ROBERT KOCH INSTITUT

http://edoc.rki.de/docviews/abstract.php?lang=ger&id=3532

Originally published as:

Grumann, D., Nübel, U., Bröker, B.M.
Staphylococcus aureus toxins - Their functions and genetics
(2014) Infection, Genetics and Evolution, 21, pp. 583-592.

AUTHOR MANUSCRIPT. © Elsevier (2013): This is the author's version of the work. It is posted here by permission of Elsevier for personal use, not for redistribution. Some changes resulting from the publishing process, such as editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Some changes may have been made to this work since being accepted for publication. A definitive version was subsequently published in Infection, Genetics and Evolution, [Volume: 21, Jan 2014] DOI: 10.1016/j.meegid.2013.03.013

1 *Staphylococcus aureus* toxins - their functions and genetics

2 Dorothee Grumann[a], Ulrich Nübel[b], Barbara M. Bröker[a, *]

3

4 [a]Institute of Immunology and Transfusion Medicine, University of Greifswald,
5 Greifswald, Germany

6 [b]Robert Koch Institute, Wernigerode, Germany

7

8 *Corresponding author:

9 Institute of Immunology and Transfusion Medicine, University of Greifswald, 17487
10 Greifswald, Germany, Phone: +49-3834-865595, Fax: +49-3834-865490, E-mail:
11 broeker@uni-greifswald.de

12

Abstract

The outcome of encounters between *Staphylococcus (S.) aureus* and its human host ranges from life-threatening infection through allergic reactions to symptom-free colonization. The pan-genome of this bacterial species encodes numerous toxins, known or strongly suspected to cause specific diseases or symptoms. Three toxin families are in the focus of this review, namely (i) pore-forming toxins, (ii) exfoliative toxins and (iii) superantigens. The majority of toxin-encoding genes are located on mobile genetic elements (MGEs), resulting in a pronounced heterogeneity in the endowment with toxin genes of individual *S. aureus* strains. Recent population genomic analysis have provided a framework for an improved understanding of the temporal and spatial scales of the motility of MGEs and their associated toxin genes. The distribution of toxin genes among clonal lineages within the species *S. aureus* is not random, and phylogenetic (sub-) lineages within clonal complexes feature characteristic toxin signatures. When studying pathogenesis, this lineage association, which is caused by the clonal nature of *S. aureus* makes it difficult to discriminate effects of specific toxins from contributions of the genetic background and/or other associated genetic factors.

Keywords

S. aureus, Pore-forming toxins, Exfoliative Toxins, Superantigens, Evolution, Epidemiology

1. Introduction

Staphylococcus (S.) aureus is notorious as the most common causative agent of hospital-acquired infections, and the spread of antibiotic resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), in hospitals challenges health care systems worldwide. Moreover, *S. aureus* strains of increased virulence, known as community-aquired MRSA (CA-MRSA), can threaten even healthy individuals in the community (Chambers and Deleo 2009; David and Daum 2010; DeLeo, Otto et al. 2010). In addition, *S. aureus* is currently being discussed as the trigger and/or enhancer of allergies of the respiratory system and the skin (Gould, Takhar et al. 2007; Bachert and Zhang 2012). Nevertheless up to now, no anti-*S. aureus* vaccine has been approved for medical practice (Schaffer and Lee 2008; Spellberg and Daum 2012). In spite of the above, the most frequent encounter of *S. aureus* with its human host is peaceful colonization, and around 20% of adults are persistent carriers of the micro-organisms, while another 60% are intermittently colonized (Wertheim, Melles et al. 2005; van Belkum, Verkaik et al. 2009). What makes the species *S. aureus* so immensely successful?

Multiple virulence factors encoded in the pan-genome of *S. aureus*

A salient feature of *S. aureus* is its variability. By indexing nucleotide sequence diversity at seven universally present genetic loci, multilocus-sequence typing (MLST) to date has revealed about 2,400 'sequence types' (ST) for *S. aureus* (see www.mlst.net). The vast majority of these diverse STs, however, are clustered in a remarkably limited number of clonal complexes (CC), each of which appears to be distributed worldwide (reviewed in (Nubel, Strommenger et al. 2011)). The predominant *S. aureus* lineages are CC1, 5, 8, 15, 22, 30, 45, 59, 80, 97 and 121 (Nubel, Strommenger et al. 2011).

About 75% of the *S. aureus* genes are shared by more than 95% of strains and hence may be considered the 'core genome' of the species. In addition, two kinds of variably present genes can be distinguished: (i) the core variable genes (~10% of genes), which are largely conserved within each of the *S. aureus* clonal complexes and constitute their respective “make up”, and (ii) mobile genetic elements (MGEs, ~15% of genes). The core variable genome includes most surface-associated genes (microbial surface components recognizing adhesive matrix molecules, MSCRAMMs) and regulator genes. Core variable genes are encoded on the bacterial chromosome and are, therefore, typically stable and transferred vertically (Lindsay, Moore et al. 2006). MGEs include bacteriophages, plasmids, *S. aureus* pathogenicity islands (SaPI), transposons, and staphylococcal chromosomal cassettes (SCC) (Feil, Cooper et al. 2003; Lindsay and Holden 2006; Lindsay, Moore et al. 2006; Lindsay 2010). They mainly encode resistance (e.g. methicillin resistance genes) and virulence genes (e.g., Panton-Valentine leukocidin (PVL) genes, superantigen (SAg) genes). MGEs can be distributed either by vertical transmission to daughter cells or by horizontal transfer (Lindsay and Holden 2006).

1 The full complement of all genes (also known as the pan-genome) of *S. aureus* encodes a
2 wide array of secreted or cell-surface-associated virulence factors (Foster 2005). These
3 include proteins that

4 (1) mediate adherence to damaged tissue, extra-cellular matrix and the surface of host
5 cells (Foster and Hook 1998),

6 (2) facilitate tissue destruction and spreading,

7 (3) promote iron uptake (Skaar and Schneewind 2004),

8 (4) bind to proteins in the bodily fluids to help evade antibody- and complement-
9 mediated immune responses, including the action of phagocytes,

10 (5) lyse host cells and

11 (6) manipulate the innate and adaptive immune responses.

12 However, a clear association between virulence genes and disease symptoms has been
13 established or is strongly suspected only for some potent *S. aureus* toxins causing, for
14 example, toxic shock syndrome (TSS), staphylococcal scalded skin syndrome (SSSS),
15 necrotizing pneumonia, or deep-seated skin infections (Jarraud, Cozon et al. 1999;
16 Dinges, Orwin et al. 2000; Jarraud, Mougel et al. 2002; Ladhani 2003; Holtfreter and
17 Broker 2005). This review focuses on such toxins, including pore-forming toxins, like
18 Panton-Valentine leukocidin (PVL) and hemolysin-α (Hla, α-toxin,), exfoliative toxins
19 (ET) and the superantigens (SAgs).They damage the membranes of host cells, degrade
20 inter-cellular junctions, or modulate the immune response by aberrant activation of
21 immune cells. Only a few *S. aureus* toxins, such as Hla and the phenol-soluble modulins
22 (PSMs), are core genome-encoded, while most of the other toxin genes are localized on
23 MGEs (Table 1). Hence, the species *S. aureus* is characterized by extraordinary
24 heterogeneity regarding the toxin gene equipment of individual clinical isolates.

25

26 **2. Pore-forming toxins**

27 *S. aureus* can produce several toxins that damage the membranes of host cells, which can
28 ultimately lead to cell lysis. At sublytic concentrations, these pore-forming toxins are
29 potent cell stressors. In synergy with other danger signals such as lipoproteins that
30 activate the toll-like receptor 2 the toxins trigger the NALP3-inflammasome response
31 resulting in release of cytokines IL1, IL18 and IL33 (Franchi, Munoz-Planillo et al. 2012).
32 Hla, hemolysin-γ (Hlg) and PVL have been shown to exert strong pro-inflammatory
33 effects in this manner (Munoz-Planillo, Franchi et al. 2009; Holzinger, Gieldon et al.
34 2012; Kebaier, Chamberland et al. 2012; Perret, Badiou et al. 2012).

35

1 **2.1 Hemolysin-α (Hla, α-toxin)**

2 Hla is released by 95% of *S. aureus* strains as a water-soluble monomer of 33kDa with
3 pore-forming and pro-inflammatory properties. The *hla* gene is not mobile. Its
4 expression is regulated by at least three global regulatory systems including the
5 accessory gene regulator (*agr*) (Xiong, Willard et al. 2006); it is therefore not surprising
6 that Hla is produced in varying amounts by *S. aureus* strains. Upon binding to a
7 membrane receptor, Hla forms heptameric pores, thereby destroying a variety of host
8 cells, including epithelial cells, erythrocytes, fibroblasts, monocytes, macrophages, and
9 lymphocytes, but not neutrophils. The Hla receptor has long remained elusive and only
10 recently ADAM10 (A disintegrin and metalloproteinase 10) has been identified as a
11 binding partner of Hla (Wilke and Bubeck Wardenburg 2010; Inoshima, Inoshima et al.
12 2011). Binding of Hla and pore formation activates the enzyme, which degrades E-
13 cadherin in the epithelial adherens junctions (Inoshima, Inoshima et al. 2011).
14 Moreover, the ADAM10-Hla complex interferes with focal adhesion complexes (Wilke
15 and Bubeck Wardenburg 2010). Both mechanisms would be able to disrupt the integrity
16 of the epithelial and endothelial layers, thereby paving the way for *S. aureus* invasion.
17 The group of Julie Bubeck-Wardenburg has used murine infection models to
18 demonstrate that Hla strongly contributes to the pathogenesis of skin infections and
19 pneumonia induced by *S. aureus*-USA300, which produces the toxin in abundance
20 (Bubeck Wardenburg, Bae et al. 2007; Bubeck Wardenburg, Patel et al. 2007; Bubeck
21 Wardenburg and Schneewind 2008; Inoshima, Inoshima et al. 2011; Wardenburg 2011;
22 Inoshima, Wang et al. 2012; Powers, Kim et al. 2012).

