II. Introduction

Members of the genus *Staphylococcus* are gram-positive cocci (0.5 to 1.5 µm in diameter) that occur in pairs, tetrads, short chain, and irregular "grape-like" clusters. Staphylococci are nonmotile, nonsporeforming, and usually catalase positive, and unencapsulated or have limited capsule formation. Most species are facultative anaerobes. The cell wall of these bacteria contains peptidoglycan and teichoic acid. The diamino acid present in the peptidoglycan is L-lysine. The interpeptide bridge of the peptidoglycan consists of oligoglycine peptides which are susceptible to the action of lysostaphin.

Strains of this bacteria are widespread in nature. Currently, there are 33 species recognized in the genus *Staphylococcus*, and about one-half of these are indigenous to human. The largest populations of human staphylococci are usually found in regions of the skin and mucous membranes surrounding openings to the body surface [1]. The most important pathogenic species are *S. aureus*, *S. epidermidis*, and *S. saprophyticus*.

1. *Staphylococcus aureus* as a human pathogen:

S. aureus as a coagulase positive species has long been recognized as a common causative pathogen in many human diseases. It has been reported that the infections caused by these bacteria have severe consequences despite antibiotic therapies [2-6]. *S. aureus* produces a wide variety of exoproteins that contribute to its ability to colonize and cause diseases in mammalian hosts. Nearly all strains secrete exoenzymes and cytotoxins which include alpha, beta, gamma and delta toxins, in addition to nuclease, protease, lipase, hyaluronidase, and collagenase. Some strains produce one or more additional exoproteins, which include toxic shock syndrome toxin-1, the staphylococcal enterotoxins, the exofoliative toxins, and leukocidin. Each of these toxins is known to have potent effects on cells of the immune system, but many of them have other biological effects as well [7, 8]. TSST-1 and staphylococcal enterotoxins are also known as pyrogenic toxin superantigens (PTSAgs). They have been implicated in the pathogenesis of several acute or chronic human diseases [9-11]. By activating autoreactive T-cell clones, PTSAgs could theoretically induce autoimmune diseases in humans. In addition, TSST-1 reactivates bacterial cell wall-induced arthritis in rats, suggesting that this toxin may play a role in recurrent arthritides [7].

It is now recognised that pathogenic Gram-positive bacteria express on their surfaces, proteins which contribute to virulence. They do so by promoting adherence to host cells and / or tissue components and by binding soluble components of plasma such as albumin and immunoglobulins to allow evasion of immune responses and avoidance of phagocytosis. In *S. aureus*, the genes for the immunoglobulin binding protein, protein A, the fibronectin binding proteins, the collagen binding protein and the fibrinogen binding protein have been sequenced. Protein A is the archetypal cell wall associated protein of *S. aureus* and generally of gram-positive bacteria. It comprises five repeated units of 58 amino acid residues each of which can bind to the Fc region of IgG [12]. It was suggested that protein A (Spa) and immunoglobulin binding proteins from other pathogens promote coating of the bacterial surface with IgG molecules attached by the Fc region which could not be recognisable by the Fc receptors on leucocytes. The coating of IgG could also mask binding sites for opsonins present in normal serum. Infection experiments in mice with the protein A-deficient mutant showed that protein A is a virulence factor [13-18]. The ability of *S. aureus* to adhere to biomaterial surfaces that have become coated with host plasma and matrix proteins such as fibrinogen and fibronectin is a major determinant for initiating a foreign body infection. Fibronectin is also found on the surface of epithelial cells and may be a factor in promoting bacterial colonization in peritonitis, invasive endocarditis and ruminant mastitis [19]. Mutants with specific insertions in *fnbA* and *fnbB* (coding for fibronectin binding proteins) had strongly inhibited the ability of *S. aureus* to bind to Fn-coated plastic surfaces [20]. *S. aureus* forms clumps in plasma due to its ability to bind fibrinogen, a multisubunit glycoprotein that is proteolytically converted into fibirin, a major component in blood clots. Fibrinogen is deposited in large quantities at wound sites and is quickly deposited on synthetic material such as catheters [21]. The clfA gene encodes a fibrinogen-binding protein is with an apparent molecular mass of 130 kDa. The putative ClfA protein has features that suggest that it is associated with the cell surface. Furthermore it contains a novel 308 residue region comprising dipeptide repeats predominantly of Asp and Ser ending 28 residues upstream from the LPXTG motif common to wall-associated proteins. Moreover, the ClfA mutants are completely defective in adherence [22]. Collagen-rich tissues, as bone and cartilage, that are the preferential sites of staphylococcal infections, are also the tissues that harbour orthopaedic implants. These can be easily coated in vivo by collagen and thus become prone to adhesion of Staphylococci strains which carry the collagen adhesin gene (cna) [23]. This protein has features at the C-terminus typical of cell wall-associated proteins. Both anti-Cna antibodies and recombinant Cna protein blocked adherence of bacteria to cartilage and to collagen

