

What Distinguishes Highly Pathogenic Staphylococci from Medium- and Non-pathogenic?

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Abstract Members of the genus *Staphylococcus* are widespread as commensals of humans and animals where they colonize the skin or mucous membranes. While this coexistence remains mostly untroubled, especially for the healthy host, the bacteria may pose a serious threat for the human or animal host when they get access to inner layers of the body through breaches in skin or membranes. Among the members of the genus a wide span exists in the ability to cope with the hostile conditions encountered in the bloodstream of the living host as a scarce supply of certain nutrients, attacks of the immune system, or anti-infective measures undertaken in the clinical field. In this respect, *Staphylococcus aureus* is by far the most versatile species of the genus. Its equipment with a huge repertoire of different virulence factors and additional supportive gene products that increase the capability to survive within the living host makes *S. aureus* the leading pathogen not only within the genus but also one of the most threatening microorganisms regarding hospital and community-acquired infections. Compared with *S. aureus*, the other virulent species of the genus like *S. epidermidis*, *S. lugdunensis*, *S. saprophyticus*, and *S. haemolyticus* have a more limited arsenal of virulence factors resulting in a specialized spectrum of diseases and a generally lower degree of pathogenicity. Besides the highly and medium-pathogenic staphylococci, the genus comprises also species like *S. carnosus*, *S. xylosus*, and *S. equorum* that are generally inconspicuous regarding clinical occurrences. Some strains of this group

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are used in the food industry and can be graded as non-pathogenic. This review aims to work out the differences between the pathogenic properties of highly and medium-pathogenic staphylococcal species and to draw a comparison between the pathogenic species and the food-grade *S. carnosus* TM300.

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1 Introduction

The genus *Staphylococcus* comprises more than 40 species that differ in their potential to endanger human and animal health, ranging from non-pathogenic food-grade members to dangerous pathogens causing severe infections and being resistant to the treatment by most of the commonly applied antibiotics.

Many staphylococci are found on humans, mammals, or birds where they are located on the skin, skin glands, or mucous membranes. They are either found to coexist indigenously as commensals or to be transiently present as colonizers of their hosts. The transitory presence of some staphylococcal cohabitants often hampers the identification of their natural host range (Götz et al. 2006).

The coexistence with living humans or animals accounts for the equipment of many staphylococci with factors which allow the inhabitation of their ecological niche on skin or mucous membranes by dealing with the existing environmental conditions. The capability to “only” colonize may be extended to elaborate survival strategies that become necessary when the bacteria are able to pass through breaches of skin or membranes and to enter the blood vessels of the host organism. The highly pathogenic *Staphylococcus aureus* strains developed an immense variety of mechanisms that enable them to specifically interact with host factors, to escape the hosts defense mechanisms, to enhance their fitness in the competition for rare substrates, to detach nutrients by disintegration of host tissue, and to cope with antibacterial compounds like antibiotics.

The impact of *Staphylococcus* on human life and health is reflected by numerous genome sequencing projects which aim at a better understanding of the molecular basis of staphylococcal pathogenicity. The so far sequenced staphylococcal species representatives are listed in Table 1.

2 Graduation of Pathogenicity in Staphylococcal Species

According to the equipment with virulence factors (or the ability to acquire them by horizontal gene transfer) and the resulting pathogenic capabilities, staphylococcal species may be arranged along a pathogenicity scale ranging from highly- to non-pathogenic.

2.1 Highly Pathogenic Staphylococci

Among the staphylococci, *S. aureus* is the species with the highest pathogenic potential. *S. aureus* is responsible for a variety of nosocomial or community-acquired infections ranging from boils, furuncles, styes, impetigo, and other superficial skin infections to more serious infections particularly in the chronically

Table 1 Completed genome sequences of staphylococcal species

Species/Strain	Characteristics	Reference
Non-<i>S. aureus</i>		
<i>S. epidermidis</i> RP62A	Biofilm former, catheter-associated sepsis	Gill et al. (2005)
<i>S. epidermidis</i> ATCC 12228	Non-biofilm, not infection associated	Zhang et al. (2003)
<i>S. lugdunensis</i> HKU09-01	Clinical isolate from human pus swab	Tse et al. (2010)
<i>S. lugdunensis</i> N920143	Clinical isolate from breast abscess	Heilbronner et al. (2011)
<i>S. haemolyticus</i> JCSC1435	Clinical strain, multidrug-resistant	Takeuchi et al. (2005)
<i>S. saprophyticus</i> ATCC 15305	Isolated from human urine specimen, uropathogenic	Kuroda et al. (2005)
<i>S. pseudintermedius</i> ED99	Canine pyoderma, coagulase-positive	Ben Zakour et al. (2011)
<i>S. pseudintermedius</i> HKU10-03	Canine pyoderma, coagulase-positive	Tse et al. (2011)
<i>S. carnosus</i> TM300	Non-pathogenic food grade organism	Rosenstein et al. (2009)
<i>S. aureus</i> strains		
04-02981	MRSA, For comparative analysis	Nubel et al. (2010)
RF 122	Mastitis in cattle	Herron et al. (2002)
11819-97	Clinical isolate from skin abscess; MRSA, ST80-IV, CA	Stegger et al. (2012)
71193	Clinical isolate, ST398	Uhlemann et al. (2012)
COL	Early MRSA isolate	Gill et al. (2005)
ECT-R 2	Multiresistant MSSA	Lindqvist et al. (2012)
ED 133	Bovine isolate	Guinane et al. (2010)
ED 98	Poultry isolate, bacterial chondronecrosis with osteomyelitis	Lowder et al. (2009)
HO 5096 0412	Neonatal MRSA outbreak	Wellcome trust sanger institute
JH1	Bloodstream isolate, MRSA vancomycin-sensitive	Mwangi et al. (2007)
JH9	Bloodstream isolate, MRSA VISA	Mwangi et al. (2007)
JKD6159	cMRSA ST93-IV, severe skin and invasive infection	Chua et al. (2010)
LGA251	Bulk milk isolate	Garcia-Alvarez et al. (2011)
M013	CA-MRSA, pvl-positive, ST59	Huang et al. (2012)
MN8	Isolate from urogenital tract, reference genome	Human microbiome project
MRSA252	HA-MRSA, EMRSA-16	Holden et al. (2004)
MSHR1132	Early-branched SA lineage, staphyloxanthin-negative	Holt et al. (2011)
MSSA476	CA-MSSA	Holden et al. (2004)
MW2	Highly virulent CA-MRSA	Baba et al. (2002)

(continued)

Table 1 (continued)

Species/Strain	Characteristics	Reference
Mu3	Hetero-VISA	Neoh et al. (2008)
Mu50	MRSA <i>van^R</i> , pus isolate	Kuroda et al. (2001)
N315	MRSA, pharyngeal smear isolate	Kuroda et al. (2001)
NCTC 8325	Prototype strain for SA molecular genetics	GenBank: CP000253; direct submission
ST398	MRSA, from human endocarditis, livestock-associated	Schijffelen et al. (2010)
T0131	MRSA ST239, clinical isolate	Li et al. (2011)
TCH60	Skin isolate, reference genome	Human microbiome project
TW20	Clinical isolate, MRSA, ST239	Holden et al. (2010)
USA300_FPR3757	CA-MRSA, carries ACME	Diep et al. (2006)
USA300_TCH1516	Sepsis isolate, CA-MRSA	Highlander et al. (2007)
USA300_TCH959	Buttock abscess isolate, CA-MSSA	Highlander et al. (2007)
VC40	Highly vancomycin-resistant, <i>vanA</i> -negative	Sass et al. (2012)
JKD6008	Bloodstream isolate, MRSA-VISA	Howden et al. (2010)
<i>ssp</i> Newbould 305	Bovine mastitis isolate	http://www.ncbi.nlm.nih.gov/bioproject/PRJNA162721
<i>ssp</i> Newman	Human infection isolate (1952), often used in pathogenesis studies	Baba et al. (2008)

ill or immunocompromised patient. The latter include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis, and meningitis. Its pathogenicity is based on a huge spectrum of virulence factors as well as on a variety of fitness factors that support the survival in the host. The use of anti-infective counter-measures in the treatment of diseases caused by *S. aureus* is hindered by rapidly spreading resistance genes that could give rise to multi-resistant *S. aureus* strains (MRSA).

2.2 Medium-pathogenic Staphylococci

Besides *S. aureus* as the leading staphylococcal pathogen, various other members of the genus, mostly belonging to the coagulase-negative staphylococci (CoNS), play roles as infectious agents for human or animal hosts. In comparison with *S. aureus*, they reveal a more restricted palette of virulence factors. Accordingly, the medium-pathogenic species are more specialized in their infective strategies and/or limited to a narrow spectrum of diseases. The clinical appearance of these infections may be characterized as more subtle with subacute or chronic clinical courses without fulminant signs and rarely being life-threatening (von Eiff et al. 2002).

The most prominent representative in this category is *S. epidermidis* (Kleeman et al. 1993; Weinstein et al. 1998). This organism is the most prevalent and

persistent staphylococcal species on human skin. Wounds or surgery may open entry ports to the host's bloodstream. In recent years, *S. epidermidis* has emerged as a common cause of hospital-acquired infections, including catheter-associated infections and septicemia, particularly in immunocompromised patients. Similar to *S. aureus*, *S. epidermidis* strains may become highly resistant to many antibiotics including penicillins and cephalosporins. In contrast to the multifaceted character of *S. aureus* infections, the pathogenic potential of *S. epidermidis* is almost exclusively based on its ability to form biofilms (Heilmann et al. 1996a, b, 1997; Mack et al. 1996) on implanted or indwelling polymeric material (Götz and Peters 2000). There are only few indications of infections caused by *S. epidermidis* without the involvement of foreign bodies (von Eiff et al. 2002).

Besides *S. epidermidis*, a number of other staphylococcal species that are rather occasionally observed as infectious agents of humans and animals, often in patients with a compromised immune system belong to the "medium-pathogenic" category. Strains of the species *S. saprophyticus*, *S. haemolyticus*, *S. lugdunensis*, and *S. pseudintermedius* may be assigned to this group. *Staphylococcus saprophyticus*, normally a commensal on human skin and mucous membranes, is now the second most common cause of acute urinary tract infections after *Escherichia coli* (Kuroda et al. 2005). *S. haemolyticus* was originally isolated from human skin. It is known to be involved in opportunistic infections associated with the implantation of foreign bodies, particularly in those with compromised immune systems (Takeuchi et al. 2005). *S. pseudintermedius* is a major veterinary pathogen and is the most important cause of pyoderma in dogs and other animals (Ben Zakour et al. 2011; Tse et al. 2011). *S. lugdunensis* has been reported as causative pathogen of skin and soft tissue infections, catheter-related bacteremia, native valve endocarditis, and osteomyelitis (Celard et al. 1997; Donvito et al. 1997; Heilbronner et al. 2011; Tse et al. 2010; Vandenesch et al. 1993).

2.3 Non-pathogenic Staphylococci

Some staphylococcal species are used in the food industry and are commonly regarded as non-pathogenic. This group is represented by *S. carnosus* and some strains of *S. xylosus* and *S. equorum* that are used in meat or cheese fermentations. *S. carnosus* (Schleifer and Fischer 1982) has been used since the 1950s as a starter culture in the food-industry and is classified as GRAS (generally recognized as safe) organism (Barriere et al. 2001; Marchesini et al. 1992; Niinivaara and Pohja 1956). Because of its food-grade quality it is used as a cloning host to study the function of particular staphylococcal genes (Götz 1990). Recently, the genome of the cloning strain *S. carnosus* TM300 has been analyzed (Rosenstein et al. 2009; Wagner et al. 1998). *S. xylosus* belongs to the novobiocin-resistant coagulase-negative species group of staphylococci and is commonly isolated from the skin of humans and animals (Devriese et al. 1985; Kloos and Musselwhite 1975). The type strain *S. xylosus* DSM20267 carries an arsenate, arsenite, and antimony III

resistance plasmid (pSX267), which has been cured (Götz et al. 1983). One of the cured strains, *S. xylosus* C2a has been molecularly characterized in more detail with respect to urease, sucrose, and catabolite repression via a new regulator, the catabolite control protein CcpA (Brückner et al. 1993; Brückner 1997; Fiegler et al. 1999; Jankovic et al. 2001; Jankovic and Brückner 2002, 2007; Wagner et al. 1993). *S. xylosus* C2a is used as a starter culture in the production of sausage and cheese. It contributes to the development of the red color characteristic of sausages through its nitrate reductase activity and to the orange color on the surface of certain cheeses, since some strains of *S. xylosus* are pigmented. Its genome is currently being sequenced (<http://www.genoscope.cns.fr/spip/-Staphylococcus-xylosus-C2a-.html>). *S. equorum* has been isolated from smear-ripened cheese (Hoppe-Seyler et al. 2004; Place et al. 2003). Some strains secrete a macrocyclic peptide antibiotic, micrococcin P(1), with anti-listerial activity which is exploited in cheese fermentation to cope with the threat of contaminations by *Listeria monocytogenes* (Carnio et al. 2000, 2001).