23

24 **2.2 Leukotoxins**

25 The bi-component (hetero-oligomeric) pore-forming leukotoxins can lyse cells of the
26 myleoid lineage, namely monocytes, macrophages, and neutrophils, which is considered
27 important for *S. aureus* immune evasion (Ventura, Malachowa et al. 2010; Dumont,
28 Nygaard et al. 2011). In different animal infection models, they contribute to disease
29 progression (not yet demonstrated for LukM/ LukF´-PV) (Choorit, Kaneko et al. 1995;
30 Morfeldt, Taylor et al. 1995; Kaneko, Kimura et al. 1997; von Eiff, Friedrich et al. 2004).

31 Leukotoxins consist of one class S and one class F protein of 32-35 kDa, whose genes are
32 either core genome- or phage-encoded. The class S and F proteins are non-toxic on their
33 own, but upon oligomerization, they form a β-barrelled pore-structure (Kaneko and
34 Kamio 2004). Five class F subunits (HlgB, LukF-PV, LukD, LukF'-PV, and LukG) and six
35 class S subunits (HlgA, HlgC, LukS-PV, LukE, LukM, and LukH) have been described
36 (Prevost, Mourey et al. 2001; Morinaga, Kaihou et al. 2003; Kaneko and Kamio 2004;
37 Ventura, Malachowa et al. 2010; Dumont, Nygaard et al. 2011). Table 2 shows that most
38 S and F subunits exhibit monogamous pairing, whereas HlgB, the F subunit of the
39 hemolysin-γ (*hlg*) gene cluster, can pair with either of two S subunits, HlgA or HlgC.

2.2.1 Panton-Valentine leukocidin (PVL; lukF-PV + lukS-PV)

Skin and soft tissue infections (SSTIs), such as furunculosis or abscesses, are the most frequent diseases caused by *S. aureus* outside the hospital setting. Since Lina et al. discovered a close epidemiological linkage between PVL and chronic or recurrent SSTIs as well as necrotizing pneumonia (Gillet, Issartel et al. 2002), its role in these diseases has remained under discussion. The genes of the F and S subunits are phage-encoded (Table 2) and can thus be acquired by horizontal gene transfer (Kaneko, Kimura et al. 1998; Masiuk, Kopron et al. 2010). PVL genes are found in most CA-MRSA strains, which exhibit increased virulence, e.g. in USA300 (Gillet, Issartel et al. 2002; Vandenesch, Naimi et al. 2003; Diep, Gill et al. 2006; Badiou, Dumitrescu et al. 2008; Diep and Otto 2008; del Giudice, Blanc et al. 2009; Carpaij, Willems et al. 2011). CA-MRSA cause typical diseases, namely skin and soft tissue infections (SSTIs), as well as necrotizing pneumonia. However, PVL is not restricted to CA-MRSA, but the toxin is also found in the majority of MSSA strains isolated from patients with community-acquired SSTIs (Monecke, Slickers et al. 2007; Masiuk, Kopron et al. 2010). In contrast, the genes are rare in other *S. aureus* strain collections such as nasal commensals or clinical isolates from blood-stream infection (0-5%) (von Eiff, Friedrich et al. 2004; Holtfreter, Grumann et al. 2007; Masiuk, Kopron et al. 2010). Thus, epidemiological evidence for a pathogenic role of PVL is very strong, but the correlation is not absolute, and PVL-negative CA-MRSA strains as well as MSSA strains associated with SSTI-patients have been isolated (Diep and Otto 2008; Masiuk, Kopron et al. 2010; Otto 2010; Monecke, Coombs et al. 2011). Animal infection models have yielded conflicting results, which has been attributed to differences between models, inoculum sizes, and, very importantly, the host species (Labandeira-Rey, Couzon et al. 2007; Bubeck Wardenburg, Palazzolo-Ballance et al. 2008; Brown, Dumitrescu et al. 2009; Montgomery and Daum 2009; Tseng, Kyme et al. 2009; Villaruz, Bubeck Wardenburg et al. 2009; Olsen, Kobayashi et al. 2010). In fact, the leukotoxic activity of PVL differs dramatically between species: human and rabbit neutrophils are lysed by very low toxin concentrations (> 40 ng/ml), whereas 1000-fold higher amounts are required for the lysis of mouse or java monkey neutrophils (Loffler, Hussain et al. 2010).

2.2.2 Other Leukotoxins (Hemolysin-γ, Hemolysin-γ2, LukDE, LukF'M, LukGH)

The *hlg* gene cluster giving rise to hemolysin-γ (Hlg) and hemolysin-γ2 (Hlg2) is encoded in the core genome; it is present in 99% of *S. aureus* strains. The toxins appear to contribute to septic arthritis and weight loss in mice (Nilsson, Hartford et al. 1999) and to endophthalmitis in rabbits (Supersac, Piemont et al. 1998). Recently, attention has been focussed on LukDE and LukGH, which, similar to PVL, are expressed by the majority of CA-MRSA strains. LukDE binds to CCR5 on immune cells, the chemokine receptor which is also involved in immune cell targeting by the human immunodeficiency virus (Alonzo, Kozhaya et al. 2013). Both leukotoxins contribute to the virulence of *S. aureus* in murine sepsis and renal abscess models (Dumont, Nygaard

et al. 2011; Alonzo, Benson et al. 2012). LukGH exhibits potent cytolytic activity towards neutrophils acting in synergy with PVL *in vitro* (Ventura, Malachowa et al. 2010; Rigby and DeLeo 2012). The relative contribution of PVL, LukDE and LukGH to community-acquired SSTI and necrotizing pneumonia remains to be determined. Finally, a PVL-variant, the prophage-encoded LukF'M, has been identified in *S. aureus* strain P83 of bovine origin (Zou, Kaneko et al. 2000). This toxin lyses bovine polymorphonuclear leukocytes (PMNs) and has been implicated in bovine mastitis (Schlotter, Ehricht et al. 2012).

2.2.3 Phenol-soluble modulins (PSMs)

PSMs are a family of small, α-helical amphipathic peptides with cytolytic and pro-inflammatory properties. Two subfamilies are described: the PSMα peptides, including the long-known hemolysin-δ (Hld) (Yoshida 1963), are 20 to 26 amino acids long, and the PSMβ peptides are 43 to 44 amino acids in length (Wang, Braughton et al. 2007). Most PSMs are core genome-encoded. All *S. aureus* strains contain the *hld* gene and the two loci with the operons coding for the *psmα* and *psmβ* genes (Wang, Braughton et al. 2007). In addition, a *psm-mec* gene is located on a chromosomal cassette, which is restricted to hospital-acquiredMRSA (HA-MRSA) strains carrying SCC*mec* types II, III, and VIII (Chatterjee, Chen et al. 2011). The *agr* is involved in the expression of PSMs (Wang, Braughton et al. 2007; Queck, Jameson-Lee et al. 2008). The production of PSMs is closely correlated with the capacity of staphylococcal species to cause invasive infections (Rautenberg, Joo et al. 2011). CA-MRSA isolates show an increased production of PSMs in comparison to HA-MRSA (Wang, Braughton et al. 2007). The group of Michael Otto has described multiple functions of PSMs in pathogenesis. Firstly, PSMα peptides effectively lyse white and red blood cells (Wang, Braughton et al. 2007; Cheung, Rigby et al. 2010). PSMα-deletion in CA-MRSA strains resulted in lower mortality and decreased ability to cause skin lesions in mice (Wang, Braughton et al. 2007). Secondly, PSMs have proinflammatory properties, and they induce chemotaxis and activation of human neutrophils, as well as cytokine expression (Wang, Braughton et al. 2007; Queck, Khan et al. 2009; Cheung, Rigby et al. 2010). This response is mediated by activation of the human formyl peptide receptor 2 (FPR2) (Kretschmer, Gleske et al. 2010). Thirdly, proteolytically processed PSMα1 and PSMα2 peptides show antimicrobial activity and interfere with competing colonizing pathogens (Joo, Cheung et al. 2011). Finally, they appear to contribute to the structuring of biofilms during *S. aureus* infection (Periasamy, Joo et al. 2012). Recently, Periasamy et al. have proposed that these functions in virulence are indicative of a primary role for PSMs in the commensal colonization of mammalian epithelia (Periasamy, Chatterjee et al. 2012).

3. Exfoliative toxins (ETs)

The three known *S. aureus* exfoliative toxins ETA, ETB and ETD are encoded on different genetic elements (Table 1): *eta* is localized in the genome on a temperate phage, whereas *etb* is found on plasmids and *etd* on a genomic island. The prevalence of *eta* and/or *etb* ranges from 0.5 to 3% in MSSA (Becker, Friedrich et al. 2003; Sila, Sauer et al. 2009; Nhan, Leclercq et al. 2011), whereas around 10% of MRSA are *eta* positive (Sila, Sauer et al. 2009). Holtfreter et al. observed a strong association of *etd* with invasive CC25 isolates (*spa* type t078 and relatives) (Holtfreter, Grumann et al. 2007). The expression of the ETs is *agr*-regulated (Sheehan, Foster et al. 1992). Functionally, ETs are isoforms of enzymes with high species-specificity. They have glutamate-specific serine protease activity and selectively cleave a single peptide bond in the extracellular region of human and mouse desmoglein 1 (Dsg1; desmosomal intercellular adhesion molecule), a keratinocyte cell-cell adhesion molecule. In this way, the ETs act as "molecular scissors" facilitating bacterial skin invasion (Nishifuji, Sugai et al. 2008). By loosening the keratinocyte junctions they cause blistering diseases known as bullous impetigo and staphylococcal scalded-skin syndrome.

