Therapeutics, Pharmacology and Clinical Toxicology
Vol XIX, Number 2, June 2015
Pages: 45 - 51
© Copyright reserved 2015

REVIEW

QUORUM SENSING AND BIOFILM FORMATION IN *STAPHYLOCOCCUS* SPECIES

Săndulescu Oana[1,2], Streinu-Cercel Anca[1,2], Săndulescu M.[1], Neguț Alina Cristina[1,2], Calistru P.I.[1], Berciu Ioana[1,2], Preoțescu Liliana Lucia[1,2], Streinu-Cercel A.[1,2]

[1] *Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[2] *National Institute for Infectious Diseases "Prof. Dr. Matei Balș", Bucharest, Romania*

Abstract. The burden associated with Staphylococcus species infections has steadily increased in clinical practice in the past few years. Understanding the mechanisms behind bacterial self-regulation is of utmost importance. In this paper, we describe the physiology of quorum sensing in Staphylococcus species, the main mechanism behind microbial cross-talk, coordination, and switching from expression of virulence traits to biofilm formation. We also review the main characteristics behind bacterial adherence and the particularities of biofilm formation in Staphylococcus species, with relevance to osteoarticular infections, dental surgery, cardiac surgery and the use of implanted foreign bodies, electronic devices, or indwelling catheters.
Key words: ???

Introduction

The burden associated with Staphylococcus species infections has been steadily increasing in clinical practice in the past few years, particularly in patients with long-dwelling endovascular catheters or implanted foreign bodies such as joint prostheses. Staphylococcus species can be present in the human body either as commensal, colonizing strains [1,2], or as virulent pathogens, with high prevalence of resistance to antimicrobials [3].

Understanding the mechanisms behind bacterial self-regulation is of utmost importance, especially when these impact the clinical evolution of staphylococcal infections. In this paper, we describe the main mechanisms behind microbial cross-talk, adherence and biofilm formation in Staphylococcus species.

Quorum sensing in *Staphylococcus* species

The pathogenesis of staphylococcal infections is in part driven by the bacteria's ability to communicate with each other and coordinate their actions. This communication process, labeled as quorum sensing, can display inter- or intra-species specificity.

Quorum sensing is a mechanism known to turn on bacterial virulence traits or stimulate bacterial aggregation within sessile populations, i.e. biofilm. It is dependent on bacterial population density, and it can also regulate bacterial morphology, plasmid transfer, or antimicrobial resistance [4]. Quorum sensing requires each bacterial cell to produce and release small molecules such as autoinducing peptides (AIP); in parallel with bacterial multiplication, these peptides accumulate and reach a certain cut-off concentration, determining generalized activation of transcription regulators in the late phase of exponential growth [5].

Staphylococcus species present particular pathways for quorum sensing regulation. For example, the methylthioadenosine/S-adenosylhomocysteine (MTA/SAH) nucleosidase recycles adenine and methionine through methylation reactions, and stimulates production of autoinducer-2 (AI-2), a peptide involved in universal inter-species quorum sensing [6], by the *S. aureus* synthase LuxS [7].

The accessory gene regulator (*agr*) system is involved in virulence control as well as quorum sensing. Its P2 promoter preferentially stimulates the expression of surface adhesins in the initial phases of infection, when bacterial population density is still low, and growth is exponential. When a higher population density is reached, the quorum sensing mechanisms are triggered, leading to a switch to the P3 promoter, and the subsequent expression of exoproteins such as δ-hemolysin, RNAIII [8], and other proteases, lipases and nucleases [9].

The *agrBDCA* operon encodes major determinants of quorum sensing in *Staphylococcus* species, whose expression is regulated by P2 [10]. The pro-peptide AgrD is initially processed by the AgrB endopeptidase

Oana Săndulescu
1 Dr. Calistrat Grozovici St., Bucharest, Romania
e-mail: oanasandulescu1@gmail.com

and then interacts with signal peptidase SpsB [10] to generate AIP [11]. In turn, AIPs bind to AgrC, which acts as both sensor and kinase, and autoinduces its phosphorylation [11] to initiate a downstream cascade regulating the transcription of 200 different genes [12].

Following activation, AgrC phosphorylates and activates AgrA, a response regulator that binds to the specific DNA pattern of the P2-P3 intergenic region [11].