3 Virulence Factors of Highly and Medium-pathogenic Staphylococci

Staphylococcus aureus represents by far the most versatile and potent pathogen among the staphylococci due to its capability to cause infectious diseases ranging from rather harmless superficial skin lesions to systemic infections with life-threatening symptoms. As a widespread colonizer of human skin and mucous membranes (Lowy 1998), *S. aureus* exhibits two lifestyles, as a tolerated commensal on one hand and a dangerous pathogen on the other hand. Its abilities as human pathogen are based on a comprehensive collection of various virulence factors and supportive fitness factors that play roles during the various steps of the infectious process, like adhesion to host tissue, forming multilayered and encapsulated biofilms, evasion of the hosts immune system, and coping with limited supply of nutrients like iron compounds. Correspondingly, the staphylococcal virulence factors may be subdivided into adhesins or soluble factors that mediate the attachment to host cells or extracellular matrix proteins, exoenzymes that are involved in the destruction of host tissues, toxins that directly exert detrimental effects to the host and a heterogeneous group comprising iron uptake systems, immune system evasion mechanisms, and other factors that enhance the fitness to survive in the host. The staphylococcal pathogenic potential is completed by a variety of genes that are mediating resistance to antibiotics and other antibacterial agents. Its opulent arsenal of factors involved in the course of infection makes *S. aureus* the outstanding pathogen within the genus and thus represents the benchmark to which the other pathogenic species have to be compared. Therefore, in the following sections the molecular components involved in staphylococcal pathogenicity will be presented based on a comparison with the infection principles detected in *S. aureus*.

3.1 Colonization Factors

The prevalence of *S. aureus* in the ability to cause infections is largely caused by a rich assortment of surface-located proteins that mediate attachment to host tissue by adherence to host plasma proteins or extracellular matrix (ECM) components. Many of these adhesins are covalently linked to the cell wall via a C-terminally located, conserved LPXTG-motif by the action of sortase (*srtA*) (Fischetti et al. 1990). Of the 28 surface proteins identified in *S. aureus*, 21 reveal LPXTG motifs (Houston et al. 2011). Although sortase-deficient mutants of *S. aureus* were hardly affected in growth, they showed a reduced virulence indicating the importance of correctly cell wall-anchored adhesins (Mazmanian et al. 2000). Two forms of sortase are present in *S. aureus*. Sortases of type A are responsible for anchoring the majority of surface proteins via the LPXTG-motif (Bentley et al. 2007; Schneewind et al. 1992) while sortase B is specialized to anchor the heme-iron uptake protein IsdC via its NPQTN-motif to the cell wall (Mazmanian et al. 2003). Due to its role in sorting of infection-related proteins to the cell surface, Sortase A is commonly regarded as a virulence factor. On the other hand, the corresponding gene is found throughout the staphylococcal genomes, also in the non-pathogenic *S. carnosus* TM300 with more than 60 % identity to Sortase A of *S. aureus*. This emphasizes that the role of *srtA* in virulence is not an exclusive one and depends on the contribution of the cognate substrate proteins to the infectious pathway. The more specialized sortase B is present in only a few staphylococcal species and, in addition to *S. aureus*, is found in *S. capitis*, *S. caprae* and *S. lugdunensis*. Since all these species have been described to be involved in invasive infections (Götz et al. 2006), it is conclusive that they carry an uptake system for the supply with heme-iron. Accordingly, the *srtB* gene found in these species is a more unambiguous marker for staphylococcal virulence than *srtA*.

3.1.1 Host Matrix Binding Proteins

The *S. aureus* repertoire of surface proteins allows interactions with virtually every structural component of the hosts extracellular matrix and with many plasma proteins. Correspondingly, *S. aureus* cells are able to adhere to fibril-forming collagens of types I, II, and III, laminin, elastin, fibronectin, vitronectin, fibrinogen, von Willebrand factor, and thrombospondin. The majority of these surface-located proteins are subsumed under the term “Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) of which most are covalently linked to the cell wall (Foster and Hook 1998).

The surface components described for *S. aureus* and those found in medium-pathogenic staphylococci are listed in Table 2. Of 21 proteins with LPXTG sequences in *S. aureus*, 10 had not been characterized initially and were collectively designated as *Staphylococcus aureus* surface (Sas) proteins (Roche et al. 2003a, b). At present, most of these still have no assigned function, with the exceptions of SasG and SasC for which roles in nasal colonization or in biofilm

Table 2 Staphylococcal adhesins and immune evasion factors

Adhesin	Type	Ligand	Species	Reference
Spa	MSCRAMM	Immunoglobulins, von Willebrand factor	<i>S. aureus</i>	Hartleib et al. (2000)
Cna	MSCRAMM	Collagen	<i>S. aureus</i>	Foster and Hook (1998)
FnBPA	MSCRAMM	Fibrinectin, fibrinogen	<i>S. aureus</i>	Wann et al. (2000)
FnBPB	MSCRAMM	Fibrinectin, fibrinogen	<i>S. aureus</i>	Wann et al. (2000)
ClfA	MSCRAMM	Fibrinogen; complement factor I	<i>S. aureus</i>	Hair et al. (2008)
ClfB	MSCRAMM	Fibrinogen, keratin	<i>S. aureus</i>	Walsh et al. (2004)
EbpS	MSCRAMM	Elastin	<i>S. aureus</i>	Park et al. (1996)
SdrC	MSCRAMM	Adhesion to nasal epithelial cells	<i>S. aureus</i>	Corrigan et al. (2009)
SdrD	–	Adhesion to nasal epithelial cells	<i>S. aureus</i>	Corrigan et al. (2009)
SdrE	–	Complement factor H	<i>S. aureus</i>	Sharp et al. (2012)
SdrG (FbeE)	MSCRAMM	Fibrinogen	<i>S. epidermidis</i>	Hartford et al. (2001)
SdrF	MSCRAMM	Collagen, abiotic surfaces	<i>S. epidermidis</i>	Arrecubieta et al. (2007, 2009)
IsdA	MSCRAMM	Fibrinogen, fibronectin	<i>S. aureus</i>	Clarke et al. (2004)
SasC	MSCRAMM	Adhesion to nasal epithelial cells	<i>S. aureus</i>	Schroeder et al. (2009)
SasG	MSCRAMM	Adhesion to nasal epithelial cells	<i>S. aureus</i>	Geoghegan et al. (2010)
Pls	–	Prevents adhesion	<i>S. aureus</i>	Savolainen et al. (2001)
Bbp	MSCRAMM	Bone sialoprotein, Fibrinogen	<i>S. aureus</i>	Tung et al. (2000), Vazquez et al. (2011)
AtlA	Autolysin/Adhesin	Heat shock protein Hsc70	<i>S. aureus</i>	Hirschhausen et al. (2010)
Aaa	Autolysin/Adhesin	Fibrinogen, fibronectin	<i>S. aureus</i>	Heilmann et al. (2005)
Aae	Autolysin/Adhesin	Fibrinogen, fibronectin, vitronectin	<i>S. epidermidis</i>	Heilmann et al. (2003)

(continued)

Table 2 (continued)

Adhesin	Type	Ligand	Species	Biofilm formation	Reference
Aap	–	Intercellular adhesion	<i>S. epidermidis</i>	Biofilm formation	Hussain et al. (1997)
AtfE	Autolysin/ Adhesin	Vitronectin	<i>S. epidermidis</i>	Primary attachment Internalization	Heilmann et al. (1997), Hirschhausen et al. (2010)
EmbP	–	Fibronectin	<i>S. epidermidis</i>	Biofilm formation, primary attachment, intercellular adhesion	Christner et al. (2010), Williams et al. (2002.)
Ebh	–	Fibronectin	<i>S. aureus</i>	Homologous to <i>Streptococcus</i> adhesin	Clarke et al. (2004)
GehD	Lipase	Collagen	<i>S. epidermidis</i>	Primary attachment	Bowden et al. (2002)
Eap map	–	–	–	–	–
P70	SERAM	Fibrinogen, fibronectin, prothrombin, vitronectin, bone sialo protein, osteopontin, prothrombin	<i>S. aureus</i>	Internalization?	Chavakis et al. (2005)
Coa	SERAM	Prothrombin, Platelet-binding	<i>S. aureus</i>	Staphylocoagulase, fibrin formation, blood clotting	Chavakis et al. (2005)
FbpA	–	Fibrinogen	<i>S. aureus</i>	–	Cheung et al. (1995)
vWbp	SERAM	Von Willebrand factor, prothrombin	<i>S. aureus</i>	Coagulase activity	Bjerketorp et al. (2002, 2004)
Efb	SERAM	Fibrinogen, complement factor C3b	<i>S. aureus</i>	Inhibition of platelet aggregation, antiphagocytic	Lee et al. (2004), Palma et al. (2001)
Ecb	–	Complement factor C3b	<i>S. aureus</i>	Antiphagocytic	Jongerijs et al. (2010)
Emp	SERAM	Fibronectin, fibrinogen, collagen, vitronectin	<i>S. aureus</i>	–	Hussain et al. (2001)
Aas	Autolysin/ Adhesin	Fibronectin	<i>S. saprophyticus</i>	Agglutination of sheep erythrocytes	Hell et al. (1998)
UafA	MSCRAMM	Adhesion to bladder cells	<i>S. saprophyticus</i>	Hemagglutination	Kuroda et al. (2005)
SdrI	MSCRAMM	Collagen	<i>S. saprophyticus</i>	–	Sakinc et al. (2006)

accumulation have been described (Roche et al. 2003a, b; Schroeder et al. 2009). Some of the surface adhesins are characterized by the presence of repeats with serine-aspartate (SD) rich sequences and correspondingly were grouped into the Sdr family of surface proteins (Josefsson et al. 1998). ClfA from *S. aureus* was the first described member of the Sdr protein family (McDevitt et al. 1994). In *S. epidermidis*, eleven cell-wall-anchored proteins have been identified of which seven had not been characterized previously and were bundled under the term *Staphylococcus epidermidis* surface (Ses) proteins (Bowden et al. 2005). Like in *S. aureus*, also in *S. epidermidis* members of the serine-aspartate-rich (Sdr) proteins were identified, of which SdrF and SdrG carry an LPXTG-motif (McCrea et al. 2000). SdrG (also termed Fbe) is known as *S. epidermidis* fibrinogen-binding surface protein (Hartford et al. 2001) while SdrF has collagen-binding activity and plays a role in the attachment to abiotic surfaces (Arrecubieta et al. 2007, 2009). Fbe shows similarity to the clumping factors ClfA and ClfB of *S. aureus*. In contrast to the tested *S. aureus* strains, the adherence to fibrinogen varies significantly in the analyzed *S. epidermidis* strains.

Compared to *S. aureus* and *S. epidermidis*, the coagulase-negative *S. saprophyticus* has a more reduced pathogenic potential that is essentially limited to urinary tract infections (UTI). The species representatives carry a number of virulence factors. The genome sequence of *S. saprophyticus* ATCC15305 revealed a gene encoding a cell-wall anchored protein (UafA) which has been identified as an adhesin that mediates hemagglutination and adherence to human bladder cells (Kuroda et al. 2005). Another cell wall-anchored protein identified in *S. saprophyticus* is SdrI. It belongs to the family of serine-aspartate-rich proteins and was shown to bind to collagen (Sakinc et al. 2006) as well as to fibronectin (Sakinc et al. 2009a, b). A surface-associated lipase, Ssp, is present in high amounts on the cell surface of clinical isolates (Sakinc et al. 2007). Recently, a plasmid-encoded cell-wall anchored *S. saprophyticus* surface protein F (SssF) has been described (King et al. 2012). The *sssF* gene is highly prevalent in *S. saprophyticus* clinical isolates; it shows similarity to the *S. aureus* surface protein SasF and like SasF it mediates resistance to linoleic acid and seems not to be involved in adhesion (King et al. 2012).

The equipment with the surface-located adhesins in *S. aureus* varies from strain to strain. While clumping factor A (ClfA) and the serin-aspartate repeat protein C (SdrC) are found in almost all clinical *S. aureus* isolates, the collagen-binding protein Cna and the colonization factor SdrD are harbored by only some strains (Bartlett and Hulten 2010). Some *S. aureus* adhesins are involved in nasal colonization of about 30 % of the population without symptoms, which illustrates the ambivalent commensal-pathogen nature of *S. aureus* (Wertheim et al. 2005).

3.1.2 SERAMS and Anchorless Adhesins

Another group of adhesins identified in *S. aureus* is composed of structurally unrelated proteins that are secreted and mediate the binding to host molecules, cells, or tissues. These factors have been grouped as “secretable expanded

repertoire adhesive molecules” (SERAM) and comprise various fibronectin- and fibrinogen-binding proteins as well as staphylocoagulase and von Willebrand factor-binding protein (Chavakis et al. 2005). Besides the MSCRAMMs and SERAMs, also proteins without secretion or anchoring signal were found to be involved in the recognition of ECM components. Like for streptococci, where anchorless adhesins and invasins have been categorized as a new class of virulence factors (Chhatwal 2002), a cell surface-located alpha-enolase of *S. aureus* has been detected as a laminin-binding protein (Carneiro et al. 2004). The alpha-enolase of *S. aureus* also functions as plasminogen receptor and probably is involved in enhancing the activity of staphylokinase (Carneiro et al. 2004; Molkanen et al. 2002). Furthermore, a binding to laminin and collagen I has been demonstrated (Antikainen et al. 2007). The alpha-enolase is well conserved among the staphylococci and, at least for *S. epidermidis* O47, a similar role for the enolase as virulence factor was indicated by binding to serum components and immune reactivity (Sellman et al. 2005).