4. Superantigens (SAgs)

The staphylococcal SAgs belong to the most potent T-cell mitogens known. Some of these toxins stimulate human T-cells at femtomolar concentrations. Originally, the SAgs of *S. aureus* were termed staphylococcal enterotoxins (SEs) because they elicit vomiting and diarrhea after oral uptake, the hallmarks of *S. aureus* food poisoning. This feature is different from their superantigenicity, however, because some of the recently identified SAgs apparently lack emetic properties. Therefore, the International Nomenclature Committee for Staphylococcal Superantigens introduced a new nomenclature in 2004 to distinguish SAgs with proven emetic activity in primates (staphylococcal enterotoxins; SEs) from those whose emetic properties remain unconfirmed (staphylococcal enterotoxin-like toxins; SE*l*s) and toxic shock-syndrome toxin 1(TSST-1) (Lina, Bohach et al. 2004). So far, 23 different staphylococcal SAgs have been described: the staphylococcal enterotoxins A-E, G-J, and R-T (SEA-SEE, SEG-SEJ, SER-SET),the staphylococcal enterotoxin-like toxins K-Q and U-X (SE*l*K-SE*l*Q, SE*l*U-SE*l*X) and TSST-1 (Proft and Fraser 2003; Lina, Bohach et al. 2004; Holtfreter and Broker 2005; Thomas, Chou et al. 2007; Ono, Omoe et al. 2008; Wilson, Seo et al. 2011).

Genetic analysis of *S. aureus* clinical isolates, including whole genome sequencing, has shown that ~80% of *S. aureus* clinical isolates harbor an average of five to six SAg genes. There is extensive heterogeneity of SAg gene patterns between *S. aureus* strains (Jarraud, Peyrat et al. 2001; Baba, Takeuchi et al. 2002; Becker, Friedrich et al. 2003; Holtfreter, Bauer et al. 2004). In addition, the *S. aureus* strains that harbor SAg genes produce varying levels of the toxins. This can been attributed to the involvement of at least four global regulators, *agr, sarA*, σ^B and *saeRS* (Tseng, Zhang et al. 2004; Andrey, Renzoni et al. 2010; Kusch, Hanke et al. 2011). Most SAgs are encoded on MGEs, such as

bacteriophages, plasmids, SaPIs, and genomic islands (Johns and Khan 1988; Fitzgerald, Monday et al. 2001; Lindsay and Holden 2006; Ono, Omoe et al. 2008), whereas the recently described SE*l*X is core genome-encoded (Wilson, Seo et al. 2011) (Table 1).

The mechanism of action of SAgs was discovered by Bernhard Fleischer and Hubert Schrezenmeier and first described in 1988 (Fleischer and Schrezenmeier 1988). In contrast to conventional peptide antigens, SAgs activate a large fraction of T lymphocytes simultaneously. Conventional antigens are taken up by antigen-presenting cells (APCs) and processed by protease digestion. The resulting antigenic peptides are bound to major histocompatibility complex (MHC) molecules and displayed on the APC surface as MHC/peptide complexes. These are recognized by T-cells via the hypervariable loops of their T-cell receptor (TCR) α and β chains. SAgs can bypass this highly specific antigen-driven interaction between T-cells and APCs. They directly cross-link certain TCR Vβ domains with conserved structures on MHC class II (MHC II) molecules expressed on professional APCs. They interact with MHC II by binding to the α-chain (antigen peptide-dependent or independent) or to a conserved histidine in the β-domain via a zinc complex (peptide-dependent) (Fraser and Proft 2008).. Furthermore, each SAg interacts with a defined TCR repertoire determined by the TCR Vβ sequences. As the human genome encodes approximately 50 TCR Vβ elements, which are unevenly represented in the T-cell pool of an individual, up to 20% of T cells can be activated by a given SAg (Proft and Fraser 2003). In contrast, conventional peptide antigens stimulate only 1 out of 10^5 to 10^6 naïve T-cells (Fraser, Arcus et al. 2000). The Vβ-restricted T-cell expansion is thus the hallmark of SAgs (Kappler, Kotzin et al. 1989; White, Herman et al. 1989; Choi, Lafferty et al. 1990) with two exceptions: (i) the SAg SEH also contacts TCR Vα chains (Petersson, Pettersson et al. 2003; Thomas, Dauwalder et al. 2009) and (ii) the staphylococcal protein A, which is universally expressed by *S. aureus*, acts as a B cell SAg targeting B-cell receptors (membrane-anchored antibodies) which use the immunoglobulin-V_H3 gene element (Silverman and Goodyear 2006). In addition, many T-cell SAgs also trigger cytokine release by the APCs, which are activated via MHC-II engagement (Proft and Fraser 2003).

4.1 Role of SAgs in staphylococcal virulence

SAgs have been implicated in a broad range of diseases. SEs are the causative agents of staphylococcal food poisoning resulting from ingestion of contaminated food. Due to their extraordinary stability in denaturing conditions, such as heat and low pH, SEs are not completely destroyed by mild cooking or digestion of food in the stomach. Nausea, emesis, abdominal pain or cramping and diarrhea ensue after a short incubation time. The disease is usually self-limiting (Thomas, Chou et al. 2007).

Staphylococcal toxic shock syndrome (TSS) is characterized by high fever, rash, desquamation, vomiting, diarrhea and hypotension, frequently resulting in multiple

organ failure. In TSS, *S. aureus* is usually localized, either at mucosal sites (vagina or nasophryx) or in abscesses (Fraser and Proft 2008), but the released SAgs act systemically, triggering large numbers of T-cells to produce massive amounts of pro-inflammatory cytokines, such as IL-2, IFN-γ and TNF. This cytokine storm causes the symptoms (Bergdoll, Crass et al. 1981; McCormick, Yarwood et al. 2001). This is followed by a state of profound T-cell unresponsiveness or anergy, where the T-cells fail to proliferate or secrete IL-2 (Rellahan, Jones et al. 1990), or they even undergo cell death (Alderson, Tough et al. 1995). It has, therefore, been proposed that SAgs might confer an evolutionary advantage to *S. aureus* by deleting T-cells that help B-cells to mount an effective antibody response against the bacteria (Fraser, Arcus et al. 2000). This view has been challenged by the observation that SAgs themselves are potent immunogens eliciting an effective and highly specific neutralizing antibody response (Holtfreter, Roschack et al. 2006; Grumann, Ruotsalainen et al. 2011).

The role of SAgs in other forms of sepsis is less well defined. In animal models, SAgs and LPS, a major component of the outer membrane of Gram-negative bacteria and highly potent stimulator of the innate immune system, most effectively synergize in the induction of lethal shock (Schlievert 1982; Blank, Luz et al. 1997). This observation prompted the development of the two-hit model of septic shock (Bannan, Visvanathan et al. 1999), which was later generalized by Holtfreter and Bröker: A first hit by SAgs or other potent T cell stimuli is potentiated by a second hit by pathogen-associated molecular patterns (PAMPs), which activate the innate immune system. This sequence of events culminates in a dramatic, often lethal activation of the whole immune system (Holtfreter and Broker 2005). The sequence of events varies in the different accounts.

Kawasaki disease is an acute febrile disease in children that resembles TSS. A role for SAgs has been suggested (Yarwood, Leung et al. 2000). Intravenous immunoglobulin therapy is highly effective when given early, suggesting that the agent is a toxin that is neutralized by anti-toxin antibodies contained in pooled human serum.

Finally, a prominent role for SAgs is being discussed in skin and airway allergies. For atopic dermatitis, a correlation between clinical severity and colonization with SEA- and SEB-producing *S. aureus* as well as with IgE specific for SEA and SEB was documented in one study, but not confirmed in others (Bunikowski, Mielke et al. 1999; Zollner, Wichelhaus et al. 2000). Bronchial asthma afflicts around 300 million people worldwide, thus belonging to the most common diseases. In allergic asthma, the triggers are known inhalative allergens (= allergy-driving antigens), while the causative agents of non-allergic or intrinsic asthma, around 10% of cases, are not known. Intrinsic asthma is often of late onset (3rd-4th decade of life) and takes a severe disease course, which is refractory to established treatment strategies. Chronic rhinosinusitis, a pronounced inflammation of the mucosal tissue of the nose and sinuses, with or without the development of polyps, is also very frequent and often accompanied by intrinsic asthma. Since many patients possess high titres of SAg-specific IgE in their serum or locally in

1 the polyps, several research groups promote the opinion that allergic reactions to
2 *S. aureus* SAgs drive or at least amplify chronic airway inflammation (Gevaert,
3 Holtappels et al. 2005; Bachert, Zhang et al. 2008; Barnes 2009; Bachert, Zhang et al.
4 2010; Zhang, Holtappels et al. 2011).