Most staphylococcal species involved in human disease display agr analogues involved in quorum sensing and virulence regulation, i.e., *S. aureus, S. epidermidis, S. intermedius, S. warneri,* and *S. lugdunensis* [5].

In *S. aureus* strains, AgrC displays allelic specificity for AIPs 1 through 4 [11]. In *S. epidermidis* however, a certain degree of interference between AIPs 1 through 3 has been described [10].

Agr activity can be self-regulated, or it can be controlled by various systems, such as the CodY global regulator, which can repress *agr* activity during the exponential growth phase, when population density and nutrient supply are low [11,13]. Under such environmental conditions, CodY can induce biofilm formation, through synthesis of polysaccharide intercellular adhesin (PIA) [13], particularly in *S. epidermidis* and to a lesser extent in *S. aureus*[14].

Prolonged exposure to sublethal concentrations for antimicrobial agents such as ciprofloxacin, mupirocin or rifampin can generate mutations in agr and impair its quorum sensing function, leading instead to a competitive fitness gain [15].

Biofilm rationale

Aggregation within a matrix of extracellular polymeric substance (EPS) allows bacterial populations to avoid the host's immune response by virtually 'hiding' bacterial cells within different layers inside this matrix. The shielded cells are thus protected from host defenses, opsonins [16] and antimicrobial agents, and thrive in an environment that enables the exchange of mobile genetic elements such as plasmids or phages.

Biofilm was classically regarded as a slimy mass of bacterial cells, clumped together and displaying metabolically active, antibiotic resistant bacterial cells on the outer layers, and a gradual decrease in bacterial metabolism and antimicrobial susceptibility as cells advance deeper into the biofilm matrix. However, this is not always the case, as sessile bacterial populations are pleomorphic, and recent studies have shown that nutrients are able to reach the deepest layers within the biofilm through the induction of channels in the extracellular matrix [17].

Biofilm formation requires the adherence of bacterial cells to a host surface or an implanted foreign body. This first step is known as primary attachment and it is followed by the adherence of bacterial cells to each other in the accumulation step, which leads to a multilayered structure that subsequently enters the maturation stage, and is then followed by detachment and dispersal, with septic emboli leading to the colonization of new surfaces where the whole biofilm forming process can start over [18].

Staphylococcus species have a marked capacity to generate biofilm either on host structures, i.e., endothelial lesions, or on foreign bodies, i.e. indwelling catheters. Interestingly, they can also associate with other species within a common biofilm matrix. For example, *S. aureus* and *Pseudomonas aeruginosa* coinfection has been associated with denser aggregates [19], and with 'bystander protection', i.e., an induction of antibiotic tolerance in *S. aureus* to drugs such as gentamicin or tetracycline [20]. *S. aureus* also displays preferential attachment to hyphal forms of *Candida albicans*, leading to synergistic polymicrobial biofilms [21].

Adherence to inert surfaces and in vitro biofilm formation of *Staphylococcus aureus*

Adhesion is a major step of biofilm formation in *S. aureus* [22] and it is enabled by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), namely fibronectin, fibrinogen and collagen [23].

Fibronectin is a host cell ligand that also facilitates the adhesion of *S. aureus* to inert surfaces such as polymethylmethacrylate [24], with increasing relevance in orthopedic surgery [25] and dentistry [26].

Staphylococcal cells classically adhere to each other to form grape-like clusters. Recent studies have identified that these macromolecular structures are encapsulated in an extracellular bacterial product, thus being isolated from the surrounding environment. This tends to happen quickly, in the first 15 minutes after inoculating planktonic bacteria on silicone surfaces [27].

S. aureus synthesizes protein and non-protein based adhesins, involved in adherence to inert surfaces, to proteins of the host cell extracellular matrix, and to other microbial cells, the latter representing a major step in the pathway of biofilm accumulation [28]. Thus, biofilm formation requires PIA [28,29], teichoic and lipoteichoic acid [28], extracellular DNA, serine-aspartate repeat protein C (SdrC), and a large set of surface proteins [29].

Cleavage products such as the N-terminal peptide of AgrD assemble in fibrillar amyloid structures and determine in vitro biofilm formation [30].

Adherence to polymer surfaces is regulated by the two-component system (TCS) autolysis-related locus *arlSR* by modulating the activity of peptidoglycan hydrolase [31].

