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Fabio Bagnoli
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Staphylococcus aureus

Microbiology, Pathology, Immunology, Therapy and Prophylaxis

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Declaration of Interest

Fabio Bagnoli and Rino Rappuoli are employees of GSK Vaccines and own GSK stocks. Fabio Bagnoli owns patents on *S. aureus* vaccine candidates. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Authorship

Fabio Bagnoli and Rino Rappuoli were involved in the conception and design of the book and approved its content before publication.

Preface

Staphylococcus aureus is a leading pathogen in surgical site, intensive care unit, and skin infections as well as health-care associated pneumonias. These infections are associated with an enormous burden of morbidity, mortality and increase of hospital length of stay and patient cost. *S. aureus* is impressively fast in acquiring antibiotic resistance and multidrug resistant strains are a serious threat to human health. It has been recently estimated that deaths attributable to antibiotic resistant infections will exceed the ones caused by cancer by 2050 (https://amr-review.org/Publications). *S. aureus*, was included among the ESKAPE pathogens (*Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) recognized as the leading cause of antibiotic-resistant infections occurring worldwide in hospitals. Due to resistance or insufficient effectiveness, antibiotics and bundle measures leave a tremendous unmet medical need worldwide. In addition there are no licensed vaccines or immunotherapies on the market despite the significant efforts done by public and private initiatives.

This book includes 16 chapters spanning from basic Microbiology and Immunology aspects to Pathology of key disease manifestations as well as a review of current standard of care. Furthermore, front-edge discoveries on therapeutic and prophylactic approaches alternative to antibiotics are reviewed.

Given the complexity of the Microbiology of this pathogen we decided to give significant emphasis to this aspect. We started describing conventional and molecular diagnostics-based identification methods of *S. aureus* in the microbiology laboratory. Rapid and more informative typization tests are likely to represent a significant benefit for improving clinical practice and containing the emergence of antimicrobial resistance. Methicillin-resistant *S. aureus* (MRSA) is a global issue causing increase of mortality and the need to use last-resource antibiotics. Predominant clones circulating worldwide and the associated antibiotic resistance are described.

Sugar and protein surface structures of the bacterium are comprehensively discussed. These components play key roles in cell viability, virulence and evasion of host defences. The major surface polysaccharides include the capsular

polysaccharide (CP), cell wall teichoic acid (WTA), and polysaccharide intercellular adhesin/poly-β(1–6)-N-acetylglucosamine (PIA/PNAG). They play distinct roles in colonization and pathogenesis and are being explored as targets for antimicrobial interventions.

Surface proteins have very diverse functions (e.g., adhesion, invasion, signalling, conjugation, interaction with the environment and immune-evasion). They have been categorized into distinct classes based on structural and functional analysis. We provide the defining features associated with cell wall-anchored surface proteins and a framework for their categorization based on the current knowledge of structure and function.

On top of surface virulence factors, *S. aureus* secretes pore-forming toxins that kill eukaryotic immune and non-immune cells. Here we provide an update on the various toxins, the identification of its receptors on host cells, and their roles in pathogenesis.

S. aureus pathogenicity is driven by the wealth of virulence factors and its ability to adapt to different environments. The latter is due to the presence of complex regulatory networks fine-tuning metabolic and virulence gene expression. One of the most widely distributed mechanisms is the two-component signal transduction system (TCS) that can reveal an environmental signal and trigger an adaptive gene expression response. It encodes a total of 16 conserved pairs of TCS that are involved in diverse signalling cascades ranging from global virulence gene regulation such as quorum sensing by the Agr system, the bacterial response to antimicrobial agents, cell wall metabolism, respiration and nutrient sensing. Herein we give an overview of the current knowledge on TCS and its influence on virulence gene expression.

The versatility of *S. aureus* is reflected by the wide range of disease that it can cause. It's a leading cause of bacteraemia, infective endocarditis, osteomyelitis, pneumonia, indwelling medical device related infections, as well as skin and soft tissue infections (SSTIs). SSTIs are among the most common infections worldwide. They range in severity from minor, self-limiting, superficial infections to life-threatening diseases requiring all the resources of modern medicine. They have variable presentations ranging from impetigo and folliculitis to surgical site infections (SSIs). Here we describe the anatomical localization of the different SSTI associated with *S. aureus*, the virulence factors known to play a role in these infections, their current epidemiology as well as the standard of care and potential prophylaxis.