5

6 **5. The evolution of the *S. aureus* toxin gene families**

7 Toxin gene clustering and sequence homologies imply evolution from ancestral genes by
8 gene duplication and variation. These features are prominent in the families of
9 leukotoxins, ETs, and SAgs. The enterotoxin gene cluster (*egc*), discovered on a
10 staphylococcal genomic island (νSaβ) by the group of Jarraud, is given here as an
11 example (Jarraud, Peyrat et al. 2001; Letertre, Perelle et al. 2003). *Egc* SAgs are the most
12 prevalent SAg genes in commensal and invasive *S. aureus* isolates, with frequencies
13 ranging between 46% and 66% in different strain collections (Becker, Friedrich et al.
14 2004; Fueyo, Mendoza et al. 2005; Holtfreter, Grumann et al. 2007; Monecke, Luedicke
15 et al. 2009). Most *egc*-positive *S. aureus* strains harbor five SAg genes (*seg*, *sei*, *selm*, *seln*,
16 and *selo*) and the pseudogenes *ψent1* and *ψent2* (Jarraud, Peyrat et al. 2001). The *egc* is
17 unusually variable. The *S. aureus* clonal cluster CC30, for example, harbors an additional
18 SAg gene, designated *selu*, a fusion product of *ψent1* and *ψent2* (Letertre, Perelle et al.
19 2003; Thomas, Jarraud et al. 2006; Holtfreter, Grumann et al. 2007). Given that the
20 members of the *egc* display considerable sequence differences and each of the *egc* SAgs
21 shows closest similarity to SAgs encoded outside the *egc*, Jarraud et al. proposed that the
22 *egc* functions as an “enterotoxin gene nursery” (Jarraud, Peyrat et al. 2001).

23

24 **6. The phylogenetic distribution and motility of *S. aureus* toxin genes**

25 MGEs and toxin genes they carry can be disseminated either by vertical transmission to
26 daughter cells upon cell replication or by horizontal gene transfer. Transduction through
27 bacteriophages commonly is considered the predominant mechanism for acquisition of
28 genetic material in *S. aureus,* whereas DNA transformation (Morikawa, Takemura et al.
29 2012) and conjugation are assumed to be less relevant (Lindsay and Holden 2006).

30 Over the past decade, studies based on sequencing a limited number of representative
31 *S. aureus* genomes (Lindsay and Holden 2006), comparative genomic hybridization to
32 microarrays (Monecke, Slickers et al. 2008; McCarthy and Lindsay 2012; McCarthy,
33 Witney et al. 2012; Shore, Brennan et al. 2012), or dedicated PCR assays (Holtfreter,
34 Grumann et al. 2007; Masiuk, Kopron et al. 2010) have provided an overview of the
35 distribution of MGEs and their associated toxin genes in the *S. aureus* population. An
36 overwhelming feature of the resulting data is that there exists remarkable variation in
37 the endowment with MGEs, both within and among clonal complexes (Holtfreter,
38 Grumann et al. 2007; Monecke, Luedicke et al. 2009; Masiuk, Kopron et al. 2010). It

further appeared that MGEs and hence the toxin genes they carried were not randomly distributed between lineages, suggesting restrictions to horizontal transfer (Holtfreter, Grumann et al. 2007; Monecke, Slickers et al. 2008; Monecke, Luedicke et al. 2009; Masiuk, Kopron et al. 2010; Shore, Brennan et al. 2012).

Due to their location on different genetic elements (Table 1), individual *S. aureus* toxin genes differ greatly in their distribution and horizontal genetic mobility. Some by definition belong to the core genome because they are present in almost all strains: *hla*, the *hlg* gene cluster, and the *psm* loci. Most are much more variably distributed, but, importantly, all are more or less closely linked to the genetic background of *S. aureus*. One extreme example is the *egc*-encoding genomic island νSAβ. Among the dominant clonal clusters, *egc* is found in all strains of CC5, CC22, and CC45 as well as in CC25. CC30 and CC121 isolates are characterized by a variant of νSAβ harboring an *egc* with a functional *selu* gene. On the other hand, CC1, CC8, CC15 and CC395 appear to lack *egc* completely (Holtfreter, Grumann et al. 2007; Masiuk, Kopron et al. 2010; Grumann, Ruotsalainen et al. 2011). Other SAgs with strong clonal complex linkage are the SaPI-encoded *tst* and *sec*-*sell* (CC45), or the plasmid-encoded *sed, sej* and*ser* (CC8). SAg genes with a broader distribution are the phage-encoded *sea*, and the SaPI-encoded *seb* (Peacock, Moore et al. 2002; Fueyo, Mendoza et al. 2005; Diep, Gill et al. 2006; Lindsay and Holden 2006; Lindsay, Moore et al. 2006; Holtfreter, Grumann et al. 2007; Monecke, Berger-Bachi et al. 2007; Monecke, Slickers et al. 2007; Masiuk, Kopron et al. 2010; Monecke, Coombs et al. 2011). The pore-forming toxin lukFS-PV genes are broadly distributed among *S. aureus* clonal lineages with the notable exception of CC45 (Monecke, Slickers et al. 2007; Goerke, Pantucek et al. 2009; Masiuk, Kopron et al. 2010) and a subclade of CC121, which in turn is associated with *eta* and/or *etb* (Masiuk, Kopron et al. 2010) (our unpublished data). In contrast, *etd* is rare and characterizes CC80 MRSA isolates as well as some CC25 strains (Holtfreter, Grumann et al. 2007; Masiuk, Kopron et al. 2010; Monecke, Coombs et al. 2011). All the studies cited above were limited, however, by the typing techniques applied (i. e., MLST or *spa* typing), and their associated discriminatory power. Because substantial variation of MGE endowment was observed within the phylogenetic groupings resolved (clonal complexes, mostly), the dynamics of MGE motility could not be investigated. In contrast, more recent work has provided novel, quantitative insights into the frequency of MGE acquisition and loss in *S. aureus*, based on extended MLST (analysing variation at >100 genetic loci; (Nubel, Roumagnac et al. 2008; Lowder, Guinane et al. 2009; Nubel, Dordel et al. 2010)) or whole-genome sequencing, respectively (Lindsay and Holden 2006; Harris, Feil et al. 2010; McAdam, Templeton et al. 2012; Holden, Hsu et al. 2013), applied to globally representative population samples. In these studies, abundant polymorphisms were ascertained in the *S. aureus* core genome, which provided robust, high-resolution phylogenetic frameworks for each of the populations investigated. One important discovery borne from these investigations was that *S. aureus* constitutes 'measurably evolving populations', accumulating detectable genetic variation over

epidemiological timescales that can be directly observed. Importantly, this feature enabled the application of coalescence-based methods for dating evolutionary events and tracking staphylococcal spatial spread on the basis of temporally structured population samples. Furthermore, the motility of variably present genomic components could be evaluated in relation to the evolutionary history of the *S. aureus* core genome.By mapping the presence of motile genomic components onto core-genome based phylogenetic trees, multiple events of gene acquisition or loss were identified. Using the accumulation of SNPs in the core genome for calibration, these genetic events could be dated and pinpointed to geographic areas in which they likely had occurredEven though genomic plasticity was not the main focus of any of these recent studies, warranting further in-depth analyses of the available data, a deepened understanding of the temporal and spatial scales of MGE motility in *S. aureus* currently emerges.

Each of the clonal complexes forms a radiation of multiple phylogenetic sub-lineages that had not been resolved by MLST, *spa* typing, or DNA macrorestriction (Nubel, Roumagnac et al. 2008; Harris, Feil et al. 2010; McAdam, Templeton et al. 2012; Holden, Hsu et al. 2013). These sub-lineages evolve mostly clonally, as homologous recombination is extremely rare (Nubel, Roumagnac et al. 2008; Holden, Hsu et al. 2013) [Castillo-Ramirez 2012; PMID: 23270620]. The most recent common ancestors of individual sub-lineages date back only few decades (Lowder, Guinane et al. 2009; Harris, Feil et al. 2010; Nubel, Dordel et al. 2010; McAdam, Templeton et al. 2012; Holden, Hsu et al. 2013). Notably, sub-lineages are equipped with specific MGEs (and associated toxin genes). Within a given clonal complex, sub-lineages commonly share several of their MGEs (e. g., SaPIs and prophages, which may have been acquired prior to the sub-lineage split), but they also show multiple differences (typically, additional prophages, SCC*mec* elements, transposons, plasmids) (Nubel, Roumagnac et al. 2008; Holden, Hsu et al. 2013) (our unpublished work).

A recently published study compared comprehensive accessory genomes derived from *de-novo* assemblies of Illumina sequencing reads from 193 *S. aureus* isolates from CC22 and mapped their components onto a high-resolution phylogeny (Holden, Hsu et al. 2013). This analysis demonstrated the accumulation over time of genetic determinants for antimicrobial resistance in a hospital-associated sub-lineage of CC22, in response to selective pressure experienced in a clinical environment. There was also evidence of occasional loss of resistance genes, prophages, and even entire SaPIs, and one example of a replacement of an SCC*mec* element through another, structurally different SCC*mec* element. Such events were rare, however, each affecting a small minority of isolates in the sample, whereas the majority of MGE appeared fully conserved within the sub-lineage (Holden, Hsu et al. 2013). Thus, once acquired, MGE presence in the genome commonly remained stably maintained throughout sub-lineage evolution (i. e., over decades), even though some minor structural variation was acquired through recombination.

In summary, the acquisition and loss of MGE appears to be a rare event, and horizontal gene transfer is not as rampant as perhaps may have been anticipated. Differences between phylogenetic sub-lineages sufficiently explain the gene content variation within clonal complexes that had been observed in previous studies. After all, the association of genetic traits with phylogenetic (sub-)lineages is to be expected from a highly clonal population, provided the genotyping procedure provides sufficient discriminatory power to resolve such lineages (Turner and Feil 2007)[Kurt et al, PLOS One 2013, in press].

The linkage of toxin genes with the genomic background has practical implications for the epidemiological investigation of *S. aureus* virulence. In most cases, the contribution of individual toxin genes to a phenotype of interest cannot be clearly separated from that of the genomic background at the species level. In order to avoid the erroneous attribution of pathophysiological effects to *S. aureus* toxins, these should be studied by comparing toxin-positive and toxin-negative isolates within a given clonal cluster or subclade and/or by using multi-factorial approaches. This will optimize the power of genetic investigations into the mechanisms of *S. aureus* pathogenesis.