Bacterial adherence is also influenced by certain characteristics of the prosthetic material. The adherence of *S. aureus* to expanded polytetrafluoroethylene (ePTFE) and teflon polymer plus aluminum reaches its peak at 30 minutes, and is then followed by detachment, while adherence to silicone-based materials tends to increase as a factor of the incubation time [32]. These findings may encourage the use of ePTFE membranes in surgical sites with a high potential of contamination, such as guided bone regenerations in the oral cavity.

Staphylococcal adherence is also higher on polyurethane than on titanium surfaces, potentially impacting the choice of materials for implantable cardiac devices [33,34].

S. aureus and *S. epidermidis* both display significantly

higher adherence to surfaces with the following characteristics: expanded polytetrafluoroethylene polymers, multifilament meshes, increased filament diameter, increased mesh weight, and smaller pore size [35].

A recent study has also found a direct correlation between bacterial inoculum density and adherence to prosthetic biomaterials of *S. aureus* and *S. epidermidis*. However, the same researchers have failed to identify a lower cut-off value that would inhibit bacterial adherence, reporting that an inoculum containing less that 10 bacterial cells is enough to display adherence to inert surfaces [36].

In vivo biofilm formation by *Staphylococcus aureus*

The intercellular adhesion (*ica*) locus drives in vitro and in vivo adherence in staphylococci, and biofilm generated by *S. aureus* displays different characteristics depending on *ica* involvement, i.e., *ica*-dependent and *ica*-independent biofilm [37].

Fibronectin binding protein (FnBP) A, promotes bacterial adherence to fibrinogen through its N2N3 subdomains, thus mediating biofilm formation [38]. The Aaa autolysin/adhesin is also involved in staphylococcal adherence to extracellular matrix proteins such as fibrinogen, fibronectin and vitronectin [39]. The MTA/SAH nucleosidase increases the transcription of the *lytM* and *atlE* genes that code for autolysins, promoting the release of extracellular DNA through autolysis, and stimulating biofilm production in *S. aureus* [40].

The extracellular adherence protein (EAP) is involved in *S. aureus* adherence and aggregation, leading to biofilm formation in the presence of human serum proteins [41], which represent important biofilm cofactors [42].

Beta-hemolysin displays biofilm-ligase activity, apart from its well described role as sfingomyelinase [43,44]. In the presence of DNA, β-hemolysin molecules covalently cross-link, generating a matrix for biofilm accumulation [45].

Biofilm-bound staphylococci display an increased expression of succinate dehydrogenase (sdh) CAB genes, favoring the non-oxidative pathway of the tricarboxylic acid cycle and thus facilitating bacterial growth in environments with suboptimal nutrient and oxygen supply [46].

RsaA is a σB (SigB)-dependent non-coding sRNA regulator that enhances biofilm formation [47]. The TCS regulator system staphylococcal respiratory response AB (*SrrAB*) controls bacterial survival under static biofilm conditions [48] while phenol soluble modulins (PSMs) regulate biofilm structuring, maturation, detachment, and recolonization by generating channels that enable nutrient delivery [17] and bacterial dispersal and dissemination to new areas where adherence and biofilm formation can take place anew [49]. As PSMs are produced via an *agr*-dependent pathway, this quorum sensing system is also involved in biofilm structuring and dispersion [4].

The staphylococcal bone sialoprotein-binding protein (Bbp) enables in vivo adherence to mineralized tissue, binding to bone sialoprotein (BSP), a glycoprotein found in the dentine extracellular matrix [50] and the bone, and thus generates persistent infections such as osteomyelitis [51].

Quantitative PCR studies [52] have identified a time-dependent fluctuation in the expression of biofilm-associated genes, i.e., the RNA expression of elastin binding protein (ebps) is increased 6-fold at 24 and 48 hours, compared to the level at 12 hours [53]; clumping factors A (ClfA) [54] and B (ClfB) [53] bind to fibrinogen in late stages of the adherence process, after an incubation of roughly 24 hours.

Adherence to inert surfaces and in vitro biofilm formation of coagulase-negative staphylococci

Similar to the data presented for *S. aureus*, in coagulase-negative staphylococci (CoNS) adherence is dependent of multiple factors, including bacterial traits, inoculum density [36] and characteristics of the prosthetic surface involved [33,35]. In CoNS however, adherence to inert surfaces correlates with virulence to a greater extent than in *S. aureus* [55].