Musculoskeletal infections, bacteremia and infective endocarditis associated to *S. aureus* infections are very difficult to treat and important causes of morbidity and mortality. Osteomyelitis can cause long-term relapses and functional deficits and bacteremia and infective endocarditis are associated with excess mortality when compared to other pathogens. Although considerable advances have been achieved in their diagnosis, prevention and treatment, the management remains challenging and impact on the healthcare system is still very high.

S. aureus can also infect several animal species (e.g., cattle, poultry and pigs) and transmission from animals to humans and vice versa has been observed. This

represents an important threat to public health, as animal strains can adapt to the human population and spread additional antibiotic resistance.

Medical need associated to *S. aureus* infections is enhanced by raising prevalence of multidrug resistant strains and acquisition of resistance to last resort antibiotics. Therefore, alternative medical interventions are urgently needed. Vaccines certainly represent one of the most important options. Unfortunately a correlate of protection against *S. aureus* is not known and this represents a significant issue for developing vaccines. Herein, we review what is known and unknown about innate and adaptive immunity against this complex pathogen. We provide an overview on the major cell types involved in innate immune defence and major differences of the immune response during colonization versus infection. Although the contribution of adaptive immunity against *S. aureus* is not yet clear, there are accumulating evidence both from animal models and from human data that T cell- and B cell-mediated adaptive immunity can control the infection. Unfortunately *S. aureus* has evolved several mechanisms to manipulate innate and adaptive immune responses to its advantage. Indeed, it expresses factors able to interfere with many critical components of the immune system and hamper proper immune functioning. In recent years research, including structural and functional studies, has fundamentally contributed to our understanding of the mechanisms of action of the individual factors.

In addition to the lack of a known correlate of protection, failure of developing an effective vaccine against this pathogen is likely due to several other reasons. Indeed. all attempts so far targeted single antigens, contained no adjuvants and efficacy trials were performed in severely ill subjects. We show the link between Phase III clinical trial data of failed vaccines with their preclinical observations and we provide a comprehensive evaluation of potential target populations for efficacy trials taking into account key factors such as population size, incidence of *S. aureus* infection, disease outcome, primary endpoints as well as practical advantages and disadvantages.

The last chapter provides an overview of a promising new therapeutic approach. Lysins are a new class of anti-infectives derived from bacteriophage, which cleave cell wall peptidoglycan causing immediate bacterial lysis. Importantly, lysins have high specificity for the pathogen and low chance of bacterial resistance.

In conclusion, this volume gives a comprehensive overview of the Microbiology, Pathology, Immunology, Therapy and Prophylaxis of *S. aureus* reviewing recent findings and knowledge on very diverse arguments and at the same time linked to each other. That is the uniqueness behind a book like this and the added value towards a search in literature databases.

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Carriage, Clinical Microbiology and Transmission of *Staphylococcus aureus*

Anna Aryee and Jonathan D. Edgeworth

Abstract *Staphylococcus aureus* is one of the most important bacterial pathogens in clinical practice and a major diagnostic focus for the routine microbiology laboratory. It is carried as a harmless commensal in up to two-thirds of the population at any one time predominantly not only in the anterior nares, but also in multiple other sites such as the groin, axilla, throat, perineum, vagina and rectum. It colonizes skin breach sites, such as ulcers and wounds, and causes superficial and deep skin and soft tissue infections and life-threatening deep seated infections particularly endocarditis and osteomyelitis. *S. aureus* is constantly evolving through mutation and uptake of mobile genetic elements that confer increasing resistance and virulence. Since the 1960s, hospitals have had to contend with emergence of methicillin-resistant *S. aureus* (MRSA) strains that spread better in hospitals than methicillin-susceptible *S. aureus* (MSSA) and are harder to treat. Since the 1980s, distinct community MRSA strains have also emerged that cause severe skin and respiratory infections. Conventional identification of MSSA and MRSA in the microbiology laboratory involves microscopy, culture and biochemical analysis that for most samples is straightforward but slow, taking at least 48 h. This delay has significant consequences for individual patient care and public health, through inadequate or excessive empiric antibiotic use, and failure to implement appropriate infection control measures for MRSA-colonized patients during those first 48 h. This unmet need has driven development of rapid molecular diagnostics that either complement or replace conventional culture techniques in the laboratory, or can be placed in the clinical environment as point-of-care (POC) devices. These new technologies provide results to clinicians anything from within an hour to 24 h, depending on sample and clinical setting, and should transform management of patients with *S. aureus* and other bacterial diseases; however, uptake is often slow due to the disruptive effect of new technologies, costs of transition and uncertainty