substrates. In addition, a null mutant in *cna* isolated by allelic replacement was less virulent than the parental strain in a mouse model for septic arthritis [24].

The expression of these virulence factors is growth phase dependent and is mediated in part by at least two global regulatory elements, the accessory gene regulator (*agr*) and the Staphylococcal accessory regulator (*sar*) [25-33]. The *agr* operon encodes four protein products (Agr A-D) comprising a quorum-sensing apparatus that is homologous to many two-component signal transduction systems found in prokaryotic organisms. AgrD is exported from the cell by the membrane bound AgrB protein. AgrD serves as a peptide pheromone and is specifically recognized by the AgrC membrane bound receptor. Once the extracellular concentration of AgrD reaches a particular level, AgrC initiates a signal transduction pathway that is believed to induce AgrA. By a mechanism that has yet to be elucidated, cytoplasmic AgrA is thought to activate the expression of the *agr* operon (RNAII) and the divergently expressed RNAIII [33, 34]. RNAIII is the effector molecule of this system, and highest expression in broth culture is found in the late-logarithmic phase [35, 36]. The RNAIII of the agr system positively regulates genes encoding some proteins produced after logarithmic phase (nuclease, staphylokinnase, lipase, phospholipase C, serine protease and hyaluronidase), and negatively regulates genes encoding the production of fibronectin-binding proteins, vitronectin-binding protein, CF, Protein A, and coagulase [37, 38]. The *sar* locus is composed of three overlapping transcripts, designated *sarA*, (0.56 kb), *sarC* (0.8 kb), and *sarB* (1.2 kb), initiated from the P1, P3, and P2 promoters, respectively. Because of this multiplicity of promotors, the activation of *sar* leading to the expression of SarA, the major *sar* regulatory molecule, is complex and may be growth phase dependent. Whereas the *sarB* transcript and the more abundant SarA transcripts are maximally expressed during the exponential phase. The transcription of *sarC* from the P3 promoter is most active during postexponential phase [39]. Molecular analysis indicates that the larger *sarB* transcript is essential for full expression of RNAII and RNAIII, while the shorter *sarA* and *sarC* transcript only partially restored *agr*–related transcription. It is likely that *agr* activation is partially mediated by the binding of the *sar* gene product (s) to the *agr* promoter [29, 33, 40].

These modulators may either directly interact with the target gene (e.g., RNAIII with *hla* mRNA) or control another regulatory molecule (e.g., *sar* regulation of the *agr* gene product) which, in turn, alter the transcription of the target gene. Alpha-hemolysin is so far the most detailed examined *S. aureus* cytotoxin. A high percentage of staphylococcal strains produce this toxin, and it is toxic to a wide range of mammalian cells. It is particularly active against rabbit erythrocytes, and it is also dermonecrotic and neurotoxic. It has been investigated that

the *sar* locus activates the synthesis of alpha-hymolysin at the transcriptional level, presumably in part by the interaction of SarA with the *agr* locus [28], but regulation of the collagen adhesin gene by *sar* is agr-independent [30]. Both transcriptional gene fusion and Western analysis revealed that sarA up-regulates both toxic shock syndrome toxin 1 gene (tst) expression and staphylococcal enterotoxin B production, respectively [41].

In bacteria, alternative sigma factors of the RNA polymerase are known to play a crucial role in regulating gene expression upon major changes in the environment. DNA sequence analysis of the *S. aureus sigB* operon revealed four complete open reading frames (*rsbU, rsbV, rsbW,*, and *sigB*) with significant predicted amino acid homology and gene arrangement to *rsbU, rsbV, rsbW,* and *sigB* in *B. subtilis* as summarized in figure 1 [42].