Staphylococcal adherence to prosthetic surfaces is important not only because it allows the establishment of chronic, hard-to-treat infections, but also because sessile populations are able to avoid the host's immune response, with decreased polymorphonuclear cells activation compared to planktonic cells [56].

The most studied member of the CoNS group is *Staphylococcus epidermidis*. It has been significantly associated with clinically relevant infections in patients with immune deficits and in those with implanted foreign bodies [57,58].

S. epidermidis adheres to inert polystyrene surfaces through adhesins such as: Bap homologue protein (Bhp) [8,22], staphylococcal surface proteins 1 and 2 (Ssp-1 and Ssp-2) [8], serine-aspartate repeat protein f (SdrF) [59], and extracellular DNA [60]. Its AtlE autolysin is involved in adherence to polymers [61].

The *ica* gene, whose role has been discussed above for *S. aureus*, also determines biofilm formation in *S. epidermidis* strains, through PIA processing and involvement of the DNA-binding staphylococcal accessory regulator A (sarA) [62,63], which induces the transcription of *icaADBC* [64]. PIA plays a major role in surface colonization [8], biofilm formation [8,65] (even for small colony variants – SCVs) [66], mediation of biofilm viscoelasticity [67], and avoidance of host immune response [8,65,68].

For *S. lugdunensis* the preliminary steps in adhesion to polystyrene are dependent on iron-regulated surface determinant C (IsdC) [69]. A homologous function is played by the AtlL autolysin in *S. lugdunensis* [70], the AtlWM autolysin in *S. warneri* [70] and the Aas autolysin/adhesin in *S. saprophyticus* [71].

In vivo biofilm formation by coagulase-negative staphylococci

To generate biofilm in vivo, *S. epidermidis* synthesizes adhesins that bind human proteins. Thus, fibrinogen is bound by the Aae autolysin [8,61], fibrinogen-binding protein (Fbe) [8,72], staphylococcal conserved antigen

(ScaA) [8], SdrG [8], and *S. epidermidis* surface protein C (SesC) [73]; fibronectin is bound by the Aae autolysin [8,61], ScaA [8], and teichoic acid [8,74]; elastin by elastin binding protein (Ebp), and collagen by glycerol ester hydrolase (GehD) and SdrF [8].

Similar to its role in *S. aureus*, PIA modulates intracellular adherence and is involved in biofilm accumulation [5]. PSMs also regulate biofilm in CoNS, particularly in S. epidermidis, through biofilm maturation and dispersion [17,49], controlled by *agr* [10].

Bacterial adherence not only represents the first step in biofilm formation, but it is also implicated in initiating the infectious process. Moreover, the same proteins that are involved in adherence can also drive the pathogenesis of staphylococcal infections, as is the case with accumulation-associated protein (Aap) [55]

Conclusions

Staphylococcus aureus and coagulase-negative staphylococci synthetize a myriad of factors involved in adherence to inert surfaces, to human plasma proteins, and to bacterial as well as human cells. They display complex mechanisms for self-regulation and bacterial cross-talk, with serious implications on the infectious process, while quorum sensing allows bacterial cells to coordinate by switching on and off various virulence traits and the capacity for biofilm formation, according to predefined cut-off values for bacterial population density.

ACKNOWLEDGEMENT: *This paper is partially supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.*

References

1. Lucia Preotescu L, Streinu-Cercel O. Prevalence of nasal carriage of S aureus in children**.** *GERMS.* **2013 Mar 5;3(2):49-51.**

2. Mimica MJ, Badue-Pereira MF. Staphylococcus aureus colonization in Brazilian children. *GERMS.* **2014 Mar;4(1):22.**

3. Vamsimohan A, Gupta S, Muralidharan S. Daptomycin resistance in methicillin-resistant Staphylococcus aureus: a report from Southern India. *GERMS.* **2014 Sep;4(3):70-2.**

4. Singh R, Ray P. Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. *Future Microbiol.* **2014;9(5):669-81.**

5. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev.* **2014 Oct;27(4):870-926.**