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of the optimal solution given successive advances. More evidence of the health economic, clinical and antimicrobial resistance benefit will help support introduction of these new technologies. Finally, preventing MRSA transmission has been a priority for healthcare organizations for many years. There have been significant recent reductions in transmission following local and national campaigns to re-enforce basic and heightened infection control interventions such as universal hand hygiene, barrier nursing, decolonization and isolation of MRSA-colonized patients detected through routine culture or screening policies. Developments in whole genome sequencing are providing greater insight into reservoirs and routes of transmission that should help better target interventions to ensure sustainable control of endemic strains and to identify and prevent emergence of new strains.

Contents

1 Clinical Microbiology

Staphylococcus aureus is a facultative anaerobe belonging to the genus *Staphylococcus* within the family of *Staphylococcae*. It is one of the most commonly identified clinically significant bacteria in a routine microbiology laboratory, and its identification by traditional techniques is a straightforward, albeit slow process, which is becoming more rapid with the introduction of molecular techniques.

Upon receipt of samples in the laboratory, Gram staining can be performed on sterile site samples such as pus and deep respiratory specimens to identify the presence of bacteria by light microscope. Staphylococci appear as irregular small clusters of Gram-positive cocci and traditionally no further information is available to the clinician on the first day. Samples are cultured on blood agar for 18–24 h when *S. aureus* colonies appear glistening, smooth and translucent, often with a golden pigment. Presumptive colonies are confirmed as *S. aureus* at this point using the techniques described below, although plates are usually re-incubated for a

further 24 h to detect slower growing colonies. Antibiotic susceptibility testing can also be set up on colonies identified at 24 h. By 48 h, colonies are approximately 1–2 mm in diameter and often exhibit a small zone of β-haemolysis. Thus, in a traditional laboratory, the clinician can expect to be told if staphylococci are present in important sterile site samples on the day of sample collection, whether *S. aureus* is present in the sample the following day, and receive a final report with antibiotic susceptibilities the day after.

A variety of biochemical tests are used to identify *S. aureus* colonies based on production of coagulase and deoxyribonuclease, presence of *S. aureus* specific antigens or the ability to ferment mannitol. The tube coagulase test is the traditional gold standard for discriminating between *S. aureus* and other staphylococci, usually referred to as coagulase-negative staphylococci (CoNS). This is a clinically important distinction because CoNS are rarely pathogenic in the absence of prosthetic material upon which they can reside in biofilm, although it is recognized that some CoNS are coagulase positive and some coagulase-negative *S. aureus* isolates have been reported (Vandenesch et al. 1993). The slide coagulase test is a more rapid test based on the presence of clumping factor, but up to 15 % of *S. aureus* isolates are negative. Latex agglutination tests detecting protein A, clumping factor and other surface antigens are also sensitive although less specific due to cross-reactivity with various CoNS.

Antimicrobial susceptibility testing is set up at the same time as identification of *S. aureus* using a number of culture-based methodologies. Disc diffusion testing is often used to assess simultaneous susceptibility to a variety of antibiotics. A key focus is to distinguish between methicillin-susceptible and methicillin-resistant *S. aureus*. This can be done using an oxacillin or cefoxitin disc, which has been shown to be an accurate surrogate marker for methicillin resistance (Skov et al. 2006). Antibiotic susceptibilities can also be performed using commercially available automated platforms such as the Vitek®2, BD Phoenix™ or MicroScan WalkAway systems.

An additional important focus for the microbiology laboratory is the specific detection of methicillin-resistant *S. aureus* (MRSA) in screening swabs from carriage and clinical sites, particularly the anterior nares, to identify colonized patients and institute infection control precautions (Coia et al. 2006). Many laboratories inoculate screening swabs directly onto selective agar, particularly chromogenic agars that provide a presumptive positive identification of MRSA within 24 h of sample receipt in the laboratory (Nahimana et al. 2006; Denys et al. 2013). Excluding presence of MRSA requires a further 24 h, and presumptively positive samples should be confirmed by antimicrobial susceptibility testing.