The similar organization of the *sigB* operons in *S. aureus* and *B. subtilis* suggests analogous roles for the *rsbV* regulatory proteins in addition to *rsbW*. In *B. subtilis*, *rsbV* is an anti-anti-sigma factor which competes with SigB for binding to anti-sigma factor rsbW a mechanism dubbed partner switching [43]. During the normal exponential phase, rsbW inactivates *rsbV* by phospholylation, promoting the formation of rsbW-SigB complex. In response to stress or starvation, on the other hand, *rsbV* is dephosphorylated and captures the rsbW to form rsbV-rsbW complexes leading to the release of active SigB [44, 45]. It has been demonstrated that *sigB* in *S. aureus* is induced during the stationary phase, upon heat shock and appears to participate directly and indirectly in the expression of different virulence genes [46, 47]. The corresponding *sigB* in *B. subtilis* is known to be itself the target of a complex regulatory network, which controls gene expression in response to certain stress and stationary phase specific signals [48]. It has been shown that transcription of the P1 promoter of *sar* locus is dependent on the primary sigma factor sigmaSA, while that of the P3 promoter is dependent on sigmaSB [27, 29]. Detailed immunoblot analysis as well as a competitive enzyme-linked immunosorbent assay of the cell extract of the *sigB* mutant with anti-SarA monoclonal antibody 1D1 revealed a higher SarA expression in the mutant than in the parental control [49].

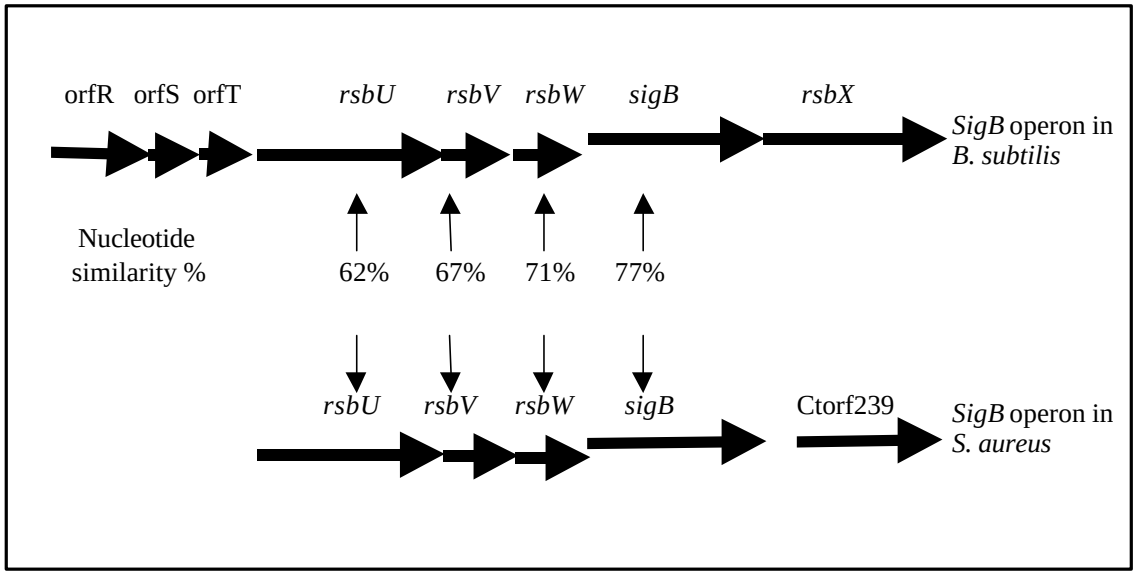

Figure 1: Genetic organization of the *S. aureus sigB* cluster and the nucleotide similarities with the *sigB operon* in *B. subtilis*.

It has been reported that, hyperproduction of alpha-hemolysin in a *S. aureus sigB* mutant was associated with elevated SarA expression [49]. The most striking difference between the *sigB* mutants and the parental strains of *S. aureus* is the drastic alteration in resistance for antibiotics. Wu et al., [42] showed a 64-fold reduction of resistance to methicillin in a *sigB* minus *S. aureus* compared to that in the parental strain. In addition to this, *sigB* plays a major role in bacterial acid tolerance. A low-pH environment is characteristic of the phagolysosome, being one of the major modes of killing by human neutrophils. *S. aureus* is able to grow over a wide range of pH levels, and changes in pH can affect production of many virulence determinants in this bacteria [50]. The *sigB* mutant showed increase sensitivity to heat shock, high salt stress, ethanol shock, and oxidative stress [51]. Moreover, inactivation of this gene in *S. aureus* revealed in addition to phenotypic changes, a marked deficiency in coagulase expression and clumping factor production [52].

