adhesion (*icaADBC)* operon, which encodes three membrane proteins (IcaA, IcaD and IcaC) with enzymatic activity and one extracellular protein (IcaB) (Djordjevic et al., 2002; Christensen et al., 1985). The *icaADBC* gene locus has also been detected in *S. aureus* and a range of other coagulase-negative staphylococci (Allignet et al., 2001; Cramton et al., 1999; Knobloch et al., 2002; McKenney et al., 1999). In addition, several surface proteins have been involved in the biofilm formation process, including biofilm associated protein (BAP) (Cucarella et al., 2001), *S. aureus* surface protein G (SasG) (Montanaro et al., 2011; Corrigan et al., 2007), Fibronectin-binding proteins (FnBPs) (Vergara-Irigaray et al., 2009; O'Neill et al., 2008) or Staphylococcal protein A (Spa). It is now suggested that protein-mediated biofilm formation under in vivo conditions is also an important virulence factor (Merino et al., 2009).

It is estimated that approximately 65% of all bacterial infections in humans are caused by biofilms (Costerton and Stewart, 2000) and Christensen et al. (1982) showed that 63% of the pathogenic strains produced slime, and only 37% of the nonpathogenic strains produced slime (Costerton et al., 1995). In the laboratory, Christensen et al. (1982) demonstrated that only one slime-producing cell per 16 000 non-slime-producing cells results in a culture that produces a gross amount of slime. Furthermore, there is increasing recognition that biofilm growth gives rise to a significant population of bacteria with a diverse set of phenotypes, often termed "variants" (Yarwood et al., 2007). This phenomenon has been explained by the ''insurance hypothesis,'' which posits that the presence of diverse subpopulations increases the range of conditions in which the community as a whole can thrive (McCann, 2000; Yachi and Loreau, 1999).

A biomaterial can be defined as any substance, natural or synthetic, used in the treatment of a patient that at some stage, interfaces with tissue (Wollin et al., 1998). Although, any medical device easily inserted and removed (catheters, contact lenses, endotracheal and nasogastric tubes) or long-term implants (cardiac valves, hip joints and intraocular lenses) represents potentially a favorable support to microbial biofilms formation. Whereas, it is now well documented that biofilms are notoriously difficult to eradicate (Diani et al., 2014) and are often resistant to systemic antibiotic therapy and removal of infected device becomes necessary (Lewis, 2001; Souli and Giamarellou, 1998). Anyway, the skin surrounding the catheter insertion site has been implicated as the most common source of central venous catheters (CVC) colonization (Raad et al., 1993).

In order to study bacterial biofilms, a large variety of experimental direct (including microscopy techniques) and indirect observation methods have been developed. The microtiter plate procedure is an indirect method for estimation of bacteria *in situ* and can be modified for various biofilm formation assays (An and Friedman, 2000). This method has been investigated using many different organisms and stains (Hobby et al., 2012; Ramage et al., 2001; Stepanovic et al., 2000; Christensen et al., 1985; Deighton and Balkau, 1990; Miyake et al., 1992) in which the optical density (OD) of the stained bacterial film is measured with an automatic spectrophotometer.

In this study, we screened our original collection of 50 clinical isolates of *S. aureus* from intravenous catheter-associated infections by the polypropylene microtiter plate method for determining their ability to form biofilm. Parallelly, it is known that the genes that are crucial for biofilm formation are a subset of the genes involved in pathogenesis. This work was realized for the first time at the university hospital of Tlemcen. Our aim was to assess biofilm-forming ability of our collection, knowing that this organism is difficult to control and causes several constraints in different services of the hospital.

MATERIALS AND METHODS

Staphylococcus aureus isolates

In a total of 240 clinical isolates of *Staphylococcus* spp. isolated from catheters from four different services (neonatology, internal medicine, pneumology, pediatric and neurology service) at the university hospital of tlemcen (North-West Algeria) during a period of two years (from 2009 to 2011), 50 strains were identified as *S. aureus* on the basis of standard and conventional microbiological techniques including Gram stain, catalase and coagulase tests. The identification was completed with API Staph gallery (bioMérieux, Marcy l'Etoile, France).

Microtiter plate assays

In the present study, we screened the fifty clinical isolates of *S. aureus* for their ability to form biofilm by microtiter plate method according to the works of Christensen et al. (1985) with some modifications.