The analysis of blood cultures differs from other samples. 10–15 ml of blood is inoculated into media bottles immediately after collection from the patient and sent to the laboratory where they are placed into automated incubators. Positive cultures are flagged when bacterial growth is detected usually by continuous monitoring of changes in pH due to CO_2 production. The time taken for automated systems to detect bacteria depends on the number of bacteria in the sample (which can be up to 200 CFU/ml for endovascular infection down to <10 bacteria per ml of blood) and

the initial viability of bacteria that may either be intracellular or dormant. For *Staphylococcus aureus* bacteriaemia (SAB), over 80 % of positive culture bottles flag within 24 h (Khatib et al. 2005). Gram staining is performed on an aliquot of a flagged bottle to identify staphylococci, although this information has only limited clinical benefit because CoNS are more frequently identified in blood cultures. Conventionally, flagged blood culture media is plated onto agar that provides identification and disc diffusion susceptibility testing results the following day.

1.1 Introduction of Rapid Molecular Detection Methodologies

The slow nature of culture and biochemical-based detection methods means that identification and antimicrobial susceptibility of *S. aureus* only becomes available about 48 h after initial key clinical management decisions are made, and this is recognized as a major clinical and public health problem. For the individual patient, if serious *S. aureus* infection was not clinically suspected, then the patient may not be started on appropriate initial antibiotic therapy, particularly if the *S. aureus* is methicillin resistant, and this delay has been associated with higher mortality in some studies (González et al. 1999; Soriano et al. 2000). At a population level, uncertainty about whether an acute illness is bacterial or the likely antimicrobial susceptibilities prompts empiric treatment with broad-spectrum antibiotics to cover a range of potential bacterial causes including MRSA. This presents a public health problem due to overuse of empiric antibiotics that drives antibiotic resistance. There is also delay in identifying MRSA-colonized patients and instituting infection control precautions, which increases the potential for nosocomial transmission.

These unmet clinical needs have driven the development of rapid molecular diagnostics throughout the patient pathway to speed up time to detection and reporting of pathogenic bacteria including *S. aureus*. In the laboratory, these molecular methods can either enhance traditional culture-based processing or completely replace culture-based techniques.

1.2 Enhancing Culture-based Techniques

This involves rapid laboratory-based molecular analysis of *S. aureus* colonies or flagged positive blood culture bottle after initial culture of specimens for 24 h or more. Many laboratories have introduced matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS), which identifies bacterial colonies by analysing the protein composition of the bacterial cell (Wieser et al. 2012). This new technology has transformed species identification in

microbiology laboratories allowing bacterial identification within minutes: it is cheaper, more accurate and usually faster than biochemical-based methodologies and can replace most traditional biochemical tests. It was initially applied to bacterial colonies but has also been successfully applied to aliquots of blood culture sample that have flagged as positive (Mestas et al. 2014). Results are available within an hour although identification of Gram-positive bacteria is less effective than Gram-negative bacteria (78 % vs. 90 %). Additionally, rapid latex agglutination tests can be performed on single colonies or positive blood culture bottles to detect PBP2a as a marker of MRSA (Brown and Walpole 2001; Chapin and Musgnug 2004).

There have also been advances in the rapid nucleic acid based detection of organisms including MSSA and MRSA from flagged positive blood culture bottles (Opota et al. 2015). The Cepheid Xpert system uses PCR to identify *S. aureus* and MRSA direct from positive blood culture samples in about 2 h. PCR correlated with culture results in 80/82 (97.5 %) flagged blood culture bottles containing GPC in clusters by microscopy (Ratnayake and Olver 2011). Nanosphere's Verigene Gram-positive blood culture test allows rapid identification of both MSSA and MRSA from positive blood culture samples in less than 3 h. Mono-microbial bacterial isolates were correctly identified in 147 of 148 flagged blood culture bottles containing Gram-positive bacteria (38 MRSA or MSSA) (Beal et al. 2013). The FilmArray Blood Culture ID panel identifies 24 organisms and 3 antibiotic resistance genes including *S. aureus* and *mec*A in positive blood culture samples in approximately 1 h. The FilmArray correctly identified 19 *S. aureus* isolates from 167 mono-microbial flagged blood culture bottles and 156 (91.6 %) isolates overall (Altun et al. 2013). These technologies reduce by about 24 h the time to provide clinicians with a *S. aureus* identification and methicillin susceptibility result from blood cultures.