6. Parveen N, Cornell KA. Methylthioadenosine/S-adenosylhomocysteine nucleosidase, a critical enzyme for bacterial metabolism. *Mol Microbiol.* **2011 Jan;79(1):7-20.**

7. Bao Y, Li Y, Jiang Q, Zhao L, Xue T, Hu B, et al. Methylthioadenosine/S-adenosylhomocysteine nucleosidase (Pfs) of Staphylococcus aureus is essential for the virulence independent of LuxS/AI-2 system. *Int J Med Microbiol.* **2013 May;303(4):190-200.**

8. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. *Seventh edition ed: Churchill Livingstone. Elsevier;* **2010.**

9. Mitchell G, Fugere A, Pepin Gaudreau K, Brouillette E, Frost EH, Cantin AM, et al. SigB is a dominant regulator of virulence in Staphylococcus aureus small-colony variants. *PLoS One.* **2013;8(5):e65018.**

10. Olson ME, Todd DA, Schaeffer CR, Paharik AE, Van Dyke MJ, Buttner H, et al. Staphylococcus epidermidis agr quorum-sensing system: signal identification, cross talk, and importance in colonization. *J Bacteriol.* **2014 Oct;196(19):3482-93.**

11. Painter KL, Krishna A, Wigneshweraraj S, Edwards AM. What role does the quorum-sensing accessory gene regulator system play during Staphylococcus aureus bacteremia? *Trends Microbiol.* **2014 Dec;22(12):676-85.**

12. O'Rourke JP, Daly SM, Triplett KD, Peabody D, Chackerian B, Hall PR. Development of a Mimotope Vaccine Targeting the Staphylococcus aureus Quorum Sensing Pathway. *PLoS One.* **2014;9(11):e111198.**

13. Majerczyk CD, Sadykov MR, Luong TT, Lee C, Somerville GA, Sonenshein AL. Staphylococcus aureus CodY negatively regulates virulence gene expression. *J.Bacteriol.* **2008 Apr;190(7):2257-65.**

14. Fluckiger U, Ulrich M, Steinhuber A, Doring G, Mack D, Landmann R, et al. Biofilm formation, icaADBC transcription, and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. *Infect Immun.* **2005 Mar;73(3):1811-9.**

15. Paulander W, Nissen Varming A, Baek KT, Haaber J, Frees D, Ingmer H. Antibiotic-mediated selection of quorum-sensing-negative Staphylococcus aureus. *MBio.* **2013;3(6):e00459-12.**

16. Cerca N, Jefferson KK, Oliveira R, Pier GB, Azeredo J. Comparative antibody-mediated phagocytosis of Staphylococcus epidermidis cells grown in a biofilm or in the planktonic state. *Infect Immun.* **2006 Aug;74(8):4849-55.**

17. Periasamy S, Chatterjee SS, Cheung GY, Otto M. Phenol-soluble modulins in staphylococci: What are they originally for? *Commun Integr Biol.* **2012 May 1;5(3):275-7.**

18. Van Mellaert L, Shahrooei M, Hofmans D, Eldere JV. Immunoprophylaxis and immunotherapy of Staphylococcus epidermidis infections: challenges and prospects. *Expert Rev Vaccines.* **2012 Mar;11(3):319-34.**

19. Kumar A, Peng TY. Presence of Pseudomonas aeruginosa influences biofilm formation and surface protein expression of Staphylococcus aureus. *Environ Microbiol.* **2015 Apr 28.**

20. DeLeon S, Clinton A, Fowler H, Everett J, Horswill AR, Rumbaugh KP. Synergistic interactions

of Pseudomonas aeruginosa and Staphylococcus aureus in an in vitro wound model. *Infect Immun.* **2014 Nov;82(11):4718-28.**

21. Zago CE, Silva S, Sanita PV, Barbugli PA, Dias CM, Lordello VB, et al. Dynamics of Biofilm Formation and the Interaction between Candida albicans and Methicillin-Susceptible (MSSA) and -Resistant Staphylococcus aureus (MRSA). *PLoS One.* **2015;10(4):e0123206.**

22. Tormo MA, Knecht E, Gotz F, Lasa I, Penades JR. Bap-dependent biofilm formation by pathogenic species of Staphylococcus: evidence of horizontal gene transfer? *Microbiology.* **2005 Jul;151(Pt 7):2465-75.**