3. Outlook: Toxins as vaccination targets

Toxins are interesting vaccine candidates because (i) they are dangerous and significantly contribute to pathogenesis and (ii) their toxic functions can be neutralized by specific antibodies. In fact, antibody-mediated protection from the effects of *S. aureus* toxins has been convincingly demonstrated in humans and in animal models (Holtfreter, Kolata et al. 2010; Cheung and Otto 2012; Daum and Spellberg 2012; Spellberg and Daum 2012). However, the extra-ordinary variability of toxins in the pan-genome of *S. aureus* constitutes an enormous challenge for the development of broad-spectrum anti-toxin vaccines. It is highly unlikely that a "one fits all"-strategy can solve the problem; an effective anti-*S. aureus* vaccine will contain multiple toxins or toxoids. Selection criteria should include their role in pathogenesis, their distribution among *S. aureus* strains and the degree of natural immunization in the human host population. In view of the pronounced heterogeneity, even within toxin families, it appears mandatory to establish in each case where the natural (or induced) human antibody response is located on a scale ranging from exquisite antigen specificity to broad cross-reactivity with a whole toxin family. Generally, immune selection may drive antigenic variation, but the impact this could have on toxin gene evolution and diversity in *S. aureus* has not been assessed systematically, considering recently accumulated, abundant genome sequence data.

1 Finally, it must be borne in mind that besides antibodies, T lymphocytes are also
2 necessary for clinical protection against *S. aureus*. Hence, any anti-toxin vaccine
3 approach should be complemented with a strategy to elicit a powerful *S. aureus*-specific
4 T cell response (Spellberg and Daum 2012).

5

6

1 **Acknowledgements**

2 We wish to acknowledge our research teams and the many scientists whose work we
3 describe in this review, and regret that many interesting contributions could not be
4 included because of space constraints. We are grateful to Kate Splieth for eliminating
5 language errors and typos from the manuscript. We thank Jodi Lindsay for helpful
6 comments on MGE nomenclature. Our work has been supported by the Deutsche
7 Forschungsgemeinschaft (TRR34, GRK840), the Bundesministerium für Forschung und
8 Technologie (HiCare, Infection genomics) and by the Alfried Krupp Wissenschaftskolleg.

Table 1: Toxin genes in the *S. aureus* pan-genome.

	Toxin genes	References
Core Genome		
	hla	(Bhakdi and Tranum-Jensen 1991)
	hld	(Janzon, Lofdahl et al. 1989)
	hlg gene cluster	(Kaneko and Kamio 2004)
	psmα, psmβ	(Wang, Braughton et al. 2007)
	selx	(Wilson, Seo et al. 2011)
Genomic Islands		
νSaβ	enterotoxin gene cluster (*egc*): *seg, sei, selm, seln, selo*, (*selu, selu2, selv*)	(Baba, Takeuchi et al. 2002; Letertre, Perelle et al. 2003; Thomas, Jarraud et al. 2006)
	lukDE	(Baba, Takeuchi et al. 2002; Barrio, Rainard et al. 2006; Lindsay and Holden 2006)
νSaγ	*etd*	(Yamaguchi, Nishifuji et al. 2002; Highlander, Hulten et al. 2007)
Plasmids		
pIB485	*sed, sej, ser*	(Bayles and Iandolo 1989; Zhang, Iandolo et al. 1998; Omoe, Hu et al. 2003)
pF5	*sej, ser, ses, set*	(Omoe, Hu et al. 2003; Ono, Omoe et al. 2008)
pGSA$_{18}$ *rep32* (pETB)	*etb*	(Yamaguchi, Hayashi et al. 2001; McCarthy and Lindsay 2012)
pGSA$_{11}$ *rep22* (SAP057A)	*etb*	(McCarthy and Lindsay 2012)
Staphylococcal Cassette Chromosomes		
SSC*mec* types II, III, VIII	*psm-mec*	(Queck, Khan et al. 2009; Chatterjee, Chen et al. 2011)
Pathogenicity Islands		
SaPIn1 (N315)/ SaPIm1 (Mu50) (νSa4 type I)	*sell, sec, tst*	(Novick and Subedi 2007)
SaPI3 (COL νSa1)	*seb, selk, selq*	(Novick and Subedi 2007)
SaPImw2	*sell, sec*	(Baba, Takeuchi et al. 2002; Lindsay and Holden 2006)
Bacteriophages		
ϕSa1	*lukFM* (φPV83)	(Choorit, Kaneko et al. 1995; Zou, Kaneko et al. 2000; Kaneko and Kamio 2004; McCarthy, Witney et al. 2012)
	eta (φETA)	(Yamaguchi, Hayashi et al. 2000; Kuroda, Ohta et al. 2001; McCarthy, Witney et al. 2012)
ϕSa2	*lukFS-PV*	(Kaneko, Kimura et al. 1998; Narita, Kaneko et al. 2001; Baba, Takeuchi et al.

		2002)
ϕSa3	*sea*; *selp*; *sea, selq, selk*	(Baba, Takeuchi et al. 2002; McCarthy, Witney et al. 2012)

Table 2: Leukotoxins.

Leukotoxins	**Components[a]**		**Localisation**	**Prevalence[b]**	**Cell specificity**
	Class F subunit	**Class S subunit**			
Hemolysin-γ (Hlg)	HlgB (Hlg1, LukF)	HlgA (Hlg2)	*hlg* gene cluster; genome	~99%	erythrocytes from humans and other mammalian species
Hemolysing-γ2 (Hlg2)/ Leukocidin (Luk)	HlgB (Hlg1, LukF)	HlgC (LukS)			human and rabbit PMN and rabbit erythrocytes
Panton-Valentine Leukocidin (PVL)	LukF-PV	LukS-PV	*pvl* locus; phage	0-5%	human and rabbit PMN
LukDE	LukD	LukE	pathogenicity island	30-87%	murine and rabbit PMN
LukF'M	LukF'-PV	LukM	phage	0%	bovine PMN
LukGH (LukAB)	LukG (LukB)	LukH (LukA)	n.d.	n.d.	human PMN

[a]Synonyms are indicated in brackets.
[b]Prevalence in clinical *S. aureus* isolates from humans.
References: (Choorit, Kaneko et al. 1995; Gravet, Couppie et al. 2001; Kaneko and Kamio 2004; von Eiff, Friedrich et al. 2004; Ventura, Malachowa et al. 2010; Dumont, Nygaard et al. 2011; Alonzo, Benson et al. 2012; Rigby and DeLeo 2012; Vandenesch, Lina et al. 2012)

References

Alderson, M. R., T. W. Tough, et al. (1995). "Fas ligand mediates activation-induced cell death in human T lymphocytes." J Exp Med **181**(1): 71-77.

Alonzo, F., 3rd, M. A. Benson, et al. (2012). "Staphylococcus aureus leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo." Mol Microbiol **83**(2): 423-435.

Alonzo, F., 3rd, L. Kozhaya, et al. (2013). "CCR5 is a receptor for Staphylococcus aureus leukotoxin ED." Nature **493**(7430): 51-55.

Andrey, D. O., A. Renzoni, et al. (2010). "Control of the Staphylococcus aureus toxic shock tst promoter by the global regulator SarA." J Bacteriol **192**(22): 6077-6085.

Baba, T., F. Takeuchi, et al. (2002). "Genome and virulence determinants of high virulence community-acquired MRSA." Lancet **359**(9320): 1819-1827.

Bachert, C. and N. Zhang (2012). "Chronic rhinosinusitis and asthma: novel understanding of the role of IgE 'above atopy'." J Intern Med **272**(2): 133-143.

Bachert, C., N. Zhang, et al. (2010). "Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma." J Allergy Clin Immunol **126**(5): 962-968, 968 e961-966.

Bachert, C., N. Zhang, et al. (2008). "Role of staphylococcal superantigens in upper airway disease." Curr Opin Allergy Clin Immunol **8**(1): 34-38.

Badiou, C., O. Dumitrescu, et al. (2008). "Panton-Valentine leukocidin is expressed at toxic levels in human skin abscesses." Clin Microbiol Infect **14**(12): 1180-1183.

Bannan, J., K. Visvanathan, et al. (1999). "Structure and function of streptococcal and staphylococcal superantigens in septic shock." Infect Dis Clin North Am **13**(2): 387-396, ix.

Barnes, P. J. (2009). "Intrinsic asthma: not so different from allergic asthma but driven by superantigens?" Clin Exp Allergy **39**(8): 1145-1151.

Barrio, M. B., P. Rainard, et al. (2006). "LukM/LukF'-PV is the most active Staphylococcus aureus leukotoxin on bovine neutrophils." Microbes Infect **8**(8): 2068-2074.

Bayles, K. W. and J. J. Iandolo (1989). "Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D." J Bacteriol **171**(9): 4799-4806.

Becker, K., A. Friedrich, et al. (2004). "Systematic survey on the prevalence of genes coding for staphylococcal enterotoxins SElM, SElO, and SElN." Mol Nutr Food Res. **48**(7): 488-495.

Becker, K., A. W. Friedrich, et al. (2003). "Prevalence of Genes Encoding Pyrogenic Toxin Superantigens and Exfoliative Toxins among Strains of *Staphylococcus aureus* Isolated from Blood and Nasal Specimens." J. Clin. Microbiol. **41**(4): 1434-1439.

Bergdoll, M. S., B. A. Crass, et al. (1981). "A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome Staphylococcus aureus isolates." Lancet **1**(8228): 1017-1021.