2. Nosocomial infections by coagulase negative Staphylococcus (CNS)

The CNS are a major component of our normal microflora, especially of the skin and the mucous membranes. Formerly, CNS were considered to be saprophytic or mildly pathogen for humans. However, several species of CNS like *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* are now documented as opportunistic human pathogens [53-56]. It is now

generally believed that the virulence of CNS is related to their ability to attach to and subsequently colonize the surfaces of implanted medical devices which can result in bacteremia. Unfortunately, nosocomial bacteremia due to CNS is a rapidly increasing problem and is responsible for significant morbidity and mortality [57, 58]. In hematooncologic patients, the effective application of antibiotic prophylaxis has facilitated the introduction of more aggressive chemotherapies that have further increased the risk of septicemia by CNS [59].

2.1 Biofilm production in *S. epidermidis*

Of all of the species of CNS, *S. epidermidis* is the most common cause of nosocomial bacteremia and is the major organism responsible for infection of implanted prosthetic medical devices such as intravenous catheter, prosthetic heart valves, artificial joints, and cerebrospinal fluid shunts [58, 60, 61] especially in immunocompromised patients, such as cancer patients and premature neonants [62, 63]. It has been suggested that the ability to form biofilms on smooth surfaces contributes significantly to the virulence of *S. epidermidis* [61]. Biofilm formation in *S. epidermidis* is assumed to occur in two stages: The first stage is a rapid initial attachment of the bacteria to polymer surfaces by nonspecific forces such as surface charge and hydrophobicity [64-66], followed by a cell proliferation process and the production of an extracellular polysaccharide substance PIA (Polysaccharide intercellular adhesin). The PIA represents a β-1,6-linked *N*-acetyl-glucosaminyl polymer which mediates the intercellular adherence of the bacteria and the accumulation of a multilayered biofilm [67-71]. Molecular studies on the nature of biofilm formation revealed that in the primary attachment of *S. epidermidis* a surface-associated protein is involved. This protein was recently identified as an autolysin [71]. The production of PIA during the accumulative phase of biofilm formation was found to be associated with the expression of the *ica* operon comprising the *ica*A, *ica*D, *ica*B, and *ica*C genes [69, 72] (Figure 2).

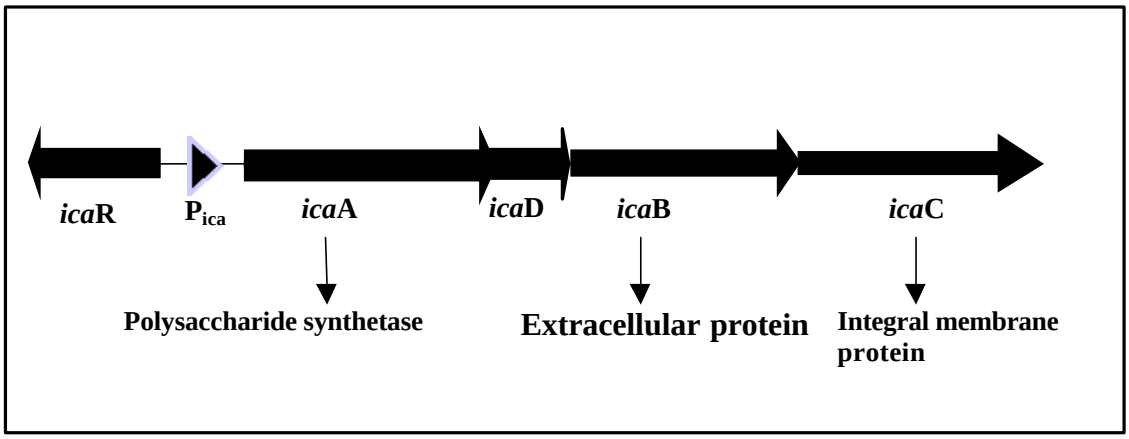

Figure 2: Genomic organization of the *ica* locus (*ica*A, *ica*D, *ica*B, and *ica*C) coding for the PIA production in *S. epidermidis.*