3.1.3 Autolysins/Adhesins

Another class of bifunctional proteins for which adhesive properties have been described comprises the autolysins/adhesins including AtlA and Aaa from *S. aureus* (Heilmann et al. 2005), AtlE and Aae from *S. epidermidis* (Heilmann et al. 1997, 2003), Aas from *S. saprophyticus* (Hell et al. 1998) and AtlC from *S. caprae* (Allignet et al. 2002). Members of this group are distinguished by a bi-functional character as autolysins with amidase/glucosaminidase activity that also have adhesive properties (Heilmann et al. 1997). The major autolysins of *S. aureus* and *S. epidermidis*, AtlA and AtlE, interact with peptidoglycan (Biswas et al. 2006; Zoll et al. 2010). They are targeted to the septum region via their repeat domains by an exclusion strategy mediated by wall teichoic acid (WTA) (Schlag et al. 2010) and the receptor at the septum is most likely lipoteichoic acid (Zoll et al. 2012). Analyses of *S. epidermidis* biofilm mutants have shown that AtlE is involved in the initial attachment to polystyrene. Furthermore, a vitronectin-binding activity has been shown for AtlE (Heilmann et al. 1997) and a role in the accumulation of extracellular DNA in *S. epidermidis* biofilms has been proposed (Qin et al. 2007). These findings indicate that AtlE might play an important role in several steps of biofilm formation. Interestingly, while no or low fibronectin-binding activity has been reported for AtlE (Heilmann et al. 1997), the homologous AtlC of *S. caprae* was shown to exhibit a pronounced binding to fibronectin (Allignet et al. 2002). Recently, another function of AtlE and AtlA in internalization of infecting staphylococcal cells to host cells based on an interaction with the host heat shock cognate protein Hsc70 has been reported (Hirschhausen et al. 2010).

Highly similar orthologues of Atl have been found in all sequenced staphylococcal genomes indicating a ubiquitous presence of the corresponding gene in staphylococci, whereas data on functions of the gene products are scarce. The conservation of the *atl* gene sequence has been exploited to develop an Atl-based

typing method that provided a phylogenetic tree comparable to those based upon 16S RNA or on the comparison of whole genome sequences (Albrecht et al. 2012).

3.1.4 Elastin-Binding Proteins

In contrast to the LPXTG-anchoring of most MSCRAMMs, EbpS, an elastin-binding protein of *S. aureus* is integrated into the cytoplasmic membrane by two transmembrane domains which are located in the central part of the primary structure (Downer et al. 2002). Binding to elastin, a major component of the human extracellular matrix, is also mediated by FnBPA and FnBPB that had already earlier been identified as adhesins with fibronectin-binding activity (Greene et al. 1995). But the elastin-binding activity differs between both adhesin types since EbpS interacts with soluble elastin and tropoelastin while FnBPA and FnBPB bind to immobilized elastin.

3.1.5 Fibronectin-Binding Proteins

Binding to soluble and insoluble fibronectin (Fn), a major component of the fibrin-matrix in blood clots and one of the plasma proteins that cover surfaces of implants, is mediated by various adhesins in *S. aureus* (see Table 2). Fibronectin-binding adhesins are also found in *S. epidermidis* (Table 2) but in contrast to *S. aureus*, the *S. epidermidis* Fn-binding proteins interact only with immobilized fibronectin (Valentin-Weigand et al. 1993). This differential adhesion behavior reflects the different occurrences of pathogenicity of both species as *S. epidermidis* exerts its virulence predominantly by adhesion to surfaces that are covered by plasma proteins followed by intercellular adhesion while *S. aureus* also acts as a wound pathogen adhering to soluble fibronectin as being present in wound exudates (Valentin-Weigand et al. 1993).

3.1.6 Factors Involved in Intercellular Adhesion During Biofilm Formation

The adhesin-mediated attachment to host tissue or surfaces of implants is the precondition for the subsequent formation of multi-layered cell communities which eventually become entrapped by exopolysaccharides (EPS) or proteinaceous intercellular material—the biofilms. The ability to adhere to surfaces mediated by adherent compounds is the prerequisite for the second stage, the accumulation phase and intercellular aggregation. This attachment either occurs directly to the abiotic surface or indirectly by adhesion to host proteins that cover the implant. There is a great number of surface adhesins known; any of the above described adhesins might contribute to binding to a specific surface. In comparison with the rich spectrum of *S. aureus* adhesins, the repertoire of *S. epidermidis* is restricted to a limited number of adhesive proteins (see Table 2).

3.2 Biofilms

The formation of biofilms is a feature shared by virulent *S. aureus* and *S. epidermidis* strains. In *S. aureus*, the formation of biofilms is one strategy among others to resist unfavorable conditions in the host as exerted by shearing forces of the bloodstream, the immune response of the host, or anti-infective measures during disease treatment. While the self-protective strategies of *S. aureus* involve additional possibilities like formation of encapsulated microcolonies mediated by the action of coagulating enzymes (Guggenberger et al. 2012), *S. epidermidis*' defensive strategy is mainly based on the formation of biofilms.

The compounds mediating intercellular adhesion and leading to multi-layered bacterial cell aggregates during the later steps of biofilm formation are limited. The most obstinate biofilms are still formed by polysaccharide intercellular adhesin (PIA) (Mack et al. 1996) encoded by the *ica* operon (Heilmann et al. 1996a, b). The accumulated cells are tightly imbedded in this polysaccharide matrix like in a chewing gum (Götz 2002; Heilmann et al. 1996a, b).

Production of PIA is dependent on gene products encoded by the *ica* locus (Cramton et al. 1999; Heilmann et al. 1996a, b) that is generally considered as major genetic component correlated with biofilm formation. This view is supported by the deficiency in biofilm formation found in *ica*-negative strains like *S. epidermidis* ATCC 12228, whereas the *ica*-positive clinical isolate *S. epidermidis* RP62A is a potent biofilm former (Gill et al. 2005; Zhang et al. 2003). PIA-dependent biofilm formation by *S. epidermidis* was shown to be a phase variable process as insertion of IS256 into the *icaC* gene (a frequent integration site) leads to inactivation of biofilm formation—a process which is reversible by precise excision of IS256 from *icaC* (Ziebuhr et al. 1999). But reversion to a biofilm-positive phenotype has also been achieved without excising IS256 indicating that *S. epidermidis* is able to form biofilms in a PIA-independent manner (Rohde et al. 2005).

This variation also seems to be achieved by genome rearrangements as indicated by a genome comparison between the *ica*-positive *S. epidermidis* RP62A and the *ica*- and biofilm-negative *S. epidermidis* ATCC12228. The *ica* operon of *S. epidermidis* RP62A is preceded by a genome segment that is inverted in the genome of strain ATCC12228 (see Fig. 3). Since the *ica* operon is located at the “break point” between the inverted fragments, it is tempting to speculate that this inversion caused the loss of the *ica* genes in ATCC 12228 thus leading to a biofilm-negative phenotype.

For a long time the *ica* operon as the genetic basis for PIA production has been regarded as mandatory for biofilm formation but more recently also an *ica*-independent formation of biofilms has been detected in *ica*-negative mutants of *S. epidermidis* (Rohde et al. 2007). The PIA-independent biofilm was demonstrated to be metaperiodate-resistant indicating a proteinaceous rather than a polysaccharide character (Rohde et al. 2005). This kind of biofilm is less compact and can be easily disrupted by proteases (Marti et al. 2010). A number of surface proteins were described that mediate an *ica*-independent intercellular adhesion thus contributing to biofilm formation.

The proteins involved in intercellular aggregation are Aap, Bap, Bhp, Embp, SasC, and SasG (Hussain et al. 1997; Macintosh et al. 2009; Rohde et al. 2005; Schumacher-Perdreau et al. 1994).

The 2276 amino acids biofilm-associated protein (Bap) from *S. aureus* is encoded on a mobile genetic element, pathogenicity island SaPIbov2, which is only found in bovine-associated *S. aureus* strains (Cucarella et al. 2001; Tormo et al. 2005). Thus, it seems to play no role in human infections by *S. aureus*. For *S. aureus*, a phase-variable expression of Bap has been reported which switches between the “on” and “off” states at a similar frequency and might be involved in the detaching of *S. aureus* cells from a biofilm (Tormo et al. 2007).

In *S. epidermidis* and other CoNS homologs of *S. aureus* Bap have been identified and their role in biofilm formation for *bap*-positive and *ica*-negative staphylococci has been reported (Tormo et al. 2005). The primary structure of Bap is characterized by domains comprising sequence repeats and Bap-homologs in *S. epidermidis*, *S. simulans*, *S. chromogenes*, *S. xylosus* and *S. hyicus* reveal different numbers of repeats in these domains (Tormo et al. 2005). In contrast to *S. aureus*, no indications for a location on a mobile element were found for the *bap*-positive non-*S. aureus* species (Tormo et al. 2005).

The strong *ica*-positive biofilm-former *S. epidermidis* RP62A is a methicillin-resistant clinical isolate from an intravascular catheter-associated sepsis (Gill et al. 2005). In the genome sequence of *S. epidermidis* RP62A another homolog of Bap, named Bhp (Bap homologous protein) is encoded that shows a more distant relationship to *S. aureus* Bap and the Bap homologs in the non-*S. aureus* species. The *bhp* gene is also found in a number of *S. epidermidis* strains of which no complete genome sequences are available at the time of writing this article (*S. epidermidis* M23864, VCU126, VCU128, VCU037, VCU045, VCU125) but it is not present in the *ica*-negative strain *S. epidermidis* ATCC 12228 or in the *ica*-positive biofilm-former *S. epidermidis* O47 (unpublished results, our lab). The Bhp proteins can also contribute to biofilm formation, play a role in bacterial infectious processes, and can occasionally be contained in mobile elements (Lasa and Penades 2006). On the other hand, the expression of Bhp was shown to be downregulated in an Aap-dependent biofilm (Hennig et al. 2007).

As a factor involved in the PIA-independent biofilm formation of *S. epidermidis* the surface protein accumulation-associated protein (Aap) was identified which has to be proteolytically processed in order to mediate intercellular adhesion (Rohde et al. 2005).

Aap is an *S. epidermidis* surface protein that was shown to mediate intercellular adhesion in PIA-negative *S. epidermidis* strains leading to an extracellular biofilm matrix of proteinaceous character (Rohde et al. 2005). Aap is a large cell wall-anchored protein which reveals a signal peptide followed by an A-domain that shares similarity with the *S. aureus* surface protein SasG and a B-domain composed of a varying number of repeats, depending on the Aap-producing *S. epidermidis* strain (Rohde et al. 2007). Aap is anchored to the cell wall via its C-terminal LPXTG signal. The intercellular adhesion activity could be assigned to the B-domain of Aap that becomes active after proteolytic excision of the A domain (Rohde et al. 2005).

While the B-domain is responsible for a later step in biofilm formation, the A-domain may have a function in the initial attachment of *S. epidermidis* cells to host tissue. Macintosh et al. demonstrated that the A-domain mediates adhesion of *S. epidermidis* to human corneocytes (Macintosh et al. 2009). Thus, Aap may act as a bi-functional protein that is involved in the initial phase in biofilm formation via the activity of the A-domain and, after proteolytic processing, in the biofilm stabilization by intercellular adhesion promoted by the B-domain. Some *S. epidermidis* strains were isolated that are genotypically *aap*-positive, whereas Aap proteins are only expressed in a subpopulation of the corresponding cultures. It is postulated that cells that do not express Aap might get detached easier from host cells and could be candidates for cells that enter the hosts' bloodstream via implanted materials (Macintosh et al. 2009).

The LPXTG-anchored proteins SasG and SasC of *S. aureus* show intercellular adhesion activity which is involved in biofilm formation. SasG shares similarity with Aap and SasC (Corrigan et al. 2007; Geoghegan et al. 2010) and contributes to nasal adhesion (Roche et al. 2003a, b). SasC conferred production of huge cell aggregates, increased adherence to polystyrene, and enhanced biofilm formation to *S. carnosus* and *S. aureus* (Schroeder et al. 2009). In *S. aureus*, an additional role for the IgG-binding protein A (Mazmanian et al. 2000) seems to be the involvement in *ica*-independent biofilm formation. The reported data indicate that protein A is not covalently linked to the cell wall when acting in biofilm formation (Merino et al. 2009).

Embp is a giant multifunctional cell-wall protein from *S. epidermidis* that mediates fibronectin-binding, biofilm accumulation, and escape from phagocytosis (Christner et al. 2010; Williams et al. 2002). Its *S. aureus* homolog, Ehb, is a 1.1 megadalton surface protein with fibronectin-binding activity (Clarke et al. 2004).

3.3 Immune Escape and Modulating Factors

The versatility of *S. aureus* as a potent pathogen is *inter alia* based on a variety of strategies to evade the hosts' defensive immune response. The factors involved in undermining the immune defense may be roughly categorized into defensive and active measures.

Defensive strategies are based on protective principles such as biofilm formation (see above) and blood clotting by coagulating factors. In this way, *S. aureus* protects itself by mechanical barriers like the intercellular matrix present in biofilms, a pseudocapsule and/or the microcolony-associated meshwork which is built up by the action of coagulase and von Willebrand factor-binding protein (Guggenberger et al. 2012). In addition, comparative transcriptome and proteome analysis revealed that biofilm-forming staphylococci may adopt a state of reduced metabolic activity due to oxygen and nutrient limitations (Beenken et al. 2004; Resch et al. 2005, 2006). The physiological heterogeneity as well as the reduced to non-growth state may be responsible for the high tolerance in biofilms (Lewis 2007). The biofilm-dependent

evasion strategy is common to *S. aureus* and the less aggressive pathogen *S. epidermidis* while the defense by encapsulation into microcolonies by a concerted action of coagulase and von Willebrand factor-binding protein (Guggenberger et al. 2012) has up to now exclusively been reported for *S. aureus*.

Classically, the determination of coagulase (staphylocoagulase, *coa*) activity is used as a distinctive feature to differentiate between *S. aureus* and most of the other staphylococcal species that are grouped as coagulase-negative staphylococci (CoNS). At the beginning of staphylococcal taxonomy, the classification as “coagulase-negative” was virtually synonymous to “non-pathogenic”. But the ascent of the coagulase-negative *S. epidermidis* as potent pathogen in the clinical field as well as the isolation of *S. aureus* strains that show no coagulase activity (Akineden et al. 2011) have softened the discriminatory power of coagulase activity as a diagnostic criterion.