23. Foster TJ, Hook M. Surface protein adhesins of Staphylococcus aureus. *Trends Microbiol.* **1998 Dec;6(12):484-8.**

24. Vaudaux P, Suzuki R, Waldvogel FA, Morgenthaler JJ, Nydegger UE. Foreign body infection: role of fibronectin as a ligand for the adherence of Staphylococcus aureus. *J.Infect Dis.* **1984 Oct;150(4):546-53.**

25. Paulus AC, Franke M, Kraxenberger M, Schroder C, Jansson V, Utzschneider S. PMMA Third-Body Wear after Unicondylar Knee Arthroplasty Decuples the UHMWPE Wear Particle Generation In Vitro. *Biomed Res Int.* **2015;2015:575849.**

26. Nakagawa K, Saita M, Ikeda T, Hirota M, Park W, Chang-Il Lee M, et al. Biocompatibility of 4-META/MMA-TBB resin used as a dental luting agent. *J Prosthet Dent.* **2015 Apr 13.**

27. Hess DJ, Henry-Stanley MJ, Barnes AM, Dunny GM, Wells CL. Ultrastructure of a novel bacterial form located in Staphylococcus aureus in vitro and in vivo catheter-associated biofilms. *J Histochem Cytochem.* **2012 Oct;60(10):770-6.**

28. Heilmann C. Adhesion mechanisms of staphylococci. *Adv Exp Med Biol.* **2011;715:105-23.**

29. Barbu EM, Mackenzie C, Foster TJ, Hook M. SdrC induces staphylococcal biofilm formation through a homophilic interaction. *Mol Microbiol.* **2014 Oct;94(1):172-85.**

30. Schwartz K, Sekedat MD, Syed AK, O'Hara B, Payne DE, Lamb A, et al. The AgrD N-terminal leader peptide of Staphylococcus aureus has cytolytic and amyloidogenic properties. *Infect Immun.* **2014 Sep;82(9):3837-44.**

31. Fournier B, Hooper DC. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of Staphylococcus aureus. *J Bacteriol.* **2000 Jul;182(14):3955-64.**

32. Brewer AR, Stromberg BV. In vitro adherence of bacteria to prosthetic grafting materials. *Ann Plast Surg.* **1990 Feb;24(2):134-8.**

33. Viola GM, Rosenblatt J, Raad, II, Darouiche RO. Comparison of bacterial adherence to titanium versus polyurethane for cardiac implantable electronic devices. *Am J Cardiol.* **2013 Jun 15;111(12):1764-6.**

34. Perez-Jorge C, Conde A, Arenas MA, Perez-Tanoira R, Matykina E, de Damborenea JJ, et al. In vitro assessment of Staphylococcus epidermidis and Staphylococcus aureus adhesion on TiO(2) nanotubes on Ti-6Al-4V alloy. *J Biomed Mater Res A.* **2012 Jul;100(7):1696-705.**

35. Sanders D, Lambie J, Bond P, Moate R, Steer JA. An in vitro study assessing the effect of mesh morphology and suture fixation on bacterial adherence. *Hernia.* **2013 Dec;17(6):779-89.**

36. Sanders DL, Kingsnorth AN, Lambie J, Bond P, Moate R, Steer JA. An experimental study exploring the relationship between the size of bacterial inoculum and bacterial adherence to prosthetic mesh. *Surg Endosc.* **2013 Mar;27(3):978-85.**

37. Ferreira FA, Souza RR, Bonelli RR, Americo MA, Fracalanzza SE, Figueiredo AM. Comparison of in vitro and in vivo systems to study ica-independent Staphylococcus aureus biofilms. *J Microbiol Methods.* **2012 Mar;88(3):393-8.**

38. Geoghegan JA, Monk IR, O'Gara JP, Foster TJ. Subdomains N2N3 of fibronectin binding protein A mediate Staphylococcus aureus biofilm formation and adherence to fibrinogen using distinct mechanisms. *J Bacteriol.* **2013 Jun;195(11):2675-83.**

39. Hirschhausen N, Schlesier T, Peters G, Heilmann C. Characterization of the modular design of the autolysin/adhesin Aaa from Staphylococcus aureus. *PLoS One.* **2012;7(6):e40353.**