In these studies, it was shown that *ica*A encodes an N-acetylglucosaminyl transferase. It is the key enzyme for PIA synthesis. The gene product of *ica*B contains a typical signal sequence and, consequently, it is secreted into the medium. Both the *ica*C and *ica*D encoded proteins exhibit properties of membrane proteins. The precise function of icaB, icaC and icaD are far from being understood. Recent data suggest, however, that the co-expression of *ica*A, *ica*D and *ica*C is absolutely required for the production of biofilm [72].

Epidemiological and experimental data strongly suggested that the biofilm production contributes to the virulence properties of specific *S. epidermidis* strains. In this respect, interesting observations have been reported recently; they show that the *ica* gene cluster is more prevalent in clinical *S. epidermidis* isolates obtained from catheter related infections than in isolates from the normal skin and mucous of a healthy, non-hospitalized control group [73, 74]. Moreover, from data obtained in a mouse model system of subcutaneous infection, it was concluded that biofilm-producing *S. epidermidis* strains are more virulent than biofilm-negative strains [75]. There are reports showing that specific properties, such as colony morphology, growth rate, antibiotic susceptibility and biofilm formation, undergo changes and differ among variants of the same parents strain [73, 76-78]. Because this phenomenon was found to be reversible, it was termed phase variation. PIA synthesis has been reported recently to undergo a phase variation process. It has been investigated that in 30% of the variants the missing biofilm was due to the inactivation of either the *ica*A or the *ica*C gene by the insertion of the insertion sequence element IS*256*. Furthermore, it was shown that the transcriptoin of IS*256* into the *ica* operon is a reversible process, and after repeated passage of the PIA-negative insertional mutant, the biofilm-forming phenotype could be restored. It has been speculated that phase variation might improve the bacterial survival and growth under

changing environmental conditions [79]. Previous reports investigating the regulation of the virulence factors in *S. epidermidis* showed that slime production by most strains of *S. epidermidis* was enhanced by conditions of iron limitation produced by the addition of ethylenediamine-di-*o*-hydroxiphenol acetic acid to the growth medium [77]. Other studies revealed that the adherence of *S. epidermidis* can be mediated by some additional factors. It has been investigated that 60% of the slime positive *S. epidermidis* produce slime-associated antigen (SAA) which represent a marker of virulence for staphylococci and the addition of 1% glucose increased the percentage of slime production [80]. Veenstra et al., [63] had described that the Staphylococcal surface proteins SSP-1 and SSP2 are involved in the attachment of *S. epidermidis* 354 to polystyrene. Recent investigation reveals that PIA and the hemagglutinin (HA) of *S. epidermidis* are closely related if not identical [69, 81]. In addition, a 140-kDa extracellular protein has been demonstrated that appear to play a role in cellular accumulation but functionally appear to be unrelated with PIA or SAA expression. The presence of this protein can be demonstrated neither in *S. carnosus* nor *S. carnosus* (pCN27) carrying the *ica* genes [82]. Sequence analysis showed that adhesion is conserved between *S. epidermidis* and *S. aureus*. Comparison of the *ica* locus of *S. epidermidis* and *S. aureus* revealed 59 to 78% identity on the amino acid level. Deletion of the *ica* locus in *S. aureus* results (as well as in *S. epidermidis*) in a loss of the ability to form biofilms, PIA, and loss of the *N*-acetyl-glucosaminyl-transferase activity in vitro [83]. The *Staphylococcus* species that cross-hybridized with *icaA* DNA probes from *S. aureus* and *S. epidermidis* were the species that are most closely related. The so called epidermidis phylogenetic group, based on DNA comparisons as well as some biochemical properties [84, 85], Includes *S. auricularis* and *S. capitis*, both of which appear to carry a copy of *icaA* gene. Other more distantly related members of this group, *S. haemolyticus*, *S. hominis*, and *S. warneri*, failed to cross-hybridize with *ica*A [83].