Rapid non-nucleic acid-based technologies are also under development. Accelerate diagnostics have a platform that uses automated digital microscopy and high-resolution growth analysis to provide identification and antimicrobial susceptibility data from blood and other sterile samples in approximately 5 h. It correctly identified all 77 MRSA and 54 MSSA mostly reference isolates in one study (Price et al. 2014a). Specific Technologies are developing a system that detects mixtures of volatile organic compounds using a colorimetric array integrated into a blood culture bottle, allowing faster detection and identification than traditional methods, although it does not provide susceptibility data (Lim et al. 2014).

1.3 Replacing Culture-based Techniques

Technologies are being developed to provide bacterial identification and genotypic prediction of antimicrobial susceptibilities directly on primary samples. Some are designed to analyse clinical samples including blood cultures and include a broad

range of bacteria. For example, Abbott's Iridica system identifies over 750 bacteria and 4 antibiotic resistance genes (including mecA) from a range of samples including whole blood and respiratory specimens in under 6 h. The Mobidiag Prove-It™ Bone & Joint StripArray system identifies over 30 Gram-positive and Gram-negative bacterial species and various genotypic resistance determinants including the *mecA* gene from synovial fluid, bone biopsy and tissue in 3.5 h from DNA extraction. In one study, 8 of 38 prosthetic joint infection samples culture positive for *S. aureus* were also identified by PCR and there was one additional PCR positive sample in a patient who had received antibiotics before sample collection that was culture negative (Metso et al. 2014). The Curetis Unyvero pneumonia platform detects 18 bacteria including *S. aureus* and the mecA gene directly from respiratory samples in 4–5 h (Jamal et al. 2014). Direct molecular analysis of clinical samples rather than a colony or suspension after culture allows same day detection of pathogens including MSSA and MRSA and rapid targeting of appropriate therapy.

Diagnostics have also been developed for the specific detection of *S. aureus* and the *mecA* gene including the Cepheid Xpert systems, the LightCycler MRSA Advanced and BD Max MRSA. These PCR tests take about 2 h (Rossney et al. 2008; Peterson et al. 2010; Widen et al. 2014) and have comparable sensitivity and specificity to enrichment and plating on different chromogenic agars (all > 92 %) whilst saving 24–72 h (Lee et al. 2013). Agreement between enriched culture and PCR was 96 % in this study. Although these *S. aureus* specific tests have been predominantly applied to MRSA screening swabs to target infection control interventions, they could also be used on clinical samples such as skin and soft tissue samples (Wolk et al. 2009) and respiratory specimens (Cercenado et al. 2012) to target early appropriate therapy.

Although molecular diagnostics dramatically reduce analysis time and can provide same day identification of MSSA and MRSA, the adoption into routine laboratory service is not straightforward. Molecular technologies are usually more expensive than traditional techniques, require a period of double running during evaluation and are then often used alongside rather than completely replacing the routine culture bench, so fixed costs remain. The time taken to transport specimens to the laboratory, particularly when the laboratory is off-site, can make a same-day test into a next-day test for a significant proportion of specimens, particularly if batch processing rather than random access platforms is used (Jeyaratnam et al. 2008). Samples submitted in the afternoon may provide results in the middle of the night when specialists who advise on result interpretation and patient management may not be available; hence, decisions may be postponed until the following day, when results would have become available using culture-based techniques: this may be particularly relevant if the advice is to narrow antibiotic spectrum. Molecular diagnostics are therefore disruptive for the laboratory and clinical teams, and adoption will require evidence of clinical benefit and cost effectiveness, and education of staff across the clinical pathway to realize the anticipated benefits of rapid diagnostics (Wassenberg et al. 2011; Van Der Zee et al. 2013).