Bhakdi, S. and J. Tranum-Jensen (1991). "Alpha-toxin of Staphylococcus aureus." Microbiol Rev **55**(4): 733-751.

Blank, C., A. Luz, et al. (1997). "Superantigen and endotoxin synergize in the induction of lethal shock." Eur J Immunol. **27**(4): 825-833.

Brown, E. L., O. Dumitrescu, et al. (2009). "The Panton-Valentine leukocidin vaccine protects mice against lung and skin infections caused by Staphylococcus aureus USA300." Clin Microbiol Infect **15**(2): 156-164.

Bubeck Wardenburg, J., T. Bae, et al. (2007). "Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in Staphylococcus aureus pneumonia." Nat Med **13**(12): 1405-1406.

Bubeck Wardenburg, J., A. M. Palazzolo-Ballance, et al. (2008). "Panton-Valentine leukocidin is not a virulence determinant in murine models of community-associated methicillin-resistant Staphylococcus aureus disease." J Infect Dis **198**(8): 1166-1170.

Bubeck Wardenburg, J., R. J. Patel, et al. (2007). "Surface proteins and exotoxins are required for the pathogenesis of Staphylococcus aureus pneumonia." Infect Immun **75**(2): 1040-1044.

Bubeck Wardenburg, J. and O. Schneewind (2008). "Vaccine protection against Staphylococcus aureus pneumonia." J Exp Med **205**(2): 287-294.
Bunikowski, R., M. Mielke, et al. (1999). "Prevalence and role of serum IgE antibodies to the Staphylococcus aureus-derived superantigens SEA and SEB in children with atopic dermatitis." J Allergy Clin Immunol **103**(1 Pt 1): 119-124.
Carpaij, N., R. J. Willems, et al. (2011). "Genetic variation in spatio-temporal confined USA300 community-associated MRSA isolates: a shift from clonal dispersion to genetic evolution?" PLoS One **6**(2): e16419.
Chambers, H. F. and F. R. Deleo (2009). "Waves of resistance: Staphylococcus aureus in the antibiotic era." Nat Rev Microbiol **7**(9): 629-641.
Chatterjee, S. S., L. Chen, et al. (2011). "Distribution and regulation of the mobile genetic element-encoded phenol-soluble modulin PSM-mec in methicillin-resistant Staphylococcus aureus." PLoS One **6**(12): e28781.
Cheung, G. Y. and M. Otto (2012). "The potential use of toxin antibodies as a strategy for controlling acute Staphylococcus aureus infections." Expert Opin Ther Targets **16**(6): 601-612.
Cheung, G. Y., K. Rigby, et al. (2010). "Staphylococcus epidermidis strategies to avoid killing by human neutrophils." PLoS Pathog **6**(10): e1001133.
Choi, Y., J. Lafferty, et al. (1990). "Selective expansion of T cells expressing V beta 2 in toxic shock syndrome." J. Exp. Med. **172**(3): 981-984.
Choorit, W., J. Kaneko, et al. (1995). "Existence of a new protein component with the same function as the LukF component of leukocidin or gamma-hemolysin and its gene in Staphylococcus aureus P83." FEBS Lett **357**(3): 260-264.
Daum, R. S. and B. Spellberg (2012). "Progress Toward a Staphylococcus aureus Vaccine." Clin Infect Dis **54**(4): 560-567.
David, M. Z. and R. S. Daum (2010). "Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic." Clin Microbiol Rev **23**(3): 616-687.
del Giudice, P., V. Blanc, et al. (2009). "Primary skin abscesses are mainly caused by Panton-Valentine leukocidin-positive Staphylococcus aureus strains." Dermatology **219**(4): 299-302.
DeLeo, F. R., M. Otto, et al. (2010). "Community-associated meticillin-resistant Staphylococcus aureus." Lancet **375**(9725): 1557-1568.
Diep, B. A., S. R. Gill, et al. (2006). "Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*." The Lancet **367**: 731-739.
Diep, B. A. and M. Otto (2008). "The role of virulence determinants in community-associated MRSA pathogenesis." Trends in Microbiology **16**(8): 361-369.
Dinges, M. M., P. M. Orwin, et al. (2000). "Exotoxins of *Staphylococcus aureus*." Clin. Microbiol. Rev. **13**(1): 16-34.
Dumont, A. L., T. K. Nygaard, et al. (2011). "Characterization of a new cytotoxin that contributes to Staphylococcus aureus pathogenesis." Mol Microbiol **79**(3): 814-825.
Feil, E. J., J. E. Cooper, et al. (2003). "How clonal is Staphylococcus aureus?" J Bacteriol **185**(11): 3307-3316.
Fitzgerald, J. R., S. R. Monday, et al. (2001). "Characterization of a Putative Pathogenicity Island from Bovine *Staphylococcus aureus* Encoding Multiple Superantigens." J. Bacteriol. **183**(1): 63-70.
Fleischer, B. and H. Schrezenmeier (1988). "T cell stimulation by staphylococcal enterotoxins. Clonally variable response and requirement for major histocompatibility complex class II molecules on accessory or target cells." J Exp Med **167**(5): 1697-1707.
Foster, T. J. (2005). "Immune evasion by staphylococci." Nat Rev Microbiol **3**(12): 948-958.
Foster, T. J. and M. Hook (1998). "Surface protein adhesins of Staphylococcus aureus." Trends Microbiol **6**(12): 484-488.
Franchi, L., R. Munoz-Planillo, et al. (2012). "Sensing and reacting to microbes through the inflammasomes." Nat Immunol **13**(4): 325-332.
Fraser, J., V. Arcus, et al. (2000). "Superantigens - powerful modifiers of the immune system." Mol Med Today **6**(3): 125-132.

Fraser, J. D. and T. Proft (2008). "The bacterial superantigen and superantigen-like proteins." Immunological Reviews **225**(1): 226-243.
Fueyo, J. M., M. C. Mendoza, et al. (2005). "Relationships between toxin gene content and genetic background in nasal carried isolates of *Staphylococcus aureus* from Asturias, Spain." FEMS Microbiol Lett **243**(2): 447-454.
Gevaert, P., G. Holtappels, et al. (2005). "Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue." Allergy **60**(1): 71-79.
Gillet, Y., B. Issartel, et al. (2002). "Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients." Lancet **359**(9308): 753-759.
Goerke, C., R. Pantucek, et al. (2009). "Diversity of prophages in dominant Staphylococcus aureus clonal lineages." J Bacteriol **191**(11): 3462-3468.
Gould, H. J., P. Takhar, et al. (2007). "The allergic march from Staphylococcus aureus superantigens to immunoglobulin E." Chem Immunol Allergy **93**: 106-136.
Gravet, A., P. Couppie, et al. (2001). "Staphylococcus aureus isolated in cases of impetigo produces both epidermolysin A or B and LukE-LukD in 78% of 131 retrospective and prospective cases." J Clin Microbiol **39**(12): 4349-4356.
Grumann, D., E. Ruotsalainen, et al. (2011). "Characterization of infecting strains and superantigen-neutralizing antibodies in Staphylococcus aureus bacteremia." Clin Vaccine Immunol **18**(3): 487-493.
Harris, S. R., E. J. Feil, et al. (2010). "Evolution of MRSA during hospital transmission and intercontinental spread." Science **327**(5964): 469-474.
Highlander, S. K., K. G. Hulten, et al. (2007). "Subtle genetic changes enhance virulence of methicillin resistant and sensitive Staphylococcus aureus." BMC Microbiol **7**: 99.
Holden, M. T., L. Y. Hsu, et al. (2013). "A genomic portrait of the emergence, evolution and global spread of a methicillin resistant Staphylococcus aureus pandemic." Genome Res.
Holtfreter, S., K. Bauer, et al. (2004). "*egc*-Encoded Superantigens from *Staphylococcus aureus* Are Neutralized by Human Sera Much Less Efficiently than Are Classical Staphylococcal Enterotoxins or Toxic Shock Syndrome Toxin." Infect. Immun. **72**(7): 4061-4071.
Holtfreter, S. and B. M. Broker (2005). "Staphylococcal superantigens: do they play a role in sepsis?" Arch Immunol Ther Exp (Warsz) **53**(1): 13-27.
Holtfreter, S., D. Grumann, et al. (2007). "Clonal distribution of superantigen genes in clinical Staphylococcus aureus isolates." J Clin Microbiol **45**(8): 2669-2680.
Holtfreter, S., J. Kolata, et al. (2010). "Towards the immune proteome of Staphylococcus aureus - The anti-S. aureus antibody response." Int J Med Microbiol **300**: 176-192.
Holtfreter, S., K. Roschack, et al. (2006). "*Staphylococcus aureus* carriers neutralize superantigens by antibodies specific for their colonizing strain: A potential explanation for their improved prognosis in severe sepsis." J Infect Dis **193**(9): 1275-1278.
Holzinger, D., L. Gieldon, et al. (2012). "Staphylococcus aureus Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome." J Leukoc Biol **92**(5): 1069-1081.
Inoshima, I., N. Inoshima, et al. (2011). "A Staphylococcus aureus pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice." Nat Med **17**(10): 1310-1314.
Inoshima, N., Y. Wang, et al. (2012). "Genetic requirement for ADAM10 in severe Staphylococcus aureus skin infection." J Invest Dermatol **132**(5): 1513-1516.
Janzon, L., S. Lofdahl, et al. (1989). "Identification and nucleotide sequence of the delta-lysin gene, hld, adjacent to the accessory gene regulator (agr) of Staphylococcus aureus." Mol Gen Genet **219**(3): 480-485.
Jarraud, S., G. Cozon, et al. (1999). "Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever." J Clin Microbiol **37**(8): 2446-2449.
Jarraud, S., C. Mougel, et al. (2002). "Relationships between *Staphylococcus aureus* Genetic Background, Virulence Factors, agr Groups (Alleles), and Human Disease." Infect. Immun. **70**(2): 631-641.