Coagulase interacts with prothrombin, leading to a complex called staphylo-thrombin. Coagulase activates prothrombin by conformational changes (not by cleavage like in the physiological pathway) eventually resulting in the conversion of fibrinogen into fibrin.

Besides staphylocoagulase, another factor with coagulating activity has been identified in *S. aureus*: the von Willebrand factor-binding protein (vWbp) (Bjerketorp et al. 2004). The von Willebrand factor plays a role in platelet adhesion and aggregation at the site of vascular damage. The *S. aureus* vWbp comprises around 500 amino acids and reveals sequence similarity (about 30 % identity / 45 % similarity between *S. aureus* N315 *Coa* and vWfbp) to coagulase in its N-terminal half and interacts also with prothrombin but in a more host-specific manner. The coagulating activity of *S. aureus* vWbp is highest with human or porcine plasma, while the activity in rabbit plasma which is used in the classical staphylocoagulase assay is significantly lower (Bjerketorp et al. 2004). Since both, vWbp and staphylocoagulase, exert coagulating activity it can be assumed that plasma coagulation by *S. aureus* can be ascribed to a concerted function of both proteins. Furthermore, it has been demonstrated that staphylocoagulase and von Willebrand factor-binding protein are necessary for abscess formation and that they have a role in the protection of *S. aureus* microcolonies against neutrophils (Cheng et al. 2010; Guggenberger et al. 2012). Of note, the observation of coagulase activity is not exclusive for *S. aureus* since various other staphylococcal species like *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. pseudintermedius*, and *S. schleiferi* have been described as coagulase-positive.

The coagulase activity observed in these species makes it tempting to speculate that they might also be able to form a microcolony-like defensive barrier based on the coagulating activity. But at least in the coagulase-positive species *S. pseudintermedius*, of which two complete genome sequences are available (Ben Zakour et al. 2011; Tse et al. 2011), the observed coagulase activity seems to be solely based on the von Willebrand factor-binding protein (Guggenberger et al. 2012), making the formation of *S. aureus*-like two-layered microcolonies unlikely. It will be of interest to see whether the coagulating activity of the other coagulase-positive non-*S. aureus* species can also be exclusively ascribed to the von Willebrand factor-binding protein.

A cell-wall anchored protein with von Willebrand factor-binding activity has been identified also in *S. lugdunensis* (Nilsson et al. 2004). But this protein deviates significantly from the *S. aureus* and *S. pseudintermedius* vWbp in size (about 500 amino acids versus about 2000 amino acids) and lacks sequence similarities with the latter. Furthermore, no coagulase-activity has been detected in *S. lugdunensis*, suggesting that its von Willebrand factor-binding protein does not exert this activity in contrast to the vWfbps in *S. aureus* and *S. pseudintermedius*.

The active strategies against the hosts' immune system involve factors that specifically point towards certain components of the immune response. *S. aureus* underlines its role as the leading staphylococcal pathogen by numerous factors that act in this field and distinguish *S. aureus* from the medium-pathogenic staphylococci. The IgG-binding protein A (Mazmanian et al. 2000) is an exclusive virulence factor of *S. aureus*. Its classical role in host invasion is the binding of immune globulin G via its Fc part, thus counteracting antibody-mediated opsonization by the host immune system. Besides this activity, Spa has also been described to bind the von Willebrand factor (Hartleib et al. 2000) as well as the receptor for tumor necrosis factor α , TNFR1. But the role of these binding activities is not clear (Smeltzer et al. 2009). Besides Spa, *S. aureus* produces various specific immune modulating proteins like the chemotaxis inhibitory protein (CHIPS, *chp*), the staphylococcal complement inhibitor (SCIN, *scn*), staphylokinase (*sak*), and enterotoxin A (*sea*). CHIPS interacts with complement factor C5 (C5aR) and formylated peptide receptor (FPR) of human neutrophils (Postma et al. 2004). SCIN inhibits the conversion of complement factor C3 into C3b thereby hindering phagocytosis of *S. aureus* by human neutrophils (Rooijakkers et al. 2005a, b). Staphylokinase has been reported to destroy defensins (Jin et al. 2004) and to exert an antiopsonic activity (Rooijakkers et al. 2005a, b). Besides its commonly known role in food-poisoning, the superantigenic enterotoxin A interacts with several chemokine receptors (Rahimpour et al. 1999). The genes encoding these immune modulators, *chp*, *scn*, *sea* and *sak*, are located within an innate immune evasion cluster (Chavakis et al. 2005) which is part of a β -hemolysin converting prophage (van Wamel et al. 2006). Also, clumping factor A (*clfA*) shows a recently identified complement modulating activity by binding to complement regulatory factor I thus increasing the inactivation of opsonin C3b (Hair et al. 2008).

The reputation of *S. aureus* as a "master of complement evasion" (Sharp et al. 2012) is corroborated by further complement modulating activities: the extracellular fibrinogen-binding protein (EfB) exerts an antiphagocytic function by interacting with the complement factor C3 (Lee et al. 2004); the extracellular complement-binding protein (Ecb) interacts in a similar way as EfB with the complement system but lacks the fibrinogen-binding activity of the former (Jongerijs et al. 2010); the superantigen-like protein 7 was shown to inhibit specifically the conversion of factor C5 into C5a thus counteracting the chemotaxis of neutrophils (Bestebroer et al. 2010). Recently, also the *S. aureus* surface protein SdrE has been identified as immune evasion factor by binding to complement regulatory factor H (Sharp et al. 2012). Also, in *S. epidermidis* a protein of the serine-aspartate-rich family has been shown to counteract the immune response:

the fibrinogen-binding protein SdrG (Fbe) prevents thrombin-induced fibrinogen clotting by interfering with the release of fibrinopeptide B; this interaction might interfere with the influx of phagocytic neutrophils (von Eiff et al. 2002).

3.3.1 Cytolytic Toxins Interfering with the Immune Response

The most aggressive component of the *S. aureus* immune evasion strategies involves the action of cytolytic toxins that actively attack cells of the host immune system (Foster 2005). In this context, the phenol soluble modulins (PSM) first described by Mehlin et al. in *S. epidermidis* (Mehlin et al. 1999) gained substantial attention during the past years due to the strong lytic activity of α -type PSMs on human neutrophils (Wang et al. 2007). One of the longest known PSM is the delta-toxin (*hld*) which is part of the *agr* regulatory system (Janzon and Arvidson 1990) and which has recently been associated together with beta-toxin with the escape from phago-endosomes of human epithelial and endothelial cells (Giese et al. 2011). The delta-toxin is produced by many staphylococcal species with an intact *agr* system but its activity is boosted by additional cytotoxins mainly present in *S. aureus*. Compared to *S. aureus*, the coagulase-negative staphylococci are commonly regarded as non-toxigenic and as more passive evaders that cope with the immune system mainly by defensive barriers like biofilms. Accordingly, factors with cytolytic activities against neutrophils had generally been assumed to be absent in the coagulase-negative staphylococci. However, this view is going to be challenged somewhat by the identification of the *S. epidermidis* δ -type PSM as potent leukocyte toxin (Cheung et al. 2010). PSM δ exerts a lytic activity towards neutrophils comparable to that of the most active phenol-soluble modulin of *S. aureus*, PSM α 3. But the overall cytolytic activity of *S. epidermidis* culture filtrates was observed to be low, probably due to a reduced production level of PSM δ (Cheung et al. 2010).

3.4 Internalization

S. aureus is generally regarded as an extracellular pathogen but it is also able to evade the attacking immune system by internalization into host cells. This invasive strategy opens the opportunity to persist within the host and consequently enhances the risk of recurrent infections. *S. aureus* is able to persist within a variety of non-professional phagocytic host cells (Hirschhausen et al. 2010). The onset of internalization of infecting *S. aureus* is based on the interaction of certain MSCRAMMs with the phagocytic host cells. The fibronectin-binding proteins FnbpA and FnbpB act in internalization in combination with bound fibronectin that interacts with the host cell integrin $\alpha_5\beta_1$ (Sinha et al. 1999) leading to the activation of a signaling pathway and to the uptake of fibronectin-bound bacteria by host cells (Bartlett and Hulten 2010). FnbpA and FnbpB can also mediate

internalization via the heat shock protein Hsp60 (Dziewanowska et al. 2000). Moreover, evidence has been provided that the Atl autolysins/adhesins of *S. aureus* and *S. epidermidis*, AtlA and AtlE, contribute to the internalization into epithelial cells via heat shock protein Hsc70 as a receptor (Hirschhausen et al. 2010). Recent reports also indicate that internalization as a strategy to persist within the host and to evade its immune system seems not to be exclusively exerted by *S. aureus* since invasion of coagulase-negative staphylococci like *S. epidermidis* into bone cells and *S. saprophyticus* into urinary bladder cells has been observed (Khalil et al. 2007; Szabados et al. 2008). As Atl homologs are present in all tested staphylococcal species (Albrecht et al. 2012), we assume that it may be involved in internalization of coagulase-negative staphylococci that do not contain the fibronectin-binding proteins FnbpA and FnBpB. The persistence of *S. aureus* may be further enhanced if they adopt the state of small-colony variants (SCV) which is accompanied by significant growth defects and other phenotypic changes like unusual colony morphology, reduced pigmentation, and hemolysis as well as auxotrophisms due to defects in electron transport pathways (see below).

3.5 Toxins—Aggressive Pathogenicity Factors with Host Cell Damaging Activity

The pathogenic capabilities of *S. aureus* are to a large extent characterized by a multifaceted repertoire of secreted proteins that act as toxins (see Table 3). Several diseases caused by *S. aureus* are associated with the production of specific toxins like the staphylococcal scalded skin syndrome caused by exfoliative toxins, food poisoning by enterotoxins, and toxic shock syndrome by TSST-1 (Smeltzer et al. 2009). The staphylococcal exfoliative disease is correlated with *S. aureus* strains producing one of four types of exfoliative toxins, ETA, ETB, ETC, and ETD. The exfoliative toxins share similarity with serine proteases and the epidermolytic effect is correspondingly caused by their proteolytic activity (Smeltzer et al. 2009). Besides their cell-damaging activity, some of these toxins exhibit superantigenic characteristics, giving rise to massive T-cell proliferation and enhanced cytokine production which can lead to toxic shock syndrome and hypotension due to capillary leakage (Schlievert et al. 2009).

Toxins with superantigenic activity are the toxic shock syndrome toxin 1 (TSST-1), the staphylococcal enterotoxins of serotypes A–D and I, enterotoxin-like toxins, G, H, J–X. All staphylococcal superantigens are encoded on mobile genetic elements like phages, plasmids, or pathogenicity islands with the exception of enterotoxin-like superantigen X that is chromosomally encoded (Brosnahan and Schlievert 2011). Another class of staphylococcal exotoxins is represented by hemolytic or cytolytic toxins. These include the hemolysins α , β , γ , and δ and the Panton-Valentine-leukocidin (PV-leukocidin). The hemolysins α , β , and δ act as single-component toxins while hemolysin γ and PV-leukocidin are active as

Table 3 Staphylococcal toxins

Toxin	Effect/activity	Features	Reference
Toxic shock syndrome toxin-1	Toxic shock	Superantigen (formerly SEF)	Dinges et al. (2000)
Enterotoxin A, B, C, D, E, G, I	Emetic effects, food poisoning	Superantigen	Dinges et al. (2000), Thomas et al. (2007)
Enterotoxin-like H, J–V	Lack emetic effects or have not been examined	Superantigen	Lina et al. (2004), Smeltzer et al. (2009)
Exfoliative toxins A, B, C, D	Only ETA and ETB associated with Skalded Skin Syndrome	homologs in <i>S. hyicus</i> : ExhA, ExhB, ExhC, ExhD	Ahrens and Andresen (2004), Smeltzer et al. (2009)
α -toxin (hemolysin)	Most active against rabbit erythrocytes; dermonecrotic and neurotoxic	Pore-forming (cylindrical heptamers),	Dinges et al. (2000)
β -toxin (hemolysin)	Highly hemolytic against sheep erythrocytes; “hot–cold” hemolysin; most prevalent in animal isolates	Sphingomyelinase (phosphorylase c activity); on sphingomyelin and lyso-phosphatidylcholine	Dinges et al. (2000)
Pantone-Valentine leukocidin	Attacks leukocytes, tissue necrosis	Bicomponent leukocidin; made of S- and F-components, made by 2–3 % of <i>S. aureus</i> strains, common among CA-MRSA	Lina et al. (1999), Szmigielski et al. (1999)
Leukocidin	Target leukocytes	LukM/F ⁺ widely found among ruminant isolates	Rainard (2007), Smeltzer et al. (2009)
γ -toxin (hemolysin γ)	Affects neutrophils, macrophages, erythrocytes (agar inhibits toxic activity)	Bicomponent leukocidin, made by most <i>S. aureus</i> strains	Dinges et al. (2000)

(continued)

Table 3 (continued)

Toxin	Effect/activity	Features	Reference
Phenol-soluble modulins α	Neutrophil activation and lysis	ca. 20 amino acids in length; associated with CA-MRSA virulence	Wang et al. (2007)
Phenol-soluble modulins β	Cytokine release	ca. 40 amino acids in length, homologs in several CoNS	Otto et al. (2004), Wang et al. (2007)
δ -hemolysin	Lyses erythrocytes, cytokine release	Similar to PSM α , synergism with β -toxin	Dinges et al. (2000), Otto et al. (2004)
SLUSH	Activity similar to δ -hemolysin	Three 43 aa peptides, synergistic activity with β -toxin	Donvito et al. (1997)

bicomponent toxins. α , γ , and PV-leukocidin act by forming pores in membranes of erythrocytes or neutrophils leading to lysis. β -hemolysin is produced predominantly by animal isolates of *S. aureus*, is highly hemolytic for sheep erythrocytes and was shown to have sphingomyelinase activity (Dinges et al. 2000). δ -toxin is a small, helical, and amphipathic peptide of 26 amino acids that is encoded within RNA III, the regulatory component of the *agr* system (Novick and Geisinger 2008). It causes membrane damage in a variety of mammalian cells and is secreted without a discernible signal peptide but it has been suggested that the whole toxin itself might have signal peptide-properties (Dinges et al. 2000). The bicomponent toxins hemolysin γ and PV-leukocidin are composed of two protein subunits, S and F, that assemble into oligomers in the host cell membrane (Dinges et al. 2000). The S components of γ -toxin are encoded by *hlgA* and *hlgC*; the F-component is encoded by *hlgB*. The components of the bipartite PV-leukocidin are encoded by *lukS-PV* and *lukF-PV*, but additional genes have been identified that code for components of bi-component leukocidins: *lukE/lukD* for S components and *luk M/lukF'*-PV code for F components (Smeltzer et al. 2009). The *hlg* genes are almost ubiquitously present in *S. aureus* strains while the genes for PV-leukocidin are located on a bacteriophage and found in only 1–2 % of *S. aureus* strains, predominantly in CA-MRSA. It is believed that PV-leukocidin is associated with severe community-acquired necrotizing pneumonia (Labandeira-Rey et al. 2007). However, this was controversial discussed due to conflicting results based on studies with different animal models. But it has recently been shown that the PV-leukocidin has a severe cytotoxic effect which is restricted to human and rabbit cells and could not be reproduced in murine or monkey cells (Löffler et al. 2010). Very recent data show that PV-leukocidin uses the complement factor C5a receptor, which is abundant in humans and rabbits but not in mice (Jos van Strijp, personal communication).