1.4 Point-of-Care Technologies

An even more radical advance for clinical microbiology is the movement of diagnostics out of the laboratory to the ward or bedside. Laboratory platforms or new point-of-care (POC) devices that can rapidly identify pathogens including MSSA and MRSA are being evaluated on the wards. Unlike laboratory-based testing, sample analysis at the bedside can influence initial empiric treatment and infection control decisions. Some studies have assessed laboratory platforms such as the Cepheid Xpert system to detect MRSA on the ICU, in general ward and in outpatient clinics (Leone et al. 2013; Parcell and Phillips 2014). Many companies are also developing small bench top devices that are specifically designed for use by non-laboratory personnel on the wards; for example, Cobas and Alere-I, Atlas Diagnostics, Enigma Diagnostics, BioCartis Idylla, Orion Diagnostica and GNA Biosolutions and clinical utility studies are being performed for some pathogens (Binnicker et al. 2015; Goldenberg and Edgeworth 2015; van den Kieboom et al. 2015). Even closer to the patient from a ward-based to bedside-based system, a hand-held device is being developed that can identify MSSA or MRSA within 30 min (www.quantumdx.com). This field is in its infancy, and the technology advancing so rapidly, it is unclear what, where and when rapid POC infectious diseases diagnostics will enter into routine clinical practice. There will be many factors to consider including training front-line staff, quality control, accreditation, regulatory and legal constraints, linking results to hospital health records, resolving discrepancies between POC- and laboratory-generated results, and having mechanisms to alert specialist teams for advice and follow-up. At an organizational level, there will need to be strong governance processes to ensure POC devices are introduced safely and consistently, recognizing that the clinical environment is more complex than the laboratory, where there is a tradition of high-quality process control. Health economic evaluations that incorporate all the costs and benefits of laboratory versus POC-based testing will be needed to support decision-making of clinicians and managers.

2 *S. aureus* Carriage

Staphylococcus aureus is part of the commensal flora of human skin and mucosal surfaces, in addition to being a pathogen capable of causing both superficial infections and invasive disease with considerable associated morbidity and mortality. The anterior nares are the main reservoir of *S. aureus* carriage in humans. Other carriage sites include the skin, perineum, pharynx, gastrointestinal tract, vagina and axillae (Wertheim et al. 2005). About one-third of the population carry *S. aureus* on skin and mucosal sites at any one time (Kluytmans et al. 1997). Some individuals harbour the same strain over an extended period of time, whereas others carry different strains. *S. aureus* may also be present at different anatomical sites with varying frequency in different populations (Wertheim et al. 2005).

The variability in the detection of *S. aureus* at carriage sites has led to the description of distinct states, with potentially distinct underlying mechanisms. In the early 1960s, carriage was designated into four groups, persistent, intermittent, occasional and non-carriage (Williams 1963), but most studies now recognize three states: persistent, intermittent and non-carriage (Nouwen et al. 2004). It has been reported that approximately 60 % of the population are intermittent carriers, whilst 20 % each are either persistent carriers or non-carriers (Kluytmans et al. 1997). A large longitudinal survey published in 1997 analysing nasal swabs from staff at a university hospital found the same strain of *S. aureus* (confirmed by PGFE) in the same individuals on two occasions eight years apart in 3 out of 17 (18 %) staff members, suggesting that persistence reflects a stable host strain relationship (VandenBergh et al. 1999). However, a longitudinal study of 109 healthy individuals over a period of up to three years found persistent carriers having a resident persistent strain for most of the time but with additional distinct strains at other times (Muthukrishnan et al. 2013).

The prevalence of transient and persistent *S. aureus* nasal carriage varies by geographical location, age, gender and ethnicity. Studies have shown carriage ranges from 9 % in Indonesia to 37 % in Mexico (Lestari et al. 2008; Hamdan-Partida et al. 2010). Carriage is highest amongst newborns (up to 70 %) but steadily decreases in childhood. It has been posited, but not proven, that this may be due to pneumococcal competition or interference by other bacteria present in the nasopharynx in childhood (Lebon et al. 2008). There is another peak at adolescence followed by a decrease in early adulthood. Persistent carriage is seen more frequently in children than adults, and a conversion from persistent to transient or non-carriage most commonly occurs in adolescence (Williams 1963; Kluytmans et al. 1997; Wertheim et al. 2005). Rates of carriage have also been found to be higher in patients with Type 1 Diabetes Mellitus, intravenous drug users, haemodialysis patients, surgical patients, AIDS patients and patients with qualitative or quantitative defects in leucocyte function (Lowy 1998).

The fact that *S. aureus* is found at multiple body sites, that many studies have only looked for nasal carriage, and that detection methodologies are of variable sensitivity complicates our understanding of the significance of carriage states. Evidence from longitudinal studies does imply that persistent carriers and persistent non-carriers are distinct and likely therefore to have an underlying biological explanation, but the significance of transient carriage is less clear. Defining the host and bacterial factors involved in carriage should help resolve this issue. A feature of persistent carriers identified in a number of studies is that they carry a higher bacterial load than intermittent carriers (Nouwen et al. 2004; Van Belkum et al. 2009). This higher bacterial load may mean that persistent carriers are also more likely to be implicated in transmission of *S. aureus*. This also has implications for autoinfection—with persistent carriers at significantly higher risk of this than transient and non-carriers (Von Eiff et al. 2001; Wertheim et al. 2004b, 2005).