Jarraud, S., M. A. Peyrat, et al. (2001). "*egc*, A Highly Prevalent Operon of Enterotoxin Gene, Forms a Putative Nursery of Superantigens in *Staphylococcus aureus*." J Immunol **166**(1): 669-677.

Johns, M. B., Jr. and S. A. Khan (1988). "Staphylococcal enterotoxin B gene is associated with a discrete genetic element." J Bacteriol **170**(9): 4033-4039.

Joo, H. S., G. Y. Cheung, et al. (2011). "Antimicrobial activity of community-associated methicillin-resistant Staphylococcus aureus is caused by phenol-soluble modulin derivatives." J Biol Chem **286**(11): 8933-8940.

Kaneko, J. and Y. Kamio (2004). "Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes." Biosci Biotechnol Biochem **68**(5): 981-1003.

Kaneko, J., T. Kimura, et al. (1997). "Panton-valentine leukocidin genes in a phage-like particle isolated from mitomycin C-treated Staphylococcus aureus V8 (ATCC 49775)." Biosci Biotechnol Biochem **61**(11): 1960-1962.

Kaneko, J., T. Kimura, et al. (1998). "Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriophage phiPVL carrying Panton-Valentine leukocidin genes." Gene **215**(1): 57-67.

Kappler, J., B. Kotzin, et al. (1989). "V beta-specific stimulation of human T cells by staphylococcal toxins." Science **244**(4906): 811-813.

Kebaier, C., R. R. Chamberland, et al. (2012). "Staphylococcus aureus alpha-hemolysin mediates virulence in a murine model of severe pneumonia through activation of the NLRP3 inflammasome." J Infect Dis **205**(5): 807-817.

Kretschmer, D., A. K. Gleske, et al. (2010). "Human formyl peptide receptor 2 senses highly pathogenic Staphylococcus aureus." Cell Host Microbe **7**(6): 463-473.

Kuroda, M., T. Ohta, et al. (2001). "Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*." Lancet **357**(9264): 1225-1240.

Kusch, K., K. Hanke, et al. (2011). "The influence of SaeRS and sigma(B) on the expression of superantigens in different Staphylococcus aureus isolates." Int J Med Microbiol **301**(6): 488-499.

Labandeira-Rey, M., F. Couzon, et al. (2007). "Staphylococcus aureus Panton-Valentine Leukocidin Causes Necrotizing Pneumonia

10.1126/science.1137165." Science **315**(5815): 1130-1133.

Ladhani, S. (2003). "Understanding the mechanism of action of the exfoliative toxins of *Staphylococcus aureus*." FEMS Immunol Med Microbiol **39**(2): 181-189.

Letertre, C., S. Perelle, et al. (2003). "Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*." J Appl Microbiol **95**(1): 38-43.

Lina, G., G. A. Bohach, et al. (2004). "Standard nomenclature for the superantigens expressed by *Staphylococcus*." J Infect Dis **189**(12): 2334-2336.

Lindsay, J. A. (2010). "Genomic variation and evolution of Staphylococcus aureus." Int J Med Microbiol **300**(2-3): 98-103.

Lindsay, J. A. and M. T. Holden (2006). "Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*." Funct Integr Genomics **6**(3): 186-201.

Lindsay, J. A., C. E. Moore, et al. (2006). "Microarrays Reveal that Each of the Ten Dominant Lineages of *Staphylococcus aureus* Has a Unique Combination of Surface-Associated and Regulatory Genes." J. Bacteriol. **188**(2): 669-676.

Loffler, B., M. Hussain, et al. (2010). "Staphylococcus aureus panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils." PLoS Pathog **6**(1): e1000715.

Lowder, B. V., C. M. Guinane, et al. (2009). "Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus." Proc Natl Acad Sci U S A **106**(46): 19545-19550.

Masiuk, H., K. Kopron, et al. (2010). "Association of recurrent furunculosis with Panton-Valentine leukocidin and the genetic background of *Staphylococcus aureus*." J Clin Microbiol **48**(5): 1527-1535.

McAdam, P. R., K. E. Templeton, et al. (2012). "Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant Staphylococcus aureus." Proc Natl Acad Sci U S A **109**(23): 9107-9112.

McCarthy, A. J. and J. A. Lindsay (2012). "The distribution of plasmids that carry virulence and resistance genes in Staphylococcus aureus is lineage associated." BMC Microbiol **12**: 104.

McCarthy, A. J., A. A. Witney, et al. (2012). "Staphylococcus aureus Temperate Bacteriophage: Carriage and Horizontal Gene Transfer is Lineage Associated." Front Cell Infect Microbiol **2**: 6.

McCormick, J. K., J. M. Yarwood, et al. (2001). "Toxic Shock Syndrome and Bacterial Superantigens: An Update." Annual Review of Microbiology **55**(1): 77-104.

Monecke, S., B. Berger-Bachi, et al. (2007). "Comparative genomics and DNA array-based genotyping of pandemic Staphylococcus aureus strains encoding Panton-Valentine leukocidin." Clin Microbiol Infect **13**(3): 236-249.

Monecke, S., G. Coombs, et al. (2011). "A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus." PLoS One **6**(4): e17936.

Monecke, S., C. Luedicke, et al. (2009). "Molecular epidemiology of Staphylococcus aureus in asymptomatic carriers." Eur J Clin Microbiol Infect Dis.

Monecke, S., P. Slickers, et al. (2008). "Assignment of Staphylococcus aureus isolates to clonal complexes based on microarray analysis and pattern recognition." FEMS Immunol Med Microbiol **53**(2): 237-251.

Monecke, S., P. Slickers, et al. (2007). "High diversity of Panton-Valentine leukocidin-positive, methicillin-susceptible isolates of Staphylococcus aureus and implications for the evolution of community-associated methicillin-resistant S. aureus." Clin Microbiol Infect **13**(12): 1157-1164.

Montgomery, C. P. and R. S. Daum (2009). "Transcription of inflammatory genes in the lung after infection with community-associated methicillin-resistant Staphylococcus aureus: a role for panton-valentine leukocidin?" Infect Immun **77**(5): 2159-2167.

Morfeldt, E., D. Taylor, et al. (1995). "Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII." Embo J **14**(18): 4569-4577.

Morikawa, K., A. J. Takemura, et al. (2012). "Expression of a cryptic secondary sigma factor gene unveils natural competence for DNA transformation in Staphylococcus aureus." PLoS Pathog **8**(11): e1003003.

Morinaga, N., Y. Kaihou, et al. (2003). "Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family." Microbiol Immunol. **47**(1): 81-90.

Munoz-Planillo, R., L. Franchi, et al. (2009). "A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome." J Immunol **183**(6): 3942-3948.

Narita, S., J. Kaneko, et al. (2001). "Phage conversion of Panton-Valentine leukocidin in Staphylococcus aureus: molecular analysis of a PVL-converting phage, phiSLT." Gene **268**(1-2): 195-206.

Nhan, T. X., R. Leclercq, et al. (2011). "Prevalence of toxin genes in consecutive clinical isolates of Staphylococcus aureus and clinical impact." Eur J Clin Microbiol Infect Dis **30**(6): 719-725.

Nilsson, I. M., O. Hartford, et al. (1999). "Alpha-toxin and gamma-toxin jointly promote Staphylococcus aureus virulence in murine septic arthritis." Infect Immun **67**(3): 1045-1049.

Nishifuji, K., M. Sugai, et al. (2008). "Staphylococcal exfoliative toxins: "molecular scissors" of bacteria that attack the cutaneous defense barrier in mammals." J Dermatol Sci **49**(1): 21-31.

Novick, R. P. and A. Subedi (2007). "The SaPIs: mobile pathogenicity islands of Staphylococcus." Chem Immunol Allergy **93**: 42-57.

Nubel, U., J. Dordel, et al. (2010). "A timescale for evolution, population expansion, and spatial spread of an emerging clone of methicillin-resistant Staphylococcus aureus." PLoS Pathog **6**(4): e1000855.

Nubel, U., P. Roumagnac, et al. (2008). "Frequent emergence and limited geographic dispersal of methicillin-resistant Staphylococcus aureus." Proc Natl Acad Sci U S A **105**(37): 14130-14135.