Due to their cytolytic activity on leukocytes, the bicomponent leukotoxins play an important role in the evasion of the host's immune system (see above and (Foster 2005)).

3.5.1 Toxins in Non-*Staphylococcus aureus* Species

For a long time the aggressive pathogenic lifestyle involving the production of cytolytic toxins has been exclusively assigned to *S. aureus*. But recently, upcoming reports on toxigenic factors or corresponding genes detected in *S. epidermidis* and other CoNS have softened this distinctive criterion (Madhusoodanan et al. 2011; Zell et al. 2008). In some strains of *S. hyicus*, exfoliative toxins have been identified (ExhA, ExhB, ExhC, and ExhD) that probably cause exudative epidermitis in pigs, a skin lesion that has several aspects in common with staphylococcal scalded skin syndrome in humans, and share sequence similarities with ETA, ETB, and ETD of *S. aureus* (Ahrens and Andresen 2004). Moreover, a comprehensive analysis of a collection of CoNS has revealed hemolytic activities and led to the identification of enterotoxins, TSST-1 and exfoliative toxin by immunoblot analysis in a significant

proportion of the tested strains (Seitter et al. 2011; Zell et al. 2008). In addition to these reports on toxins in CoNS, another class of small peptides, termed phenol soluble modulins, is presently gaining more attention as virulence factors with cytotoxic activity (see above).

Small peptides with cytolytic activity have also been described for some strains of the coagulase-negative species *S. lugdunensis*. They exert a synergistic hemolytic activity and have been named SLUSH (*S. lugdunensis* synergistic hemolysin). The synergistic activity of SLUSH in combination with β -hemolytic activity resembles that of δ -toxin of *S. aureus*. The hemolytic activity has been assigned to three peptides of 43 amino acids, which share high sequence identity to each other (Donvito et al. 1997). As already indicated by their size, the SLUSH peptides resemble PSMs and comparably act in the attraction and stimulation of human leukocytes (Rautenberg et al. 2011). The similarity in neutrophil response to PSM-like peptides via the formyl peptide receptor 2 led to the assumption that the production of PSM-like peptides by staphylococci might be a criterion to discriminate between virulent and commensal staphylococcal species (Rautenberg et al. 2011).

4 Fitness Factors Involved in Infection

The pathogenic properties of virulent staphylococcal species would not be complete without the sustaining activity of fitness factors that support survival under hostile conditions in the host. These factors are often not discernible from housekeeping genes that are conserved throughout the genus and thus their distinctive power concerning the categorization as virulence factor is ambiguous.

The fitness factors that play an accessory role in virulence comprise exoenzymes involved in degradation of host tissues and compounds, iron uptake systems, enzymatic functions of more specialized pathogens like the urease of *S. saprophyticus*, and additional physiological pathways such as the arginine deiminase pathway encoded by the ACME element found in *S. epidermidis* ATCC 12228 and *S. aureus* USA 300 that may provide selective advantages in the colonization of the hosts.

4.1 Exoenzymes

Degradative exoenzymes like nucleases, proteases, and lipases generally support the growth of staphylococci by the pulping of polymeric substrates in order to get hold of nutrients and components for biosynthetic purposes. Besides this housekeeping character, some of these exoenzymes may also be involved in infectious processes by sustaining the survival in confrontation with hostile conditions and shortage of certain nutrients.

4.1.1 Nuclease

The thermostable nuclease secreted by *S. aureus* is known for decades and is highly conserved within the species which is exploited in the specific detection of *S. aureus* in blood cultures (Kiedrowski et al. 2011; Lagace-Wiens et al. 2007). Besides its habitual activity concerning the metabolic digestion of nucleic acids it seems to play an additional role in the regulation of biofilm formation (Kiedrowski et al. 2011). Reports emerged on extracellular DNA as a component of the *S. aureus* biofilm matrix (Izano et al. 2008). In this context, it is consistent that the *S. aureus* thermonuclease negatively influences the biofilm formation in *S. aureus* (Kiedrowski et al. 2011) and contributes to escape from neutrophil extracellular traps (Berends et al. 2010).

4.1.2 Protease

Since a protein-based matrix has also been proposed for *S. aureus* biofilms, some of the various extracellular proteases produced by this species could have an influence on switching between biofilm-associated and free living cells. This was shown for two *S. aureus* exoproteases, Aur and SspA (Marti et al. 2010). It can be assumed that also the protein-dependent biofilm formation of *S. epidermidis* is influenced by proteases in a similar way as in *S. aureus*. As mentioned above, proteolytic activity also is involved in the processing of the *S. epidermidis* autolysin and adhesin Aap in order to activate its intercellular adhesion activity (Rohde et al. 2005). Proteases may play a further role in the inactivation of host defense mechanisms like antibodies and platelet microbicidal proteins and in the destruction of host proteins (von Eiff et al. 2002). In *S. epidermidis*, an extracellular metalloprotease with elastase activity was described (Teufel and Götz 1993) and a cysteine protease which degrades immunoglobulins, serum albumin, fibrinogen, and fibronectin has been reported (Sloot et al. 1992). Furthermore, a serine protease is involved in the processing of the antibiotic epidermin (Geissler et al. 1996). Antimicrobial peptides like epidermin and related compounds are produced by many staphylococcal species and their antimicrobial activity makes it inviting to speculate that they may be important for competition with other microorganisms during skin or mucous membrane colonization but direct evidence for this hypothesis is missing (Otto 2010a, b).

4.1.3 Lipase

The lipase or glycerol ester hydrolase is a secreted enzyme which is biosynthesized as a pre-pro-enzyme that is stepwise processed by removing signal- and propeptide during and after secretion to finally achieve its mature form (Rosenstein and Götz 2000). Besides the nutritional function by hydrolyzation of exogenous substrates a role for lipases in the colonization of the skin by the release of fatty acids which may

promote adherence has been proposed (Gribbon et al. 1993). Apart from GehC, a second lipase, GehD, has been identified in *S. epidermidis* (Longshaw et al. 2000). GehD is well conserved among the *S. epidermidis* strains and homologs are also found in *S. aureus*, *S. haemolyticus*, *S. capitis*, *S. caprae*, and *S. warneri*. Lipases are regarded as possible virulence factors in the pathogenesis of a number of localized infections such as boils or abscesses but a corresponding role has not been clearly determined (Longshaw et al. 2000). Accordingly, a surface-associated lipase of *S. saprophyticus*, Ssp, turned out to be necessary for persistence in bladder and kidney in a murine infection model but its true role in infection remains to be elucidated (Kline et al. 2010). Recent findings indicate a more concrete contribution of staphylococcal lipase in pathogenesis since *S. epidermidis* GehD has been identified as a collagen binding adhesin. Also, for the other *S. epidermidis* lipase, GehC, a role in collagen binding has been proposed but is hitherto unexplored (Bowden et al. 2002).

4.2 Iron Acquisition

The acquisition of iron is an indispensable need in microbial life and all staphylococcal species are outfitted with a variety of iron uptake systems that either work siderophore-mediated or by direct uptake of iron via surface proteins. On the other hand, the lifestyle of invasive pathogens involves a competition with the host iron uptake systems for the rare mineral compound. The number of iron uptake systems is not much different in *S. aureus* and the medium- or non-pathogenic staphylococci indicating that successful competition for iron is also important in other habitats than in the mammalian host. A genomic comparison of *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and the non-pathogenic *S. carnosus* (Rosenstein et al. 2009) yielded only the *isd* system as exclusively present in *S. aureus*. The *isd* (iron-responsive surface determinant) genes encode an iron-regulated ATP-driven uptake machinery for heme-iron, which is a preferred iron source for *S. aureus* in the initial stage of infection, while iron carried by siderophores appears to be the preferred source during later stages of infection (Skaar et al. 2004). Since inactivation of the *isd* locus has no detrimental effect on the consumption of heme-iron as sole iron source it became clear that *S. aureus* does not rely on a single system for supply with heme-bound iron (Mazmanian et al. 2003). Skaar et al. identified the *htsABC* transport system as being also involved in heme-iron uptake by *S. aureus* (Skaar et al. 2004). But it has been reported that *hts* may not exclusively be involved in uptake of heme and recent results with *isd-hts* double mutants indicate that further heme-iron uptake systems may exist in *S. aureus* (Wright and Nair 2012). Another uptake system, most probably specific for inorganic iron, is encoded by the *fepABC operon* which comprises the genes for an iron-binding lipoprotein, an iron-dependent peroxidase (that is translocated by the flanking TatA/C system) and an iron permease (Biswas et al. 2009a, b). This system is present in *S. aureus*, *S. carnosus*, and *S. haemolyticus* but is missing in a

number of other staphylococcal species and was shown to contribute to fitness and virulence during infection (Biswas et al. 2009a, b).

4.3 ACME

The arginine catabolic element (ACME) is located on a mobile genetic element and comprises the *arc* genes which encode an arginine deiminase pathway. ACME has been identified with a high prevalence in *S. epidermidis* and in some community-acquired *S. aureus* strains like USA300 (Diep et al. 2006). The ACME element was found to be integrated into *orfX* of *S. aureus* USA300 at the same attachment site as *SCCmec* and, at a corresponding attachment locus, in the genome of *S. epidermidis* ATCC12228 (Diep et al. 2006). Arginine deiminase has been described as a virulence factor in *Streptococcus pyogenes* (Degnan et al. 1998) and seems to be involved in bacterial survival at low pH values. Furthermore, depletion of arginine by the enzymatic activity of arginine deiminase inhibits nitric oxide production from L-arginine which might be advantageous for the bacteria since nitric oxide is a molecule used in immune responses against microbial infections (Diep et al. 2006). The *arc* genes are also found on the chromosomes of the ACME-containing strains and it is hypothesized that the duplication of the encoded biosynthetic pathway might enhance the ability to survive within the host (Shore et al. 2011). The high prevalence of ACME in *S. epidermidis* isolates indicates that this element might also play an essential role for these species and it is assumed that it might have been horizontally transferred from *S. epidermidis* to *S. aureus* (Diep et al. 2006).

4.4 Urease of *Staphylococcus saprophyticus*

S. saprophyticus is a coagulase-negative *Staphylococcus* belonging to the medium-pathogenic species and is of clinical relevance as a frequent cause of uncomplicated urinary tract infections (UTI) (Wallmark et al. 1978). Its specialization with regard to disease and infection locus is reflected by a restricted genetic equipment with virulence and fitness factors. Only a single LPXTG-containing protein has been identified in the genome of *S. saprophyticus* ATCC 15305 that was shown to mediate adherence to human bladder cells (Kuroda et al. 2005). The urease of *S. saprophyticus* seems also to be involved in virulence since the hydrolyzation of urea by urease is known to be an important factor in urinary tract infection (Gatermann et al. 1989). Although the urease of *S. saprophyticus* is not exclusively present in this species, it shows a remarkably higher activity than its homologs in *S. aureus* or *S. epidermidis*, indicating a regulatory phenomenon that causes an adaptation to persistence in the urinary tract environment based on activity of urease as a fitness factor (Kuroda et al. 2005).

4.5 D-Serine Deaminase of *Staphylococcus saprophyticus*

The urinary tract-pathogenic species *S. saprophyticus* appears to be well adapted to this site of infection as it is resistant to D-serine in contrast to other staphylococcal species. Human urine contains a relatively high concentration of D-serine, which is toxic to several non-uropathogenic bacteria, but can be utilized or detoxified by uropathogenic *Escherichia coli* (UPEC). *S. saprophyticus* contains a D-serine deaminase gene (*dsdA*) which is homologous to the corresponding gene in UPEC. The gene is absent in *S. xylosus* and *S. cohnii*, phylogenetically close relatives of *S. saprophyticus*, and is also not found in isolates of *S. aureus*, *S. epidermidis* and 13 other staphylococcal species (Sakinc et al. 2009a, b). It is proposed that D-serine utilization and detoxification may be a general property of uropathogenic bacteria.