Studies have also specifically investigated carriage of MRSA in hospitalized patients, to determine the optimal sites for screening programmes. Screening is often performed at the anterior nares alone but this can miss up to a third of

MRSA-colonized patients (Meurman et al. 2005), particularly those with throat or rectal carriage, and the latter may be particularly important for hospital transmission (Boyce et al. 2007). Screening programmes in high-risk areas often take swabs from multiple carriage sites to ensure colonized patients are detected (Batra et al. 2008). It is, however, unclear whether MRSA has a differential propensity for carriage at particular sites compared with MSSA.

A number of studies have attempted to identify human genetic factors associated with carriage. A study in 2007, conducted as part of the Rotterdam study (a prospective, population-based study of the incidence and risk factors of disease in an elderly population), sought to identify polymorphisms in host inflammatory response genes associated with susceptibility to *S. aureus* carriage and infection. They found the Interleukin 4 (IL4)–524 C/C host genotype was associated with increased risk of *S. aureus* carriage, irrespective of organism genotype. They also found that individuals with the C-reactive protein (CRP) haplotype 1184C; 2042C; 2911C were less likely to be colonized, and that individuals with boils were more likely to be carriers of the *CFH* Tyr402 variant and the *CRP* 2911 C/C genotype (Emonts et al. 2008). A study carried out in 2006 and 2008 compared the genetics of *S. aureus* strains, epidemiological risk factors, antibiotic exposure and allelic polymorphisms of human genes posited to be involved in carriage of persistent carriers as compared to those of volunteers in an isolated population of adult Wayampi Amerindians living in an village in the Amazonian forest. The authors concluded that a specific set of host genetic polymorphisms were the main determinants of *S. aureus* persistent nasal carriage, namely single nucleotide polymorphisms (SNPs) for CRP genes (C2042T and C1184T) and *IL4* genes (*IL4 C524T*) (Ruimy et al. 2010). A further study published in 2006, also as part of the Rotterdam study, examined the role of host polymorphisms in the glucocorticoid receptor gene in persistent *S. aureus* carriage. They found GG homozygotes of the exon 9β polymorphism had a 68 % reduced risk of persistent carriage, whereas carriers of the codon 23 lysine allele had 80 % increased risk (Van den Akker et al. 2006).

3 *S. aureus* Transmission

The high prevalence of transient or persistent carriage with genetically diverse *S. aureus* strains in all human populations makes the epidemiology of *S. aureus* complex. Most attention has focused on transmission during outbreaks, particularly with MRSA or clones that are associated with more frequent and severe disease; however, it is important to also focus on transmission of endemic MSSA clones not least to define the mechanistic basis for successful and outbreak strains. Our understanding of *S. aureus* transmission has advanced dramatically with recent developments in whole genome sequencing (WGS) supported by advances in bioinformatics, mathematical modelling and social network analysis. Sequencing and interpreting hundreds of bacterial genomes is now feasible in some centres

within a reasonable time frame, initially months but now weeks and even days, and at ever-decreasing cost (Price et al. 2013). Traditional phenotypic and genotypic typing techniques such as phage typing, pulsed field gel electrophoresis (PFGE), spa typing and multi-locus sequence typing (MLST) lacked the necessary discriminatory ability to infer possible chains of transmission. This was a particular issue for MRSA, given the limited number of dominant clones in any geographical area (Enright et al. 2002). Consequently, the clinical benefit of molecular typing to support infection control practice was limited, apart from outbreaks with newly introduced clones that were distinct from endemic clones (Edgeworth et al. 2007). In contrast, WGS allows analysis of the entire core genome sequence to identify SNP differences between isolates. The range seen is from complete identity, isolates with perhaps a few tens of SNPs differences, to those that have hundreds or thousands of SNP differences (Harris et al. 2010). With knowledge of the mutation rate, which for *S. aureus* is about 2–5 SNPs per megabase per year (Young et al. 2012; Golubchik et al. 2013), it is theoretically possible to link related isolates to a recent transmission event in a healthcare setting that would indicate a lapse in infection control practice and an opportunity to target training and other interventions (Harris et al. 2013). Sequences of both epidemiologically linked isolates and those with no prior suspected linkage can be compared, allowing both exclusion of an epidemiologically suspected transmission event and inclusion of other cases in a potential chain of transmission that were epidemiologically unsuspected (Harris et al. 2013). However, individuals do not just have one core genome sequence type, but more commonly carry multiple related isolates that can vary up to 20 or even more SNPs. Indeed, in one study a long-stay patient admitted with MRSA to an ICU in Thailand had 99 ST239 MRSA isolates sequenced over a 64-day stay on ICU which revealed 147 SNP differences between sequenced isolates (Tong et al. 2015). There is also evidence that SNP accumulation can occur faster in invasive disease (Young et al. 2012). These observations complicate the linkage of cases based solely on SNP analysis. Nevertheless, the potential of WGS to identify transmission events and therefore target education and infection control interventions in real time justifies the considerable efforts being made to translate this technology from the research setting into clinical practice. The application of WGS to *S. aureus* transmission research and then on to routine clinical practice is a fast-moving field and beyond the scope of further discussion here.