Strains from fresh agar plates were inoculated in 3 ml of brain heart infusion (BHI) with 1% glucose (Mathur et al., 2006) and incubated for 24 hours at 37°C in stationary conditions and diluted 1 in 20 with fresh medium. Individual wells of sterile, propylene, 96 well Microplate were filled with 200 µl of the diluted cultures and 200 µl aliquots of only BHI + 1% glucose were dispensed into each of eight wells of the column 12 of microtiter plate to serve as a control (to check non-specific binding and sterility of media). After incubation (24 h at 37°C), the microtiter plates content of each well was removed by tapping the bottom plates. The wells were washed four times with 200 µL of phosphate buffer saline (1 ×PBS pH 7.2) to remove planktonic bacteria. The plates were then inverted and blotted on paper towels and allowed to air dry for 15 min (Broschat et al., 2005). Adherent organisms forming-biofilms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v) (Borucki et al., 2003; Mathur et al., 2006) and allowed to incubate at room temperature for 15 min. After removing the crystal

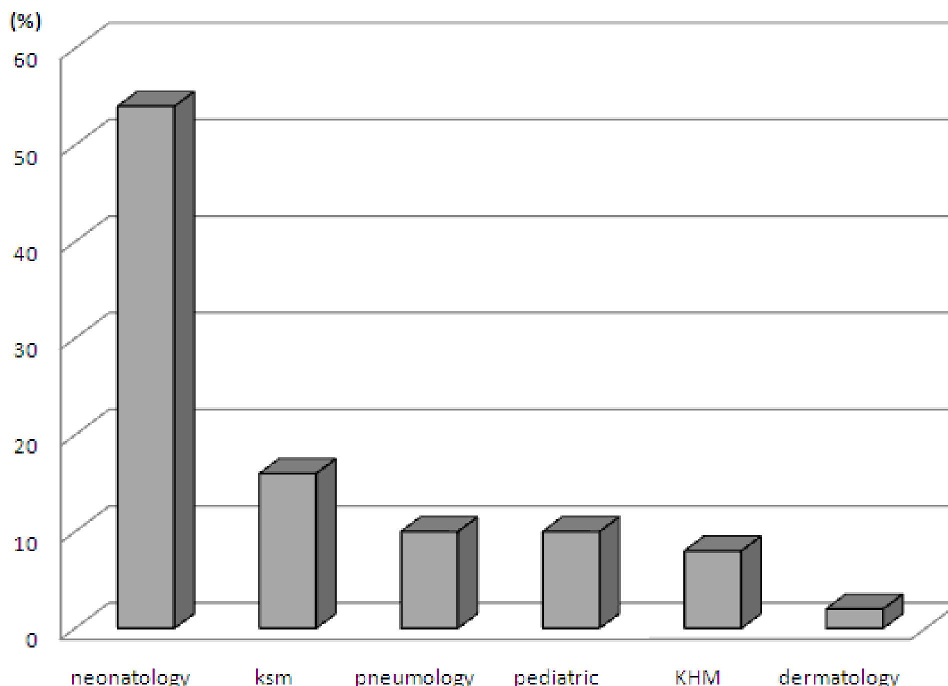

Figure 1. Distribution of the fifty studied clinical isolates of *S. aureus* according to different services of the university hospital of Tlemcen during a period of two years.

violet solution, wells were washed three times with 1 × PBS to remove unbound dye. Finally, all wells were filled by 200 µl ethanol (95%) to release the dye from the cells. Optical density (OD) of stained adherent bacteria was determined with an Absorbance Microplate Reader (model EL×800) at wavelength of 630 nm. To correct background staining, the OD values of the eight control wells were averaged and subtracted from the mean OD value obtained for each strain. The experiment was repeated three times separately for each strain and the average values were calculated with standard deviation (SD).

Classification of adherence

The mean values of OD obtained for blank tests were subtracted from the mean values of OD obtained for each test strain to correct the background staining of microtiter plate. The Absorbance Microplate Reader (model EL×800) used in this study has a dynamic range from 0 to 3.0 OD. According to the classification of Christensen et al. (1985) using the microtiter-plate, strains are divided into three categories: non-adherent, weakly adherent and strongly adherent. However, our clinical isolates were classified into four categories (Stepanovic et al., 2000): non-adherent (OD < ODc); weakly-adherent (ODc < OD < 2xODc); moderately-adherent (2xODc < OD < 4xODc); strongly-adherent (4xODc < OD); with ODc: the cut-off OD (three standard deviations above the mean OD of the blank test). The averaged OD values and standard deviations were made by Excel computer software.