Several reports have described the capacity of *S. epidermidis* to adhere to immobilized fibrinogen [86-89]. However, precoating of surfaces in vitro with various plasma proteins has also been shown to have a blocking effect on early adhesion for several strains of *S. epidermidis* [90]. The gene encoding fibrinogen binding in *S. epidermidis*, termed *fbe*, which encodes a protein has a calculated molecular mass of ~119 kDa. Sequence analysis of this gene shows similarities to an Fg-binding in *S. aureus* [91].

2.1.1 Regulation of the virulence factors in *S. epidermidis* by the agr system

The production of many virulence factors in *S. epidermidis* is likely regulated by the agr system. The sequence of the *agr* operon comprising the *agr*A, *agrC*, *agrD*, *agrB* and RNAIII in *S. epidermidis* was analysed, which is very similar to the analogous sequence in *S. aureus* and *S. lugdunensis* [92] (figure 3).

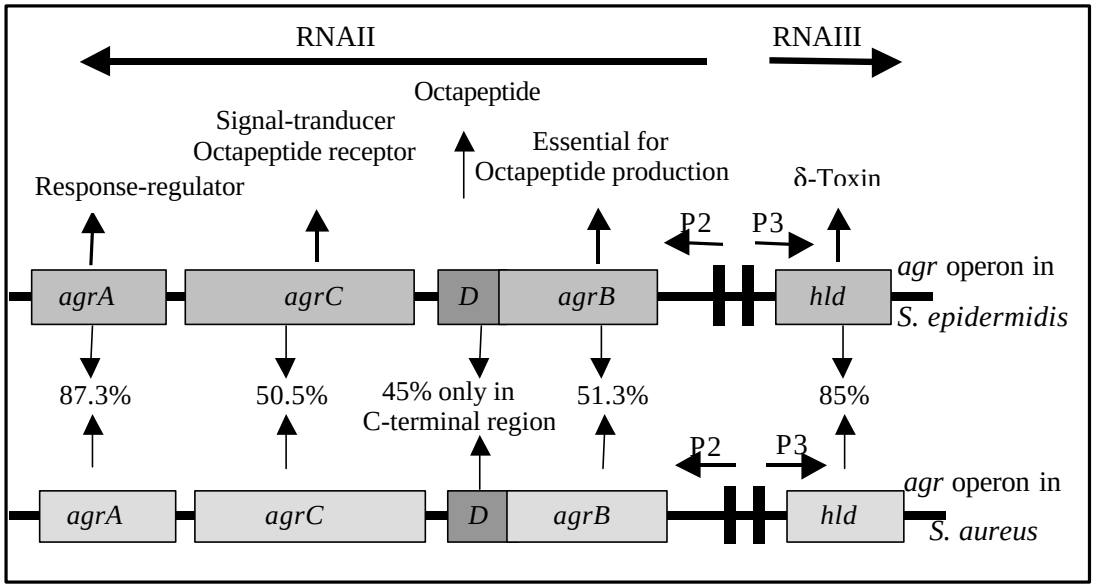

Figure 3: Genetic organization of the accessory gene regulator (*agr*) in *S. epidermidis* and the amino acid similarity to the *agr* operon in *S. aureus*.

The response regulator AgrA shows the highest identity to that of *S. aureus* (87.3%). The histidin kinase AgrC (overall identity to *S. aureus* AgrC 50.5%) has pronounced similarity to *S. aureus* in the C-terminal part of the protein, whereas the N-terminal part shows only low similarity. Comparison of the *S. aureus* and *S. lugdunensis* AgrC sequences had already led to speculation that the N-terminal portion might represent the region that binds the structurally divergent phermon peptides. Whereas the C-terminal region interacts with the highly conserved response regulator AgrA [93]. The AgrD sequence reveals a pronounced similarity in the region directly C-terminal to the phermone peptide (45%) (figure 4).