40. Bao Y, Zhang X, Jiang Q, Xue T, Sun B. Pfs promotes autolysis-dependent release of eDNA and biofilm formation in *Staphylococcus aureus. Med Microbiol Immunol.* **2014 Sep 4.**

41. Thompson KM, Abraham N, Jefferson KK. *Staphylococcus aureus* extracellular adherence protein contributes to biofilm formation in the presence of serum. *FEMS Microbiol Lett.* **2010 Apr;305(2):143-7.**

42. Wagner C, Aytac S, Hansch GM. Biofilm growth on implants: bacteria prefer plasma coats. *Int J Artif Organs.* **2011 Sep;34(9):811-7.**

43. Foster TJ. The Staphylococcus aureus "superbug". *J Clin Invest.* **2004 Dec;114(12):1693-6.**

44. Bronner S, Monteil H, Prevost G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. *FEMS Microbiol Rev.* **2004 May;28(2):183-200.**

45. Huseby MJ, Kruse AC, Digre J, Kohler PL, Vocke JA, Mann EE, et al. Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. *Proc Natl Acad Sci USA.* **2010 Aug 10;107(32):14407-12.**

46. Gaupp R, Schlag S, Liebeke M, Lalk M, Gotz F. Advantage of upregulation of succinate dehydrogenase in Staphylococcus aureus biofilms. *J Bacteriol.* **2010 May;192(9):2385-94.**

47. Fechter P, Caldelari I, Lioliou E, Romby P. Novel aspects of RNA regulation in *Staphylococcus aureus.*

FEBS Lett. **2014 Aug 1;588(15):2523-9.**

48. Kinkel TL, Roux CM, Dunman PM, Fang FC. The *Staphylococcus aureus* SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. *MBio.* **2013;4(6):e00696-13.**

49. Li S, Huang H, Rao X, Chen W, Wang Z, Hu X. Phenol-soluble modulins: novel virulence-associated peptides of staphylococci. *Future Microbiol.* **2014;9(2):203-16.**

50. Tung H, Guss B, Hellman U, Persson L, Rubin K, Ryden C. A bone sialoprotein-binding protein from Staphylococcus aureus: a member of the staphylococcal Sdr family. *Biochem J.* **2000 Feb 1;345 Pt 3:611-9.**

51. Vazquez V, Liang X, Horndahl JK, Ganesh VK, Smeds E, Foster TJ, et al. Fibrinogen is a ligand for the Staphylococcus aureus microbial surface components recognizing adhesive matrix molecules (MSCRAMM) bone sialoprotein-binding protein (Bbp). *J Biol Chem.* **2011 Aug 26;286(34):29797-805.**

52. Horosanu GC. Evaluation of bacterial internalization using qRT-PCR. GERMS. 2014 Jun;4(2):46.

53. Atshan SS, Shamsudin MN, Karunanidhi A, van Belkum A, Lung LT, Sekawi Z, et al. Quantitative PCR analysis of genes expressed during biofilm development of methicillin resistant Staphylococcus aureus (MRSA). *Infect Genet Evol.* **2013 Aug;18:106-12.**

54. Ythier M, Resch G, Waridel P, Panchaud A, Gfeller A, Majcherczyk P, et al. Proteomic and transcriptomic profiling of Staphylococcus aureus surface LPXTG-proteins: correlation with agr genotypes and adherence phenotypes. *Mol Cell Proteomics.* **2012 Nov;11(11):1123-39.**

55. Schaeffer CR, Woods KM, Longo GM, Kiedrowski MR, Paharik AE, Buttner H, et al. Accumulation-associated protein (Aap) enhances Staphylococcus epidermidis biofilm formation under dynamic conditions and is required for infection in a rat catheter model. *Infect Immun.* **2014 Oct 20.**

56. Riber U, Espersen F, Kharazmi A. Comparison of adherent and non-adherent staphylococci in the induction of polymorphonuclear leukocyte activation in vitro. *APMIS.* **1995 Jun;103(6):439-46.**

57. Rahman A, Hossain MA, Paul SK, Sultana S, Haque N, Kabir MR, et al. Methicillin-resistant coagulase-negative staphylococci (MRCoNS) by disk diffusion method. *Mymensingh Med J.* **2013 Apr;22(2):229-31.**