RESULTS

As can be shown in Figure 1, of the fifty (20.83%) clinical strains of *S. aureus*: 27 (54%), 9 (18%), 5 (10%), 5 (10%) and 4 (8%), were respectively isolated from the following services: Neonatology, pneumology, pediatric, neurology, and internal medicine.

The results of microtiter plate assay used for assessment of biofilm-forming ability of the fifty clinical isolates of *S. aureus* are presented in Figure 2. The method applied in this study allowed us to measure biofilm formation after growth in BHI 1% glucose for 24 h at 37 °C. Spectrophotometric measurement of optical densities (OD) of adherent cells enabled us to classify our clinical isolates collection into four categories (Figure 2); non adherent (OD ≤0.2), weakly (0.2<OD≤0.4), moderately (0.4<OD≤0.8) and strongly (0.8<OD) adherent strains (Figure 3). Of the 50 clinical isolates studied, 16 (32%) were designated as non adherent, 20 (40%) as weakly 10 (20%) as moderately and 4 (8%) as strongly adherent.

DISCUSSION

The *Staphylococcus* genus acquires a huge importance in implant-related infections (Campoccia et al., 2006). Elsewhere, the number of diseases caused by *S. aureus* continues to grow. One of the reasons why *S. aureus* is such a ubiquitous pathogen is that it colonizes the anterior nasopharynx in 10 to 40% of humans and can be easily transferred to the skin (Williams, 1963). Biofilm-forming ability is one of the crucial ways that enable this microorganism to express it pathogenecity. It was found

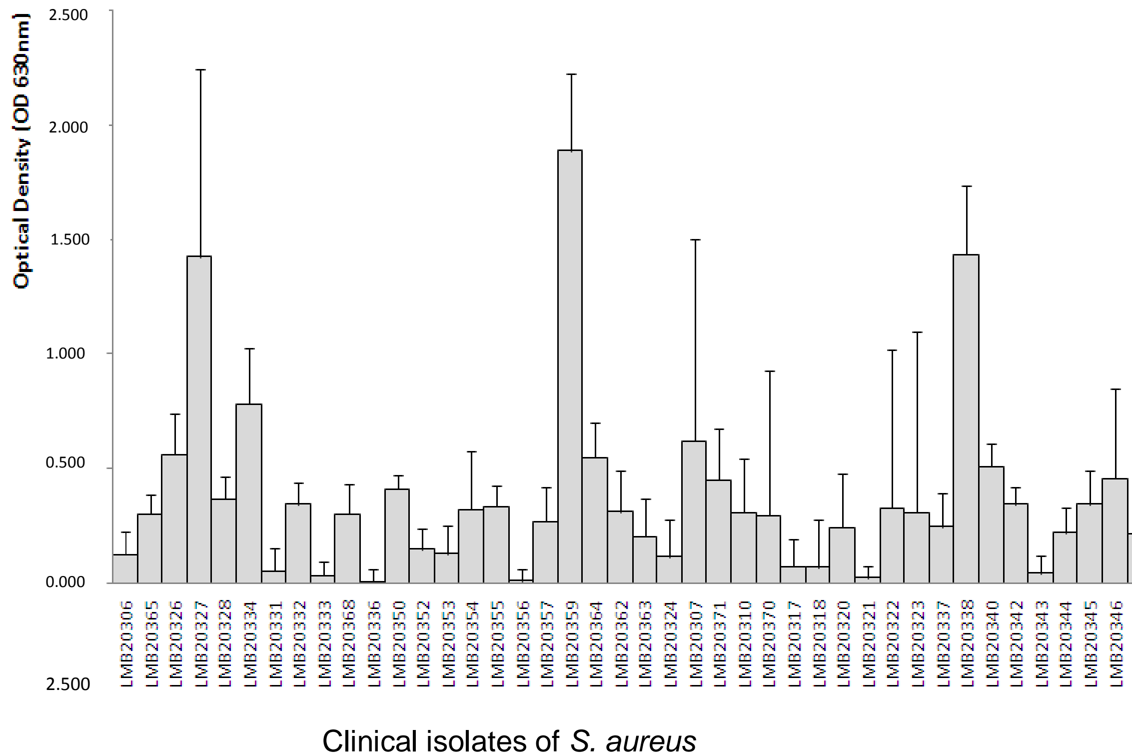

Figure 2. Biofilm-forming ability on polypropylene microtiter plate of the fifty clinical isolates of *S. aureus* following growth for 24 h at 37°C in brain heart infusion 1% glucose. Bars represent mean values of OD (measured at wavelength of 630 nm) and their standard deviations.