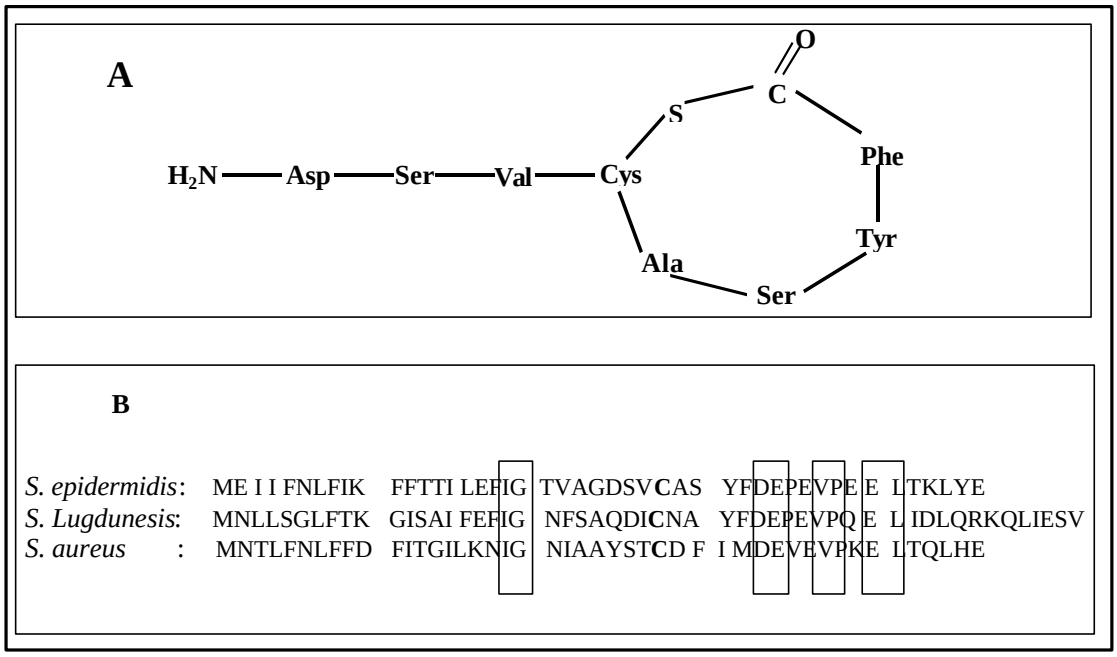

Figure 4: (a): Structure of the *agr* peptide phermone of *S. epidermidis*. It consists of the peptide DSVCASYF containing a thiolester linkage between the central cystein and the C-terminal carboxyl group. **(b):** Comparison of the AgrD protein of *S. epidermidis*, *S. lugdunesis*, and *S. aureus*. The AgrD protein comprises the sequence of the peptide phermone, which is cleaved from AgrD and posttranslationally modified by AgrB [93]. Identical amino acid boxes within AgrD sequence in the N-terminal and C-terminal vicinity of the peptide phermone sequence are shown. The sequence directly C-terminal to the phermone sequence is more conserved than the N-terminal sequence [92].

This region might represent a structural element important for the modifying reaction presumably carried out by AgrB. With the exception of the central cysteine residue, the sequence of the *S. epidermidis* peptide phermone is not related to any known *S. aureus* subgroup, nor to *S. lugdunesis*. In the agr type of phermones, only the central cysteine and its distance to the C-terminal is conserved. These conserved structural features are probably important for thiolactone formation. AgrB shows a relatively high identity to those of *S. aureus* (51.3%) and *S. lugdunesis* (53.7%). The peptide sequence of the *S. epidermidis* δ-toxin has already been known for some time [94]. It appears to be encoded within the RNAIII region with 85% identity to *S. aureus* δ-toxin region. In contrast, the RNAIII region of *S. lugdunesis* encodes no δ-toxin-like peptides [95]. It has been shown that the *S. epidermidis* pheromone can activate the agr system in *S. epidermidis*, on the other side it may acts as a competent inhibitor of the *S. aureus* agr system and of the agr-controlled expression of

virulence factors in *S. aureus* as shown for the inhibition of α-toxin and δ-toxin or the increase in the production of the surface associated protein A [32].