5 Physiological Properties Involved in Virulence and Fitness

The virulence of staphylococci is influenced by certain physiological properties that may impact the interplay with competing pathogens, enable persistence in the infected host, mediate resistance to antimicrobial compounds like lysozyme and reactive oxygen substances, and allow for evasion from neutrophil killing. Examples for these physiological influences are presented in the following sections.

5.1 Cyanide-sensitive Cytochrome bd Oxidase Prevalent in Pathogenic Species

Pseudomonas aeruginosa and *S. aureus* are opportunistic pathogens and frequently coinfect the lungs of cystic fibrosis patients. With increasing age of the patients the number of *S. aureus* cells declines slightly and that of *P. aeruginosa* increases. One of the main reasons for the decrease in *S. aureus* is its susceptibility to respiratory inhibitors excreted by *P. aeruginosa*, like pyocyanin, hydrogen cyanide, or quinoline N-oxides that may act against the commensal flora as well as against host cells. *S. aureus* and other pathogenic species contain a pyocyanin- and cyanide-sensitive cytochrome bd quinol oxidase, CydAB, while the non-pathogenic species such as *S. carnosus*, *S. piscifermentans* and *S. gallinarum* have a pyocyanin- and cyanide-resistant cytochrome bd quinol oxidase with the subunit CydB determining the resistance (Voggu et al. 2006). On the other hand it was also shown that a subpopulation of *S. aureus* survives these respiratory toxins adopting the small-colony variant (SCV) phenotype (Biswas et al. 2009a, b).

5.2 *Small-Colony Variants as a Survival Strategy Under Certain Selective Pressure*

Small-colony variants (SCVs) can be produced most likely by all facultative anaerobic microorganisms. In *S. aureus*, they have been recognized for many years and the small-colony variant phenotype is associated with persistent and recurrent infections. Clinical *S. aureus* SCVs are frequently auxotrophic for compounds involved in the biosynthesis of the electron transport chain, like menadione, thymidine, or hemin (Proctor et al. 2006). A stable *S. aureus hemB* mutant showed typical characteristics of clinical SCVs, such as slow growth, decreased pigment formation, low coagulase activity, reduced hemolytic activity, and resistance to aminoglycosides (von Eiff et al. 1997). Furthermore, the mutant was able to persist within cultured endothelial cells due to decreased alpha-toxin production. In Northern and Western blot analyses a markedly reduced expression of alpha-toxin and protein A at the mRNA and protein level was shown. The SCV phenotype of the *hemB* mutant was reversed by growth with supplemented hemin or by complementation with intact *hemB* (von Eiff et al. 1997). Hence, a defect in the electron transport system allows *S. aureus* SCVs to resist aminoglycosides and to persist intracellularly. The SCV phenotype is the answer of pathogenic staphylococcal species to certain antibiotics, to respiratory toxins as produced in the co-infection with *P. aeruginosa* or during respiratory burst in phagocytic cells.

5.3 *Structural Alteration of Peptidoglycan and Resistance to Lysozyme*

S. aureus belongs to the few bacterial species that are completely resistant to lysozyme, which greatly contributes to their persistence and success in colonizing the skin and mucosal areas of humans and animals. The reason for the lysozyme resistance is mainly based on the O-acetylation of the peptidoglycan structure at the C6-OH position of the muramic acid (Bera et al. 2005). The peptidoglycan-specific O-acetyltransferase is encoded by the *oatA* gene. Interestingly, this *oatA* gene is only present in pathogenic lysozyme-resistant staphylococci (e.g., *S. aureus*, *S. epidermidis*, *S. lugdunensis*, and others). All non-pathogenic species are lysozyme-sensitive. They can be divided into sensitive (e.g. *S. carnosus*, *S. gallinarum*, and *S. xylosum*) and hypersensitive species (e.g. *S. equorum*, *S. lentus*, and *S. arlettae*). In all lysozyme-sensitive species, the analyzed peptidoglycan was de-O-acetylated (Bera et al. 2006). Besides O-acetylation of peptidoglycan, also the wall teichoic acid and a high degree of murein cross-linking play a role in lysozyme resistance (Bera et al. 2007). It turned out that lysozyme acts not only by its muramidase activity but also as an antimicrobial peptide (Herbert et al. 2007).

5.4 Staphyloxanthin

In former times the pigmentation was a main criterion to distinguish *S. aureus* from *S. epidermidis* (originally called *S. albus*) (Götz et al. 2006). The major pigment produced by *S. aureus* is the deep-yellow carotenoid 4,4'-diaponeurosporene which is, after prolonged cultivation, in part converted into the orange pigment staphyloxanthin (Marshall and Wilmoth 1981; Wieland et al. 1994). The staphyloxanthin biosynthesis genes are organized in an operon, *crtOPQMN* which is controlled by a sigma(B)-dependent promoter (Pelz et al. 2005). The first step in the biosynthesis of staphyloxanthin is the head-to-head condensation of two molecules of farnesyl diphosphate catalyzed by the dehydrosqualene synthase CrtM to form dehydrosqualene. Dehydrogenation of dehydrosqualene by CrtN yields the yellow pigment 4,4'-diaponeurosporene. The subsequent conversion into staphyloxanthin is catalyzed in further steps involving oxidation (CrtP) and esterification reactions (CrtQ and CrtO) (Pelz et al. 2005). The membrane-bound staphyloxanthin plays a role in the resistance to reactive oxygen species (ROS) and mediates the evasion from neutrophil killing (Clauditz et al. 2006).

6 Antibiotic Resistances

Besides the fitness factors that support survival under hostile conditions, the quick adaptation to selective pressure exerted by antibiotic treatment represents an indispensable capability of pathogenic staphylococci. Staphylococcal resistance to antibiotics is mediated by genes that are in most cases located on mobile genetic elements thereby allowing their rapid spreading by lateral gene transfer. The archetypical resistance to the β -lactam derivative methicillin is encoded by the *mec* gene carried by the staphylococcal cassette chromosome *SCCmec* (see below). Methicillin-resistant *S. aureus* strains (MRSA) soon occurred after the introduction of the semi-synthetic penicillins (Barber 1961). The application of alternative antibiotics in the treatment of staphylococcal infections has been quickly counteracted by the appearance of the corresponding resistances thus leading to the synonymous use of the term MRSA for multiple resistant *S. aureus*. Most of the antibiotic resistance genes are carried by plasmids and transposons (Malachowa and DeLeo 2010) leading to the quick spreading of resistance genes under the pressure of antibiotic treatment. A comprehensive and up-to-date compilation of the numerous resistance determinants of staphylococci is presented in a review by Malachowa and DeLeo (Malachowa and DeLeo 2010). The increasing resistance of staphylococci left only a few antibiotics being effective in the treatment of infections. But also the use of glycopeptides, particularly vancomycin, as so-called last-resort antibiotics, runs the risk of getting ineffective since *S. aureus* strains with reduced susceptibility to glycopeptides are emerging. The versatility of *S. aureus* in coping with the threat of being attacked by these

antibiotics is demonstrated by different strategies to counteract the effect of the cell-wall antibiotic vancomycin. An intermediary resistance to Vancomycin is achieved by changes in the cell wall and in metabolic pathways (Appelbaum and Bozdogan 2004), while a high level vancomycin resistance is mediated by acquisition of the resistance determinant *vanA* that seems to originate from an enterococcal source (Rehm and Tice 2010). Other antibiotics like macrolides, lincosamides, aminoglycosides, and quinolones were introduced but soon became also compromised by the emergence of resistances (Cameron et al. 2011) rendering the antibiotic resistances an ever-increasing threat in the treatment of staphylococcal infections.

7 Regulation of Virulence Determinants

The versatility and quick adaptability of staphylococcal pathogens depends on the regulated expression and the concerted action of many exoproteins in response to changes in the hostile environment in the host. Several global regulatory systems have been reported for staphylococci of which some are involved in the regulation of virulence factors like the two-component regulatory systems *agr*, *sae*, *arlRS*, *srrAB* and *lytRS* and the transcription factors *sarA* and its homologs *sarR*, *sarT* and *sarS* (Bronner et al. 2004; Novick 2003). Other more specialized regulatory systems, *vra* and *gra*, are involved in the resistance to antibiotics (Gardete et al. 2006; Meehl et al. 2007) or, like *aps*, in the resistance to antimicrobial peptides (Li et al. 2007). The global regulatory systems are well conserved among staphylococci and most of them are also present in the non-pathogenic *S. carnosus* TM300 (Rosenstein et al. 2009). Their role in the regulation of virulence factors has been confirmed by numerous studies on the attenuation for virulence of mutants in the regulatory systems (for a detailed overview of the regulatory systems, see the excellent reviews by Novick (2003) and Bronner et al. (2004). The *rot* and *sar* systems seem to be specific for *S. aureus* and may exclusively function as virulence regulators (Bronner et al. 2004). The *agr* system, albeit ubiquitously present in staphylococci, reveals a significant amount of genetic diversity resulting in various *agr* types of *S. aureus* which were found to correlate with certain diseases (Feng et al. 2008).

8 Genomic Aspects Concerning Staphylococcal Virulence

Due to their impact in infection, staphylococci are also of great interest in genomic studies. In the course of the flourishing genomic research during the past decade, numerous staphylococcal genome sequences have been determined and due to the highly effective next generation sequencing methods the number of staphylococcal genome projects is ever increasing. According to its leading role as human

pathogen, most of the determined staphylococcal genome sequences are derived from various *S. aureus* strains. Currently (April 2012), 34 completed genome sequences of different *S. aureus* strains or isolates are available in the public databases (Table 1) and a multiple of this is listed as ongoing bioprojects. Based on this overwhelming amount of sequence information, important aspects concerning the impact of *S. aureus* as leading pathogen could be analyzed. Thus, the genomic changes leading from a vancomycin-susceptible bloodstream isolate of *S. aureus* to an intermediately vancomycin-resistant *S. aureus* (VISA) could be monitored based on the corresponding genome sequences (Mwangi et al. 2007). Another study reconstructed the jump of an *S. aureus* strain from human to poultry more than 30 years ago (Lowder et al. 2009). Genomic studies helped also to explain the increasing epidemiological success of community-acquired *S. aureus* strains (Diep et al. 2006; Highlander et al. 2007; Huang et al. 2012). Last but not least, the genome analysis of an early-branched, non-pigmented *S. aureus* strain yielded valuable information about *S. aureus* genome evolution (Holt et al. 2011).

Compared to the wealth of genomic information available for *S. aureus*, the amount of sequence data for non-*S. aureus* genomes is sparse. According to their clinical relevance, two genomes of *S. epidermidis* and *S. lugdunensis* strains have been published (Gill 2009; Heilbronner et al. 2011; Tse et al. 2010; Zhang et al. 2003). The determined genome sequences of *S. haemolyticus* and *S. saprophyticus* offered insights into the genetic basis for a multi-resistant phenotype and the virulence of a causative agent of urogenital tract infections (Kuroda et al. 2005; Takeuchi et al. 2005). Recently, the genomes of two canine *S. pseudintermedius* strains were added to the database providing the first genome data on coagulase-positive non-*S. aureus* strains (Ben Zakour et al. 2011; Tse et al. 2011).

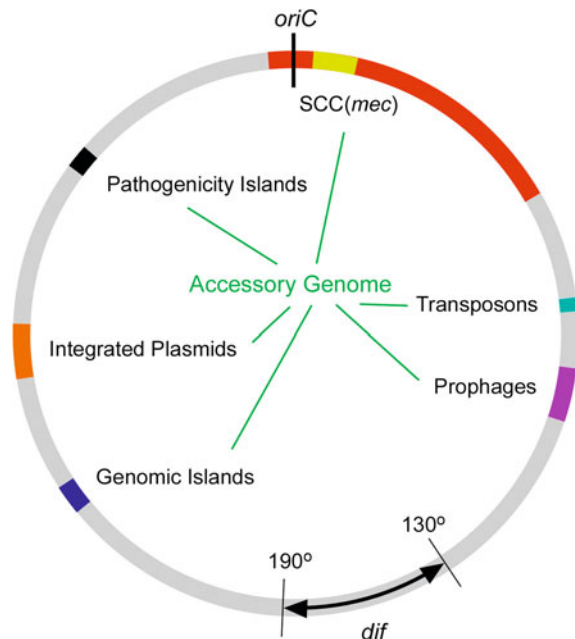
Concerning the non-pathogenic staphylococcal species, still only one sequence represented by the genome of the food-grade organism *S. carnosus* TM300 is available which furnished the basis for differential genome studies of pathogenic and non-pathogenic staphylococci (Rosenstein et al. 2009; Rosenstein and Götz 2010).