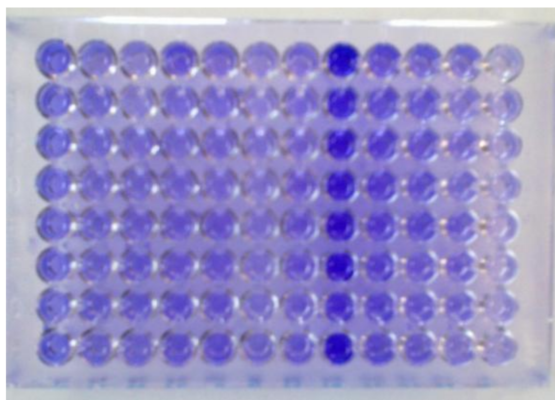

Figure 3. Screening of biofilm formation with crystal violet staining by the 96 well microtiter plate: (I) high, (II) moderate (III) weak and (IV) non adherent.

that the virulence of the organism does indeed vary with its ability to adhere to plastic tissue culture plates (Baddour et al., 1984). Furthermore, as the process of adherence is the initial event in the microbial pathogenesis of infection, failure to adhere will result in removal of the microorganism from the surface of an implanted medical device and avoidance of device-related infection (Ofek and Beachey, 1980). Moreover, biofilm formation by *S. aureus* is influenced by environmental factors like sugars (glucose and/or lactose) or proteases present in the growth medium and depends also on the genetic make-up of a particular *S. aureus* isolate (Melchior et al., 2009). Therefore, according to several researches it was supposed that assessing for biofilm formation could be a useful marker for the pathogenicity of staphylococci. Their active adhesion mechanisms are currently regarded as crucial virulence factors and frequently considered for the characterization of the clinical isolates in studies of molecular pathogenesis and epidemiology (Campoccia et al., 2006). However, some authors considered that there is a little or no correlation between biofilm formation *in vitro* and the clinical outcome of the infection (Kotilainen, 1990; Perdreau-Remington et al., 1998).

In this study, the largest number of clinical isolates of *S. aureus* was collected from neonatology services (n=27), followed by internal medicine (n=9), pneumology and pediatric services (n=5) and finally the neurology services (n=4) (Figure 1). Furthermore, investigation of the correlation between the isolation sites and biofilm-forming ability was not highlighted in this work but it would be efficient to note that among the four strains of *S. aureus* recognized as strongly adherents, two are from the neonatology services.

Various methods have been used to quantify adhesion

of microorganisms to different surfaces. Direct methods allow the *in situ* observation of microbial colonization, including microscopy techniques (laser-scanning confocal, transmission electron and scanning electron microscopy) and indirect methods such as microtiter plate assay, Tube method (TM) and Congo red agar (CRA). Among these various methods, we have used in this study a simple *in vitro* microtiter pate method to quantify the biofilm formation of 50 clinical isolates of *S .aureus*. This method has the advantage of enabling researchers to rapidly analyze adhesion of multiple bacterial strains or growth conditions within each experiment (Djordjevic et al., 2002).

It is known that the direct observation by microscopic techniques is the most important method to study adhesive cells and biofilms, but we think that the microtiter plate assay can be used alternatively as an accurate, rapid, reproducible and inexpensive primer screening method. Thus, this simple quantitative method enables us to assess simultaneously a big number of strains for their biofilm-forming ability. However, in order to complete and enhance the final results obtained in this study, it would be efficient to carry out other experiments, such as PCR for detection of *ica*ADBC genes in the isolates and comparison with the microtiter plate assay results; and animal infection test especially among the four strongly adherent stains to assess the relationship between the biofilm formation and the pathogenicity.