58. Ojo SK, Sargin BO, Esumeh FI. Plasmid curing analysis of antibiotic resistance in beta-lactamase producing Staphylococci from wounds and burns patients. *Pak J Biol Sci.* **2014 Jan 1;17(1):130-3.**

59. Toba FA, Visai L, Trivedi S, Lowy FD. The role of ionic interactions in the adherence of the Staphylococcus epidermidis adhesin SdrF to prosthetic material. *FEMS Microbiol Lett.* **2013 Jan;338(1):24-30.**

60. Qin Z, Ou Y, Yang L, Zhu Y, Tolker-Nielsen T, Molin S, et al. Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis. Microbiology.* **2007 Jul;153(Pt 7):2083-92.**

61. Heilmann C, Thumm G, Chhatwal GS, Hartleib J, Uekotter A, Peters G. Identification and characterization of a novel autolysin (Aae) with adhesive properties from *Staphylococcus epidermidis. Microbiology.* **2003 Oct;149(Pt 10):2769-78.**

62. Satorius AE, Szafranski J, Pyne D, Ganesan M, Solomon MJ, Newton DW, et al. Complement c5a generation by staphylococcal biofilms. *Shock.* **2013 Apr;39(4):336-42.**

63. Tormo MA, Marti M, Valle J, Manna AC, Cheung AL, Lasa I, et al. SarA is an essential positive regulator of Staphylococcus epidermidis biofilm development. *J Bacteriol.* **2005 Apr;187(7):2348-56.**

64. Beenken KE, Blevins JS, Smeltzer MS. Mutation of sarA in Staphylococcus aureus limits biofilm formation. *Infect Immun.* **2003 Jul;71(7):4206-11.**

65. Fredheim EG, Granslo HN, Flaegstad T, Figenschau Y, Rohde H, Sadovskaya I, et al. Staphylococcus epidermidis polysaccharide intercellular adhesin activates complement. *FEMS Immunol Med Microbiol.* **2011 Nov;63(2):269-80.**

66. Al Laham N, Rohde H, Sander G, Fischer A, Hussain M, Heilmann C, et al. Augmented expression of polysaccharide intercellular adhesin in a defined Staphylococcus epidermidis mutant with the small-colony-variant phenotype. *J Bacteriol.* **2007 Jun;189(12):4494-501.**

67. Ganesan M, Stewart EJ, Szafranski J, Satorius AE, Younger JG, Solomon MJ. Molar mass, entanglement, and associations of the biofilm polysaccharide of *Staphylococcus epidermidis. Biomacromolecules.* **2013 May 13;14(5):1474-81.**

68. Vuong C, Voyich JM, Fischer ER, Braughton KR, Whitney AR, DeLeo FR, et al. Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. *Cell Microbiol.* **2004 Mar;6(3):269-75.**

69. Missineo A, Di Poto A, Geoghegan JA, Rindi S, Heilbronner S, Gianotti V, et al. IsdC from Staphylococcus lugdunensis induces biofilm formation under low-iron growth conditions. *Infect Immun.* **2014 Jun;82(6):2448-59.**

70. Hussain M, Steinbacher T, Peters G, Heilmann C, Becker K. The adhesive properties of the Staphylococcus lugdunensis multifunctional autolysin AtlL and its role in biofilm formation and internalization. *Int J Med Microbiol.* **2014 Nov 27.**

71. Hell W, Meyer HG, Gatermann SG. Cloning of aas, a gene encoding a Staphylococcus saprophyticus surface protein with adhesive and autolytic properties. *Mol Microbiol.* **1998 Aug;29(3):871-81.**

72. Guo B, Zhao X, Shi Y, Zhu D, Zhang Y. Pathogenic implication of a fibrinogen-binding protein of Staphylococcus epidermidis in a rat model of intravascular-catheter-associated infection. *Infect Immun.* **2007 Jun;75(6):2991-5.**

73. Shahrooei M, Hira V, Stijlemans B, Merckx R, Hermans PW, Van Eldere J. Inhibition of Staphylococcus epidermidis biofilm formation by rabbit polyclonal antibodies against the SesC protein. *Infect Immun.* **2009 Sep;77(9):3670-8.**

74. Holland LM, Conlon B, O'Gara JP. Mutation of tagO reveals an essential role for wall teichoic acids in Staphylococcus epidermidis biofilm development. *Microbiology.* **2011 Feb;157(Pt 2):408-18.**