8.1 Genome Structure

A comparison of the staphylococcal genome sequences revealed a structure which is common to all genomes (Fig. 1). Each genome reveals a region that comprises about 75 % of the genome size and predominantly carries genes that are conserved throughout the genus and reveal a high degree of synteny. Accordingly, this part is designated as the conserved core region (Gill et al. 2005). More pronounced differences among the staphylococcal core regions are mostly due to mobile elements like prophages, genome, and pathogenicity islands, transposons, IS elements, and integrated plasmids (Baba et al. 2002). A comprehensive comparison of dominant sequence type (ST) lineages of *S. aureus* showed that the core genome is interspersed with regions that are highly variable between the lineages: thus, the core

Fig. 1 General structure of staphylococcal genomes. The *light-gray* section represents the conserved core region, the *red* part corresponds to the variable genome region (*oriC* environ). The components of the accessory genome are named accordingly.

oriC = origin of replication; *dif* = replication termination locus. The *double-headed arrow* indicates the span comprising the replication termination loci in the various staphylococcal genomes (see Fig. 2)



genome has been subdivided in the stable core and the core variable (CV) genome region (Lindsay et al. 2006). Many core variable genes are toxins, superantigens, exoenzymes, and regulatory elements involved in virulence. Furthermore, these genes are distinguished by a higher rate of evolutionary accepted mutations and many repeat regions (Feng et al. 2008). It should be noted that the core variable genes are defined by the comparison of closely related strains of one species. In a genome comparison of different species where the syntenic character of the conserved core has been blurred by the accumulation of evolutionary changes, a core variable region would be much less confined. The remaining 25 % of the genomes correspond to a region that exhibits a high degree of variability even between closely related species. This variable region carries the majority of the species-specific genes like for example the genes encoding protein A (Mazmanian et al. 2000) and staphylocoagulase in *S. aureus* and thus is an important factor in shaping genomic characteristics of species or strains. Since this variable region is located next to the chromosomal replication origin, *oriC*, the term “*oriC* environ” has been coined (Takeuchi et al. 2005).

Whereas the relative composition concerning conserved and variable genome regions is similar in the staphylococcal genomes sequenced so far, a remarkable difference exists regarding the lengths of the replicated genome halves (replichores) as determined by the locations of *oriC* and the replication termination locus which is near the conserved *dif* site (Hendrickson and Lawrence 2007). While all *S. aureus* genomes reveal almost perfectly balanced genomes with replichores of

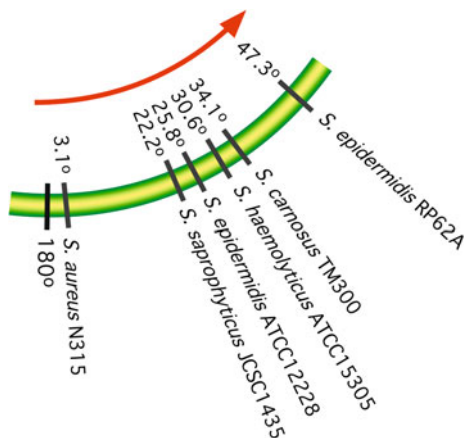


Fig. 2 Positions of the replication termination loci in selected staphylococcal genomes. The 180° indicates the position directly opposite to the origin of replication (at 0°). For each genome sequence the deviation from the 180° position is indicated

about equal lengths, the genomes of non-*S. aureus* species show a significant asymmetry as shown by a location of the *dif* region that remarkably deviates from the 180° coordinate (referring to the location of *oriC* at 0°) (see Fig. 2).

The reason for the differences in genome symmetry is unknown but we hypothesize that it could have arisen from an inversion event during the staphylococcal evolution that comprised the origin of replication in a balanced ancestor genome as can be observed between the genomes of *S. epidermidis* strains RP62A and ATCC12228 (see Fig. 3). If *oriC* were placed at a decentral position in the inverted fragment, the inversion would have led to positioning the replication origin closer to the replication terminator thereby resulting in different replicore lengths in the descendant genome. This might also be the reason for the different replicore lengths of *S. epidermidis*, RP62A and ATCC12228 (Fig. 3).

The *S. aureus* genomes reveal a more stable character with less obvious signs of recombinatorial events than the non-*S. aureus* species. This is assumed to be a consequence of a preservation of the clonal character of the *S. aureus* lineages by restriction-modification systems that control the acquisition of foreign DNA. On the other hand, it has been reported that the reversible inversion of a large chromosomal fragment provokes a switch between small colony and normal colony variants in *S. aureus* Mu50Ω (Cui et al. 2012). Of note, the inverted fragment comprises about one genome half of *S. aureus* Mu50Ω indicating that the symmetry concerning the replicore lengths might not be disturbed.

In contrast to *S. aureus*, the evolution of the *S. epidermidis* lineages has been proposed to be based mainly on recombinatorial events and lateral transfer of genetic material which is supported by the lack of corresponding restriction-modification systems (Feng et al. 2008).

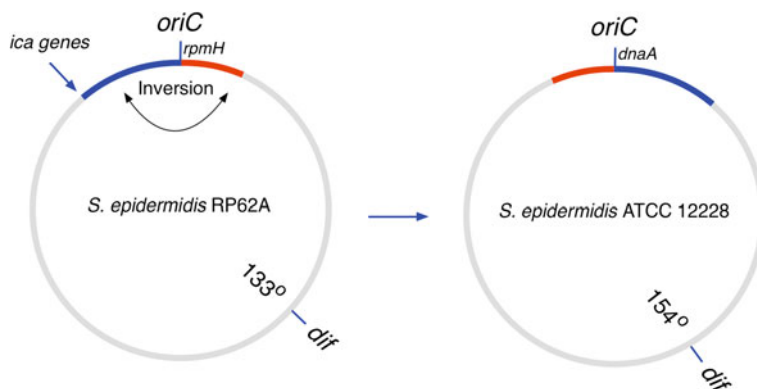


Fig. 3 Inversion of a chromosomal fragment observed between *S. epidermidis* strains RP62A and ATCC 12228. In order to emphasize the decentral position of *oriC* in the inverted fragment, the region downstream of *oriC* (referring to the RP62A genome) is colored red while the upstream region is labeled in dark blue. The position of the *ica* genes (that are not present in strain ATCC 12228) is indicated. In order to compensate for the inversion, different start genes were chosen by the annotators: *rpmH* in strain RP62A and *dnaA* in strain ATCC 12228. The differing distances between *oriC* and *dif* (given in angular degrees) in both genomes are probably caused by the asymmetric inversion around *oriC*

8.2 Genome Rearrangements

The variable region around *oriC* seems to be a preferred region for chromosomal rearrangements. Genome comparisons indicate inversions comprising the *oriC* region in some staphylococcal genomes which led to an inverted orientation of the conserved genes next to the replication origin: *dnaA* to *gyrA* on one side of *oriC* and *rpmH* to *gidB* on the other side. All *S. aureus* genomes sequenced so far show the same orientation of these conserved genes. In contrast, a pronounced heterogeneity exists among the non-*S. aureus* genomes concerning the orientation of the *oriC* region. Since it is a kind of unwritten rule to annotate *dnaA* as the first gene in newly determined genome sequences, considerable inconvenience may occur in genome alignments as some genomes appear to be inverted with respect to others resulting from inversions around *oriC* and the concomitant insistence on annotating *dnaA* as gene number 1. In order to compensate for this, some staphylococcal genome sequences have been annotated with *rpmH* as the first gene (see Fig. 3). Interestingly, different orientations of *oriC* even occur within one species as revealed by the alignments of *S. epidermidis*, RP62A and ATCC 12228. As mentioned above, this rearrangement is accompanied by the absence of the *ica* genes in strain ATCC12228 and a biofilm-negative phenotype. This example emphasizes the role of chromosomal rearrangements in the evolution of staphylococcal genomes. This is also demonstrated by the different locations of *att* sites with the same core sequences in *S. haemolyticus* and *S. saprophyticus* in comparison with *S. aureus*, which is presumably caused by genome rearrangements (Novick and Subedi 2007).

8.3 Mobile Genetic Elements in Staphylococcus

In summary, the comparative studies on staphylococcal genome sequences revealed a high flexibility corresponding to the capability to adapt quickly to the varying challenges exerted by an environment that could drastically change in the course of switching from commensalic to infectious lifestyle due to host immune defenses, anti-infective treatments, or the appearance of competing bacteria. Soon after the first staphylococcal genomes had been sequenced it became clear that the differences in pathogenic potential and the adaptability to environmental changes are to a large extent determined by mobile genetic elements which have been detected in almost all staphylococcal genomes. Their acquisition by horizontal transfer allows the rapid spreading of genetic information in order to cope with selective environmental challenges. Genomic comparisons showed that particular virulence factors are coupled to certain mobile genetic elements and that different degrees of pathogenicity correlated with the presence or absence of these elements. Nearly all toxins that cause specific diseases like pneumonia, toxic shock syndrome, necrotizing fasciitis, and food poisoning are encoded by mobile genetic elements (Novick et al. 2010). The gene coding for the PV-leukocidin is located on prophage Φ SA2pvl that is predominantly found in community-acquired (CA) MRSA (Otto 2010a, b). In *S. aureus*, also the biofilm-associated protein Bap is located on a pathogenicity island, SaPIbov2, that is exclusively present in strains associated with bovine mastitis (Tormo et al. 2005; Ubeda et al. 2003). In contrast, in other staphylococcal species *bap*-homologous genes are not located on mobile genetic elements demonstrating the remarkable genetic variability among the staphylococcal species (Tormo et al. 2005).

In *S. epidermidis*, integrated plasmids play a significant role in providing strain-specific properties. In strain RP62A the plasmid ν Se1 and in strain ATCC12228 the plasmid ν Se2 carry the genes for a strain-specific sortase and two strain-specific MSCRAMMs (Gill et al. 2005; Zhang et al. 2003). The mobile elements contribute to a large extent to the variable or accessory part of the staphylococcal genomes and comprise bacteriophages, genomic islands, pathogenicity islands, plasmids, transposons, and the staphylococcal cassette chromosome (SCC) (Lindsay and Holden 2006).

8.3.1 Pathogenicity Islands

Virulence factors or resistance determinants are often encoded on prophages or genomic islands. According to their carrying of virulence genes the corresponding genomic islands are referred to as pathogenicity islands. Some uncertainty exists concerning the definition and nomenclature of pathogenicity islands. Therefore, it has been proposed to use the term “pathogenicity island” only for those elements that share a conserved functional and genetic organization and insertion site specificity and to name them “SaPI” in the case of *S. aureus* pathogenicity islands and with a corresponding species-dependant derivation for non-*S. aureus* islands (Novick and Subedi 2007). Correspondingly, for SaPI-type islands detected in *S. haemolyticus*

(originally designated vSh2) (Takeuchi et al. 2005) and *S. saprophyticus* (originally designated vSs15305) (Kuroda et al. 2005) the names ShPI2 and SsPI15305 have been proposed, respectively (Novick and Subedi 2007).

The pathogenicity islands are widespread among the *S. aureus* genomes and related elements found also in *S. saprophyticus* and *S. haemolyticus* (Novick and Subedi 2007). The genetic organization of the pathogenicity islands resembles that of temperate phages; their integration occurs integrase-dependent at specific chromosomal sites and their mobilization is mediated by helper phages like 80 α or Φ 13 (Maiques et al. 2007). As implied by their designation, the pathogenicity islands are a major source of pathogenicity factors encoded by mobile elements in staphylococci.

Staphylococcal superantigens are often encoded by genes located on pathogenicity islands with the toxic shock syndrome toxin being the toxin most frequently encoded by SaPIs (Novick and Subedi 2007). As mentioned above, also the *S. aureus* biofilm associated protein Bap is encoded on a pathogenicity island, SaPI bov2 (Ubeda et al. 2003).

8.3.2 Genomic Islands

Besides the well-defined SaPIs, also other less strictly defined genomic islands are known which comprise clusters of genes that probably have been obtained by horizontal gene transfer. Many of these gene clusters are flanked by direct repeats and could have been mobilized by self-coded transfer functions (Novick et al. 2010). These elements often have obviously become immobilized in the genomes thus representing only remnants of formerly active elements.

Two islands, vSaz und vSa β , are found in nearly all *S. aureus* isolates (Fitzgerald et al. 2003). vSaz carries a cluster of genes encoding superantigen-like proteins, the *set*-cluster (or, according to a new nomenclature, *ssl* cluster) and a lipoprotein (*lpl*) cluster (Feng et al. 2008; Lina et al. 2004). The genomic island vSa β encodes a serine protease cluster (*spl*) and an enterotoxin cluster (Feng et al. 2008).

8.3.3 Prophages

Prophages play an important role in the differential genome compositions of staphylococci and often carry genetic information that contributes significantly to the virulence traits of a certain strain. Prominent virulence factors provided by prophages are enterotoxin A (involved in food poisoning), exfoliative toxin A (localized scalded skin syndrome) and PV-leukocidin which is implicated in the pathogenesis of community-acquired MRSA (Ben Zakour et al. 2008; Löffler et al. 2010). Other prophages provide their host genomes with means to avoid the hosts' immune responses like the chemotactic inhibitory protein (CHIPS), staphylokinase or the staphylococcal complement inhibitor (SCIN) (van Wamel et al. 2006).

8.3.4 Staphylococcal Cassette Chromosome

The staphylococcal cassette chromosome (SCC) represents a unique type of mobile genetic element in *Staphylococcus* that carries the gene for methicillin resistance, *mecA*, and reveals specificity for integration at a specific site downstream to the conserved gene *orfX* that is located near the origin of replication (Katayama et al. 2000). Spreading of the *mec*-containing SCC-elements is the cause for rapidly turning MSSA into MRSA under the corresponding selective pressure.

Besides the *mecA* gene, the SCC element carries genes responsible for mobilization, cassette chromosome recombinase (*ccr*) genes, and interjacent DNA, formerly designated junkyard (J-) DNA whereas the “J” is now standing for “joining” (Turlej et al. 2011). The J-region may function as target for additional mobile elements like plasmids or transposons that could add further resistance genes to the SCC element (Turlej et al. 2011). This region thus represents a hot spot for recombination resulting in several structural variants of SCC*mec*. Thus, various combinations of *mec*, *ccr* and J regions have been described leading to numerous types and subtypes of SCC elements (Turlej et al. 2011). Some SCC types also carry restriction-modification systems but these are only present in a small proportion of *S. aureus* strains (Feng et al. 2008). In addition to *S. aureus*, SCC*mec* has also been described in several coagulase-negative staphylococci (Garza-Gonzalez et al. 2010).