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REFERENCES

Allignet J, Aubert S, Dyke KG, El Solh N (2001). *Staphylococcus caprae* strains carry determinants known to be involved in pathogenicity: a gene encoding an autolysin-binding fibronectin and the *ica* operon involved in biofilm formation. Infect. Immun. 69:712-718.

An YH, Friedman RJ (2000). Handbook of bacterial adhesion: principles, methods, and applications. Humana Press, Totowa, N.J.

Baddour LM, Christensen GD, Hester MG, Bisno AL (1984). Production of experimental endocarditis by coagulase-negative staphylococci: variability in species virulence. J. Infect. Dis. 150:721-727.

Begun J, Gaiani JM, Rohde H, Mack D, Calderwood SB, Ausubel FM, Sifri CD (2007). Staphylococcal biofilm exopolysaccharide protects against Caenorhabditis elegans immune defenses. PLoS Pathog. 3:e57.

Borucki MK, Peppin JD, White D, Loge F, Call DR (2003). Variation in Biofilm Formation among Strains of Listeria monocytogenes. Appl. Environ. Microbiol. 69:7336-7342.

Broschat SL, Call DR, Kuhn EA, Loge FJ (2005). Comparison of the reflectance and Crystal Violet assays for measurement of biofilm formation by Enterococcus. Biofilms. 2:177-181.

Campoccia D, Montanaro L, Renata Arciolaa C (2006). The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials. 27:2331-2339.

Christensen GD, Simpson WA, Bisno AL, Beachey EH (1982). Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. 37:318-326.

Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, Beachey EH (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22:996-1006.

Corrigan RM, Rigby D, Handley P, Foster TJ (2007). The role of *Staphylococcus aureus* surface protein SasG in adherence and biofilm formation. Microbiol. 153:2435-2446.

Costerton JW, Stewart PS (2000). Biofilm and device-related infections. In: Persistent Bacterial Infections (Eds. Nataro, JP, Blaser, MJ and Cunningham- Rundles, S), ASM Press, Washington, pp. 423-439.

Costerton JW, Lewandowski Z, Caldwell D, Korber DR, Lappin-Scott HM (1995). Microbial biofilms. Ann. Rev. Microbiol. 49:711-745.

Cramton SE, Gerke C, Schnell NF, Nichols WW, Goötz F (1999). The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect. Immun. 67:5427-5433.

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

Deighton MA, Balkau B (1990). Adherence measured by microtiter assay as a virulence marker for *Staphylococcus epidermidis* infections. J. Clin. Microbiol. 28:2442-2447.

Diani M, Esiyok OG, Nima Ariafar M, Yuksel FN, Altuntas EG, Akcelik N (2014). The interactions between *esp*, *fsr*, *gelE* genes and biofilm formation and pfge analysis of clinical *Enterococcus faecium* strains. 8(2):129-137.

Djordjevic D, Wiedmann M, McLandsborough LA (2002). Microtiter plate assay for assessment of Listeria monocytogenes biofilm formation. Appl. Environ. Microbiol. 68:2950-2958.

Donlan RM, Costerton JW (2002). Biofilms. Survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15:167-193.

Dunne WM (2002). Bacterial adhesion:Seen any good biofilms lately. *Clin Microbiol Rev*.15:155-166.

Hobby GH, Quave CL, Nelson K, Compadre CM, Beenken KE, Smeltzer MS (2012). Quercus cerris extracts limit *Staphylococcus aureus* biofilm formation. J. Ethnopharmacol. 144:812-815.

Knobloch JKM, Horstkotte MA, Rohde H, Mack D (2002). Evaluation of different detection methods for biofilm formation in *Staphylococcus aureus*. Med. Microbiol. Immunol. 191:101-106.

Kotilainen P (1990). Association of coagulase-negative staphylococcal slime production and adherence with the development and outcome of adult septicemias. J. Clin. Microbiol. 28: 2779-2785.

Lewis K (2001). Riddle of biofilm resistance. Antimicrob. Agents Chemother. 45:999-1007.

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

Martí M, Trotonda MP, Tormo-Más MA, Vergara-Irigaray M, Cheung AL, Lasa I, Penadés JR (2010). Extracellular proteases inhibit protein-dependent biofilm formation in *Staphylococcus aureus*. Microbes Infect. 12:55-64.

McCann KS (2000). The diversity-stability debate. Nature. 405:228-233.