Nubel, U., B. Strommenger, et al. (2011). "From types to trees: reconstructing the spatial spread of Staphylococcus aureus based on DNA variation." Int J Med Microbiol **301**(8): 614-618.
Olsen, R. J., S. D. Kobayashi, et al. (2010). "Lack of a major role of Staphylococcus aureus Panton-Valentine leukocidin in lower respiratory tract infection in nonhuman primates." Am J Pathol **176**(3): 1346-1354.
Omoe, K., D.-L. Hu, et al. (2003). "Identification and Characterization of a New Staphylococcal Enterotoxin-Related Putative Toxin Encoded by Two Kinds of Plasmids." Infect. Immun. **71**(10): 6088-6094.
Ono, H. K., K. Omoe, et al. (2008). "Identification and Characterization of Two Novel Staphylococcal Enterotoxins, Types S and T." Infect. Immun. **76**(11): 4999-5005.
Otto, M. (2010). "Basis of virulence in community-associated methicillin-resistant Staphylococcus aureus." Annu Rev Microbiol **64**: 143-162.
Peacock, S. J., C. E. Moore, et al. (2002). "Virulent Combinations of Adhesin and Toxin Genes in Natural Populations of *Staphylococcus aureus*." Infect. Immun. **70**(9): 4987-4996.
Periasamy, S., S. S. Chatterjee, et al. (2012). "Phenol-soluble modulins in staphylococci: What are they originally for?" Commun Integr Biol **5**(3): 275-277.
Periasamy, S., H. S. Joo, et al. (2012). "How Staphylococcus aureus biofilms develop their characteristic structure." Proc Natl Acad Sci U S A **109**(4): 1281-1286.
Perret, M., C. Badiou, et al. (2012). "Cross-talk between Staphylococcus aureus leukocidins-intoxicated macrophages and lung epithelial cells triggers chemokine secretion in an inflammasome-dependent manner." Cell Microbiol **14**(7): 1019-1036.
Petersson, K., H. Pettersson, et al. (2003). "Staphylococcal enterotoxin H induces V alpha-specific expansion of T cells." J Immunol **170**(8): 4148-4154.
Powers, M. E., H. K. Kim, et al. (2012). "ADAM10 mediates vascular injury induced by Staphylococcus aureus alpha-hemolysin." J Infect Dis **206**(3): 352-356.
Prevost, G., L. Mourey, et al. (2001). "Staphylococcal pore-forming toxins." Curr Top Microbiol Immunol **257**: 53-83.
Proft, T. and J. D. Fraser (2003). "Bacterial superantigens." Clin Exp Immunol **133**(3): 299-306.
Queck, S. Y., M. Jameson-Lee, et al. (2008). "RNAIII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in Staphylococcus aureus." Mol Cell **32**(1): 150-158.
Queck, S. Y., B. A. Khan, et al. (2009). "Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA." PLoS Pathog **5**(7): e1000533.
Rautenberg, M., H. S. Joo, et al. (2011). "Neutrophil responses to staphylococcal pathogens and commensals via the formyl peptide receptor 2 relates to phenol-soluble modulin release and virulence." Faseb J **25**(4): 1254-1263.
Rellahan, B. L., L. A. Jones, et al. (1990). "In vivo induction of anergy in peripheral V beta 8+ T cells by staphylococcal enterotoxin B." J Exp Med **172**(4): 1091-1100.
Rigby, K. M. and F. R. DeLeo (2012). "Neutrophils in innate host defense against Staphylococcus aureus infections." Semin Immunopathol **34**(2): 237-259.
Schaffer, A. C. and J. C. Lee (2008). "Vaccination and passive immunisation against Staphylococcus aureus." Int J Antimicrob Agents **32 Suppl 1**: S71-78.
Schlievert, P. (1982). "Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C." Infect Immun **36**(1): 123-128.
Schlotter, K., R. Ehricht, et al. (2012). "Leukocidin genes lukF-P83 and lukM are associated with taphylococcus aureus clonal complexes 151, 479 and 133 isolated from bovine udder infections in Thuringia, Germany." Vet Res **43**(1): 42.
Sheehan, B. J., T. J. Foster, et al. (1992). "Osmotic and growth-phase dependent regulation of the eta gene of Staphylococcus aureus: a role for DNA supercoiling." Mol Gen Genet **232**(1): 49-57.
Shore, A. C., O. M. Brennan, et al. (2012). "DNA microarray profiling of a diverse collection of nosocomial methicillin-resistant staphylococcus aureus isolates assigns the majority to the correct sequence type and staphylococcal cassette chromosome mec (SCCmec) type and

results in the subsequent identification and characterization of novel SCCmec-SCCM1 composite islands." Antimicrob Agents Chemother **56**(10): 5340-5355.
Sila, J., P. Sauer, et al. (2009). "Comparison of the prevalence of genes coding for enterotoxins, exfoliatins, panton-valentine leukocidin and tsst-1 between methicillin-resistant and methicillin-susceptible isolates of Staphylococcus aureus at the university hospital in olomouc." Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub **153**(3): 215-218.
Silverman, G. J. and C. S. Goodyear (2006). "Confounding B-cell defences: lessons from a staphylococcal superantigen." Nat Rev Immunol **6**(6): 465-475.
Skaar, E. P. and O. Schneewind (2004). "Iron-regulated surface determinants (Isd) of Staphylococcus aureus: stealing iron from heme." Microbes Infect **6**(4): 390-397.
Spellberg, B. and R. Daum (2012). "Development of a vaccine against Staphylococcus aureus." Semin Immunopathol **34**(2): 335-348.
Supersac, G., Y. Piemont, et al. (1998). "Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of Staphylococcus aureus." Microb Pathog **24**(4): 241-251.
Thomas, D., S. Chou, et al. (2007). "Diversity in Staphylococcus aureus enterotoxins." Chem Immunol Allergy **93**: 24-41.
Thomas, D., O. Dauwalder, et al. (2009). "Staphylococcus aureus superantigens elicit redundant and extensive human Vbeta patterns." Infect Immun **77**(5): 2043-2050.
Thomas, D. Y., S. Jarraud, et al. (2006). "Staphylococcal enterotoxin-like toxins U2 and V, two new staphylococcal superantigens arising from recombination within the enterotoxin gene cluster." Infect Immun **74**(8): 4724-4734.
Tseng, C. W., P. Kyme, et al. (2009). "Staphylococcus aureus Panton-Valentine leukocidin contributes to inflammation and muscle tissue injury." PLoS One **4**(7): e6387.
Tseng, C. W., S. Zhang, et al. (2004). "Accessory gene regulator control of staphyloccoccal enterotoxin d gene expression." J Bacteriol **186**(6): 1793-1801.
Turner, K. M. and E. J. Feil (2007). "The secret life of the multilocus sequence type." Int J Antimicrob Agents **29**(2): 129-135.
van Belkum, A., N. J. Verkaik, et al. (2009). "Reclassification of Staphylococcus aureus Nasal Carriage Types." J Infect Dis **199**(12): 1820-1826.
Vandenesch, F., G. Lina, et al. (2012). "Staphylococcus aureus Hemolysins, bi-component Leukocidins, and Cytolytic Peptides: A Redundant Arsenal of Membrane-Damaging Virulence Factors?" Front Cell Infect Microbiol **2**: 12.
Vandenesch, F., T. Naimi, et al. (2003). "Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence." Emerg Infect Dis **9**(8): 978-984.
Ventura, C. L., N. Malachowa, et al. (2010). "Identification of a novel Staphylococcus aureus two-component leukotoxin using cell surface proteomics." PLoS One **5**(7): e11634.
Villaruz, A. E., J. Bubeck Wardenburg, et al. (2009). "A point mutation in the agr locus rather than expression of the Panton-Valentine leukocidin caused previously reported phenotypes in Staphylococcus aureus pneumonia and gene regulation." J Infect Dis **200**(5): 724-734.
von Eiff, C., A. Friedrich, et al. (2004). "Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of *Staphylococcus aureus*." Diagn Microbiol Infect Dis. **49**(3): 157-162.
Wang, R., K. R. Braughton, et al. (2007). "Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA." Nat Med **13**(12): 1510-1514.
Wardenburg, J. B. (2011). "Host-Pathogen Interactions in Staphylococcus Aureus Lung Disease." Pediatric Pulmonology: 137-138.
Wertheim, H. F., D. C. Melles, et al. (2005). "The role of nasal carriage in *Staphylococcus aureus* infections." Lancet Infect Dis **5**(12): 751-762.
White, J., A. Herman, et al. (1989). "The V beta-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice." Cell **56**(1): 27-35.

Wilke, G. A. and J. Bubeck Wardenburg (2010). "Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury." Proc Natl Acad Sci U S A **107**(30): 13473-13478.
Wilson, G. J., K. S. Seo, et al. (2011). "A novel core genome-encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia." PLoS Pathog **7**(10): e1002271.
Xiong, Y. Q., J. Willard, et al. (2006). "Regulation of Staphylococcus aureus alpha-toxin gene (hla) expression by agr, sarA, and sae in vitro and in experimental infective endocarditis." J Infect Dis **194**(9): 1267-1275.
Yamaguchi, T., T. Hayashi, et al. (2000). "Phage conversion of exfoliative toxin A production in *Staphylococcus aureus*." Mol Microbiol **38**(4): 694-705.
Yamaguchi, T., T. Hayashi, et al. (2001). "Complete nucleotide sequence of a Staphylococcus aureus exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C." Infect Immun **69**(12): 7760-7771.
Yamaguchi, T., K. Nishifuji, et al. (2002). "Identification of the *Staphylococcus aureus etd* pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B." Infect Immun **70**(10): 5835-5845.
Yarwood, J. M., D. Y. Leung, et al. (2000). "Evidence for the involvement of bacterial superantigens in psoriasis, atopic dermatitis, and Kawasaki syndrome." FEMS Microbiol Lett **192**(1): 1-7.
Yoshida, A. (1963). "Staphylococcal delta-hemolysin. I. Purification and chemical properties." Biochim Biophys Acta **71**: 544-553.
Zhang, N., G. Holtappels, et al. (2011). "Mucosal tissue polyclonal IgE is functional in response to allergen and SEB." Allergy **66**(1): 141-148.
Zhang, S., J. J. Iandolo, et al. (1998). "The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej)." FEMS Microbiol Lett **168**(2): 227-233.
Zollner, T. M., T. A. Wichelhaus, et al. (2000). "Colonization with superantigen-producing Staphylococcus aureus is associated with increased severity of atopic dermatitis." Clin Exp Allergy **30**(7): 994-1000.
Zou, D., J. Kaneko, et al. (2000). "Prophage, phiPV83-pro, carrying panton-valentine leukocidin genes, on the Staphylococcus aureus P83 chromosome: comparative analysis of the genome structures of phiPV83-pro, phiPVL, phi11, and other phages." Biosci Biotechnol Biochem **64**(12): 2631-2643.