3. Antibiotic resistance in staphylococci

Soon after the introduction of antimicrobial agents for clinical use it was evident that microorganisms can effectively respond to antimicrobial agents. The emergence of antibiotic resistance is one of the most dangerous phenomena in medicine of the last 20 years, and antibiotic resistance has increased in virtually every species of bacteria examined. In the 1990s, increasing rates of resistance to antimicrobials among a number of gram-positive and gram-negative organisms were reported from many parts of the word. Moreover, there are increasingly frequent reports of clinical problems caused by bacteria resistant to multiple antibiotics. Nosocomial infections caused by methicillin-resistant Staphylococci are a growing problem for many health care institutions [1, 54, 58]. Methicillin-resistant Staphylococci (MRS) are resistant to all penicillins, including semisynthetic penicillinase-resistant congers, penems, carbapenems, and cephalosporin. The basis of this resistance is conferred by an additional penicillin-binding protein, PBP-2a [96, 97]. The PBP-2a gene *mecA* is located within a large chromosomal region which is absent in the methicillin-susceptible *S. aureus* chromosome [98]. The region, designated *mec* DNA, is speculated to have originated from the genome of another bacterial species and become integrated into the chromosome of the *S. aureus* cell in the past. It has been reported that *mec* DNA is found to be 51,669 bp long, including terminal inverted repeats of 27 bp and a characteristic pair of direct repeat sequences of 15 bp each: one is situated in the right extremity of *mec* DNA, and the other is situated outside the *mec* DNA and abuts the left boundary of *mec* DNA. The integration of *mec* DNA was found to be located in an open reading frame of unknown function, designated *orfX* [99]. It is found that mec is a novel mobile genetic element and designated as staphylococcal cassette chromosome mec (SCCmec) [100]. Many reports showed the clinical significance of bacteremia due to vancomycin-heteroresistant staphylococci [101]. It has been demonstrated that glycopeptide resistance in *S. aureus* is associated with increased production of penicillin binding protein, and increased production of peptidoglycan cell wall material in turn binds a large amount of vancomycin. This results in blockage of cell autolysin, depletion of vancomycin in the medium, and steric hindrance so that free vancomycin in the environment is not able to penetrate the cell wall to bind to the A-

alanyl-DNA-alanine termini of new muropeptides [102]. Studies where high levels of resistance have been observed are from areas of the hospital where cross infection are common [103].

There are also other mechanisms which are involved in the bacterial resistance to antimicrobial agents. For example a slight modification in the DNA-dependent RNA polymerase, the target of rifampicin, can alter drug binding and produce bacterial resistance [104]. The alteration of the target enzymes (DNA gyrase, and topoisomerase IV) and increase in quinolone efflux from bacterial cells caused by the membrane protein NorA are two principal mechanisms by which *S. aureus* acquires quinolone resistance [105]. In addition, a 2.4-kb Staphylococcus epidermidis plasmid, pSK108, encodes a qacC multidrug resistance determinant. The pSK108 plasmid is a member of the pC194 family of rolling circle replicating plasmids, suggesting that the DNA segment containing qacC, which is bounded by the replication nick site and the minus origin palA, represents a resistance gene cassette that has undergone horizontal genetic exchange [106]. It has been indicated that the prevalence of *ermA* over the *ermC* gene as opposed to the widely held opinion of the *ermC* gene being the most dominant resistance determinant gene. In poultry, erythromycin resistant staphylococal strains were found to contain either *ermA* alone (50%) or a combination of *ermA* (100%) and *ermC* (50%). While the clinical strains contained either *ermA* (94.5%) or *ermC* (5.5%) but never both [107]. A large number of studies have examined rates of fusidic acid resistance in staphylococci. Resistance to fusidic acid is determined by a number of mechanisms. The best described are alterations in the elongation factor G, which appear in natural mutants that are harboured at low rates in normal populations of staphylococci (10^6 to 10^8). The *S. aureus* plasmid gene, *vgaB*, was described to confer resistance to streptogramin (SgA) and related compounds (PIIA, viriginamycin M, mikamycin A, synergistin A, Dalfopristin). This gene potentially encodes a 522 aminoacid-protein, VgaB, of 61327 Da, which exhibits a significant similarity to the ATP-binding domains of numerous proteins [108, 109]. Defects in the biosynthesis or in corporation of respiratory components, including menadione or cytochromes decreased the sensitivity of *Staphylococcus aureus* small colony variants to aminoglycoside, and enhanced intracellelar survival within cultured bovine aortic endothelial cells [110, 111]. Moreover, The activity of many antimicrobial agents strictly depends on the phase of bacterial growth [112, 113]. The MICs and MBCs are significantly higher for most drugs if tests are performed with partly adherent bacteria [114, 115]. It has been investigated that extracts of exopolysaccharide from slime positive strain of *S. epidermidis* antagonized the antimicrobial activity of vancomycin, teicoplanin, and perfloxacin (< 15% decrease). This

could explain the increased level of vancomycin resistance of organisms embedded in a biofilm [6, 116, 117].