McCann MT, Gilmore BF, Gorman SP (2008). *Staphylococcus epidermidis* devicerelated infections: pathogenesis and clinical management. J. Pharm. Pharmacol. 60:1551-1571.

Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A (2006) Detection Of Biofilm Formation Among The Clinical Isolates Of Staphylococci: An Evaluation Of Three Different Screening Methods. Indian J. Med. Microbiol. 24(1):25-29.

McKenney D, Pouliot KL, Wang Y, Murthy V, Ulrich M, Doring G, Lee JC, Goldmann DA, Pier GB (1999). Broadly protective vaccine for *Staphylococcus aureus* based on an in vivo-expressed antigen. Sci. 284:1523-1527.

Melchior MB, van Osch MHJ, Graat RM, van Duijkeren E, Mevius DJ, Nielen M, Gaastra W, Fink-Gremmels J (2009). Biofilm formation and genotyping of *Staphylococcus aureus* bovine mastitis isolates: Evidence for lack of penicillin resistance in Agr-type II strains. Vet. Microbiol. 137:83-89.

Merino N, Toledo-Arana A, Vergara-Irigaray M, Valle J, Solano C, Calvo E, Lopez JA, Foster TJ, Penades JR, Lasa I (2009). Protein A-

mediated multicellular behavior in *Staphylococcus aureus*. J. Bacteriol. 191:832-843.

Miyake Y, Fujiwara S, Usui T, Suginaka H (1992) Simple method for measuring the antibiotic concentration required to kill adherent bacteria. Chemother. 38:286-290.

Montanaro L, Speziale P, Campoccia D, Ravaioli S, Cangini I, Pietrocola G, et al (2011). Scenery of *Staphylococcus* implant infections in orthopedics. Future Microbiol. 6:1329-1349.

Ofek I, Beachey EH (1980). General concepts and principals of bacterial adherence in animals and man. In Bacterial Adherence, Ed. E.H. Beachey. Chapman and Hall, London.pp.1-29.

O'Neill E, Pozzi C, Houston P, Humphreys H, Robinson DA, Loughman A, Foster TJ, O'Gara JP (2008). A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J. Bacteriol. 190:3835-3850.

Perdreau-Remington F, Sande MA, Peters G, Chambers HF (1998). The abilities of a *Staphylococcus epidermidis* wild-type strain and its slime-negative mutant to induce endocarditis in rabbits are comparable. Infect. Immunol. 66:2778–2781.

Raad I (2000). Management of intravascular catheter–related infection. J Antimicrob. Chemother. 45:267-270.

Raad II, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP (1993). Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. J. Infect. Dis. 168:400-407.

Ramage G, Vande Walle K, Wickes BL, Lôpez-Ribot JL (2001). Standardized method for in vitro antifungal susceptibility testing of Candida albicans biofilms. Antimicrob. Agents Chemother. 45:2475-2479.

Souli M, Giamarellou H (1998). Effects of Slime produced by clinical isolates of coagulase negative staphylococci on activities of various antimicrobial agents. Antimicrob. Agents Chemother. 42:939-941.

Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods .40:175-179.

Trampuz A, Widmer AF (2006). Infections associated with orthopedic implants. Curr Opin Infect Dis. 19:349-356.

Vergara-Irigaray M, Valle J, Merino N, Latasa C, Garcia B, Ruiz de Los Mozos I, Solano C, Toledo-Arana A, Penades JR, Lasa I (2009). Relevant role of FnBPs in *Staphylococcus aureus* biofilm associated foreign-body infections. Infect. Immun. 77: 3978-3991.

Williams R (1963). Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol. Rev. 27:56-71.

Wollin TA, Tieszer C, Riddell JV, Denstedt JD, Reid G (1998). Bacterial biofilm formation, encrustation, and antibiotic adsorption to ureteral stents indwelling in humans. J. Endourol. 12:101-111.

Yachi S, Loreau M (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. USA, 96:1463-1468.

Ziebuhr W, Lößner I, Krimmer V, Hacker J (2001). Methods to detect and analyse phenotypic variation in biofilm-forming staphylococci. Methods Enzymol. 336:195-203.

Yarwood JM, Paquette KM, Tikh IB, Volper EM, Greenberg EP (2007). Generation of Virulence Factor Variants in *Staphylococcus aureus* Biofilms. J. Bacterol. 189:7961-7967.