8.4 Repeat Sequences

Besides the presence of various mobile genetic elements, the adaptive flexibility of bacterial genomes is mirrored by repetitive DNA sequences. There are various types of repeat sequences identified in staphylococcal genomes. They may play a role for genome diversification due to recombinatorial events that might be important for the rapid adaptation to the environment (Aras et al. 2003).

One type of sequence repeats is represented by the CRISPR (Clustered regularly interspaced short palindromic repeats) elements which are present in roughly 50 % of bacterial genomes and in most archae. The CRISPRs are composed of repeats with lengths between 20 and 50 nucleotides which are interspersed by unique spacer sequences (Bhaya et al. 2011; Marraffini and Sontheimer 2008). Recently, it has been recognized that the CRISPR elements act in cooperation with flanking CRISPR-associated (*cas*) genes as an adaptive immune mechanism against foreign DNA like phages and plasmids, which is comparable to eukaryotic RNA interference systems (Wiedenheft et al. 2012). According to the literature, CRISPR elements are seldom found in *S. aureus* strains (Holt et al. 2011; Otto 2012) with only two reported CRISPR-carrying *S. aureus* strains: the livestock-associated sequence-type 398 (Golding et al. 2010) and the early-branched lineage

strain MSHR1132 (Holt et al. 2011). In contradiction to this, the database of CRISPR elements, CRISPRdb (Grissa et al. 2007), lists most *S. aureus* genome with at least one predicted CRISPR element. But both sources congruently state that *S. saprophyticus*, *S. haemolyticus*, and *S. carnosus* reveal no CRISPR (Grissa et al. 2007; Holt et al. 2011). A CRISPR element is accordantly reported also for *S. epidermidis* RP62A where it is involved in the maintenance of genetic identity by preventing horizontal gene transfer (Marraffini and Sontheimer 2008). Also, both sequenced *S. lugdunensis* strains (Heilbronner et al. 2011; Tse et al. 2010) and *S. pseudintermedius* ED99 (Ben Zakour et al. 2011) are listed in the CRISPRdb with CRISPR elements.

Another type of repeat element, STAR (= *Staphylococcus Aureus* Repeat), has been detected in *S. aureus* and seems to be present also in some other species of the genus, like in *S. epidermidis*, as indicated by hybridization (Cramton et al. 2000). These repeats are found in intergenic regions as in the region between the *geh* (lipase) gene and *icaC* gene of the *ica*-operon (Cramton et al. 2000). The STAR-comprising intergenic regions differ significantly between various *S. aureus* strains indicating a high genetic variability, probably due to recombinatorial events.

The STAR-containing intergenic region seems to provide also a breakpoint in the rearrangement of chromosomal fragments as indicated by the large inversion observed between the genomes of *S. epidermidis* RP62a and *S. epidermidis* ATCC12228 (see Fig. 3). Since the intergenic region between *icaC* and *geh* flanks the large chromosomal fragment that has been inverted according to the genome comparison of *S. epidermidis* RP62A and *S. epidermidis* ATCC 12228, it can be assumed that this rearrangement caused the concomitant loss of the *ica*-operon in *S. epidermidis* ATCC12228. Since sequence repeats could facilitate genomic diversification by recombinational events (Aras et al. 2003) it is tempting to speculate that the STAR repeats might be prone to recombinatorial events.

8.5 Single Nucleotide Polymorphisms

Also, Single Nucleotide Polymorphisms (SNP) play a role in the variation of virulence among *S. aureus* strains. Of note, this finding is due to the wealth of genomic data available for various *S. aureus* strains or isolates. Since only one or two genome sequences are available for the other non-*S. aureus* species, the role of SNPs still remains uncertain for these. A striking example for the impact of single nucleotide exchanges in *S. aureus* is represented by the study on the decrease of vancomycin susceptibility in an *S. aureus* isolate in the course of the antibiotic treatment of an endocarditis patient. The increasing vancomycin resistance of the infecting strain was found to correlate with the accumulation of 35 point mutations in the isolates taken between the start and end of treatment (Mwangi et al. 2007).

9 The Non-pathogenic *Staphylococcus carnosus* in Comparison with the Other Staphylococcal Species

S. carnosus is a non-pathogenic member of the genus that is applied in starter cultures in the food industry and has been classified as GRAS = generally recognized as safe (Götz 1990; Schleifer and Fischer 1982). Its genome sequence has been determined and provided the means for comparative studies of pathogenic and non-pathogenic staphylococci (Rosenstein et al. 2009; Rosenstein and Götz 2010).

The *S. carnosus* genome is characterized by many features that result from an adaptation to constant environmental conditions as they are present in a defined meat starter culture environment. Phenotypically, *S. carnosus* TM300 displays various metabolic functions that favor its application in meat fermentation, like nitrate/nitrite reduction (Neubauer and Götz 1996; Neubauer et al. 1999), high osmotic stress tolerance, and two catalase genes (Rosenstein et al. 2009). On the other hand, the long-standing habitation of a nutrition-rich environment led to the loss of many functions that are required by staphylococci that live in more challenging surroundings. *S. carnosus* exhibits no noteworthy exoenzyme activities like lipase, protease, and nuclease which are commonly observed in other staphylococci. These signs of a degenerative evolution become also apparent in a comparative analysis of the *S. carnosus* genome data and those of virulent staphylococci. The GC content of nearly 35 % significantly surmounts that of the other staphylococci (Rosenstein et al. 2009). In addition, the relatively small genome size and a significant number of truncated genes including important functions like global regulators (*agr*, *sae*), the signal recognition particle *ffh* and various exoenzyme genes are a clear indication for an adaptation to a nutritionally stable habitat (Rosenstein et al. 2009). Similar signs of degenerative accommodation are also observed in other bacteria that are applied in the food industry (van de Guchte et al. 2006). While the genome data of staphylococcal species that are confronted with quickly changing and challenging habitats present in the living human or animal host are distinguished by a remarkable genome plasticity, *S. carnosus* reveals a rather static genome with few repetitive sequences, few traces of ancient mobile elements like an inactive prophage and a remnant of a genome island. All staphylococcal genomes determined so far carry also an SSC element downstream of the conserved open reading frame *orfX*. In contrast, *S. carnosus* reveals only a *pbp4*-homologous gene as a faint sign of a former SCC in the corresponding region of its genome. Taking these observations together, *S. carnosus* reveals the lowest genome plasticity of all staphylococcal genomes sequenced so far.

On the other hand, also the comparably static *S. carnosus* genome comprises a variable region next *oriC* (*oriC* environ) with an abundance of species-specific genes. A functional categorization of the gene products encoded within the *S. carnosus* *oriC* environ exhibits a clear prevalence of genes involved in amino acid transport and metabolism, again indicating an adaptation to the specific

nutrition-rich environment for a meat fermentation starter bacterium. The remnants of a prophage and a genomic island as well as the faint indication for a former SCC element suggest an ancestor that revealed a higher genome plasticity than *S. carnosus* at the present evolutionary stage. The *S. carnosus* genome data also revealed some genes with weak similarities to virulence factors like hemolysin and exotoxins and to hypothetical proteins found in *S. aureus* pathogenicity islands (Rosenstein et al. 2009).

It is thus tempting to speculate that the non-pathogenic character of *S. carnosus* is the consequence of a degenerative evolution from a pathogenic progenitor as indicated by the genetic relicts in its genome. The original habitat of *S. carnosus* is unknown but the isolation of *S. carnosus* TM300 from fermented meat suggests a former habitation of animals. Since the genetic repertoire necessary for the habitation of humans or animals most likely also involves factors classified as virulence genes it is not surprising that *S. carnosus* reveals signs of a putative pathogenic ancestor.

10 Conclusions

Albeit being implicated by the title of this article, a clearly differentiated characterization of staphylococcal pathogenicity by arbitrary and crude categories like “highly”, “medium-” and “non-pathogenic” appears not to be adequate in the face of the tremendous genetic and phenotypic flexibility and variability of staphylococcal virulence. The graduation of staphylococcal pathogenicity based on qualitative and quantitative criteria is hampered by the lack of a universally valid definition of what has to be regarded as a virulence factor. The difficulty to differentiate gene products that are exclusively involved in pathogenicity from those that may have dual roles in supporting bacterial life during infection as well as in “daily life” metabolic functions is evoked by the complex and multivalent nature of pathogenicity itself. The multitude of studies on the multifaceted aspects of staphylococcal pathogenicity of which only the “tip of the iceberg” could be addressed in this summarizing article, create a view of a variegated interplay of specialized virulence factors, ambivalent fitness factors, genetic potential, regulatory phenomena, and the resulting versatility to cope with quickly changing environmental stimuli. Consequently, if we focus only on obvious virulence genes in genomes, we might overlook the more subtle but powerful factors that lead to chronic and persistent infections, cause colonization of specific sites of body regions including implant materials, contribute to high genetic adaptability to environmental changes, or represent fitness factors with impact on physiological diversity and growth rate.

Examples for the multivalent character of pathogenicity are numerous in staphylococcal pathogens. For instance the janus-headed character of the urease of *S. saprophyticus* as a metabolic enzyme on one hand, and as a virulence factor involved in the urogenital tract infections on the other hand (Kuroda et al. 2005). Another example comes from differential analyses between pathogenic and food-

associated staphylococci that reveal numerous univocal virulence factors in the latter by immunoblot analysis (Zell et al. 2008). This at least demonstrates that a simplified approach of “counting the virulence genes” will only partially cover the complex phenomenon of staphylococcal pathogenicity. In addition, the overwhelming amount of clinical data on *S. aureus* have shown that also among the various isolates and sequence types of the leading staphylococcal pathogen the impact on human or animal health varies significantly. Here, the remarkable in vivo study on the development of vancomycin resistance in an *S. aureus* blood-stream isolate has to be mentioned again which has shown that variations in virulence could be related to subtle changes on the level of single nucleotide polymorphism even between different isolates of one strain (Mwangi et al. 2007). Moreover, this study underlines that the staphylococcal genomes are highly dynamic and that genomic studies have to consider that they are based on “genome snapshots” that have been taken under certain conditions with regard to time and environment.

There is no easy answer to discriminate pathogenic from non-pathogenic staphylococci. The role of *S. aureus* as the leading staphylococcal pathogen is by all means undisputed according to its wide and diverse spectrum of pathogenicity factors involved in destruction of host cells and tissues as well as escaping the immune defense. They could be regarded as the benchmark for the other staphylococcal pathogens. Correspondingly, the prevalence of *S. aureus* can be based on an assortment of various factors that are exclusively present in this species like toxins, gene products involved in interaction with the hosts immune defense, adhesins, represented by molecular hallmarks like protein A, thermonuclease, or staphylococagulase. But recent studies on the virulence of non-*S. aureus* pathogens indicate that at least some of these distinctive criteria will be taken over by the other staphylococci. Thus, the hitherto exclusive position of *S. aureus* as toxicogenic pathogen is attacked by the fortifying importance of phenol-soluble modulins in *S. epidermidis* as cytolytic toxins (Cheung et al. 2010) and by recent observations of CoNS as being able to internalize during infection (Hirschhausen et al. 2010).

10.1 Staphylococcus Between Commensalism and Pathogenicity

Another feature concerning the potentially harmful impact of staphylococcal pathogens on human and animal health that withstands a clear and distinctive categorization is the dualism between a commensalic and a pathogenic lifestyle that is common to the staphylococcal pathogens. Staphylococci that coexist with their hosts are well equipped with the factors that enable them to colonize their niches in the anterior nares or on the skin of humans or animals. The ambivalent nature of this coexistence is reflected by a possible advantage for the host as the bacterial colonizers might prevent other bacteria from gaining a foothold. While on the one hand this advantage is possibly gained by the risk of getting infected, the benefit of eradication of *S. aureus* from the carriers in order to prevent an

infection remains questionable on the other hand (Wertheim et al. 2005). Furthermore, it has been shown that non-*S. aureus* carriers have an increased mortality rate in comparison with nasal carriers (Wertheim et al. 2004). This ambivalent character is also reflected on the molecular level, as some adhesins which might be important in the colonization of the host might also have a role in the adhesion to host cells in the course of an infection (Corrigan et al. 2009). Also, the arginine deiminase pathway encoded by the *arc* operon on the ACME element falls into this category as it might support skin colonization on the one hand and might also be involved in getting rid of nitric oxide compounds during infection on the other hand (Diep et al. 2006).

Last but not least, the manifold occurrences of staphylococcal virulence might be regarded as a consequence of the environment inhabited by the particular species. The adaptation to the conditions of the peculiar habitat clearly correlates with the pathogenic potential. This is demonstrated by comparing the two extremes on the staphylococcal virulence scale. The tremendous versatility and flexibility of *S. aureus* as leading pathogen is surely one consequence of its habituation of the moist squamous epithelium of the anterior nares, an area that is defended by innate and induced immune responses (Foster 2009) and thus may be regarded as a front-line between commensalism and pathogenicity. On the other hand, the non-pathogenic, food-grade *S. carnosus* TM300 reveals striking signs of an adaptation to a static environment as present in sausage meat (Rosenstein et al. 2009) while the gene relics observable in its genome indicate that it might descend from a pathogenic ancestor.

Also, this converse example of staphylococcal adaptability underlines that the different manifestations of staphylococcal virulence and the versatile switching between commensalic and infectious lifestyles reflect the variably challenging conditions present in the peculiar ecological niches occupied by staphylococcal species on their human or animal hosts and thus supports that staphylococcal pathogenesis “is best viewed as an adaptation to the hostile environment of the host and its formidable antibacterial defences” (Novick 2003).

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