3.1 MRSA Transmission in the Hospital

MRSA was first identified in the UK in 1961, following which a number of distinct dominant clones emerged to spread worldwide. During the 1960s and 1970s, prevalence of methicillin resistance was often reported as being up to about 20 % of all *S. aureus* isolates, and there were many reports of outbreaks (Brumfitt and Hamilton-Miller 1989). There was a general consensus that eradicating MRSA once it had become endemic was almost impossible (Thompson et al. 1982), and some

proposed that attempting control caused more problems than it solved (Barrett et al. 1998). Nevertheless, there were encouraging reports of successful control of endemic MRSA; for example, Denmark had levels of 15 % between 1967 and 1971 that fell to 0.2 % in the 1980s in response to a national control programme (Rosdahl and Knudsen 1991). The Netherlands and Scandinavia implemented an effective national "search and destroy" policy before MRSA became endemic that has been associated with low rates of healthcare-associated (HA) MRSA to this day (Vandenbroucke-Grauls 1996; Wertheim et al. 2004a). During the 1980s and 1990s, prevalence increased further in many countries to between 30 and 50 %, often linked with emergence of a few highly successful geographically restricted HA-clones (e.g. ST5, ST8, ST22, ST36, ST239 and ST247). It is unclear whether this increase in prevalence was due to dominant clones becoming progressively better adapted to spread in the hospital environment (Holden et al. 2010, 2004), or a failure to implement and sustain effective infection control programmes during the first few decades.

3.2 Preventing MRSA Transmission

Colonized patients are the main reservoir of MRSA in hospitals with transmission predominantly occurring from colonized to non-colonized patients via healthcare worker hands that become transiently colonized during delivery of routine care (Thompson et al. 1982; Pittet et al. 2006). Patients are also thought to acquire MRSA from the environment that has become contaminated by shedding of MRSA by colonized patients (Bernard et al. 1999; Bhalla et al. 2004; Sexton et al. 2006; Otter et al. 2011) or from staff carriers, but these are generally considered minor routes in most settings.

Comprehensive guidelines are available providing evidence and recommendations for preventing transmission of multi-drug-resistant bacteria particularly MRSA (Coia et al. 2006; Yokoe et al. 2008). They comprise non-targeted interventions that have an effect on transmission of all pathogens and targeted interventions that are directed specifically against MRSA-colonized patients. Non-targeted interventions include universal hand hygiene, environmental cleaning and reduction in antimicrobial use. Targeted interventions comprise contact precautions with gloves and aprons whilst delivering care, isolation or cohorting of MRSA patients in a side-room, bay or ward with use of dedicated equipment and facilities (e.g. stethoscopes, commodes) and decolonization using surface acting or systemic agents to suppress MRSA. Decolonization is also been used as a non-targeted intervention in the ICU and can be effective against MRSA transmission (Huang et al. 2013). Targeted methods are dependent on identification of MRSA-colonized patients, either through identification in routine clinical specimens or from a risk factor based or universal screening programme.

Infection control interventions are generally implemented as part of a bundle and although there is debate about the relative importance of each intervention, their

heightened implementation at national and institutional over the past 10 years has been associated with a dramatic reduction in endemic levels of MRSA in many countries (Jarlier et al. 2010; Johnson et al. 2012). The national MRSA control programme in England was particularly effective and has led to a greater than 80 % reduction in MRSA in many hospitals. Interestingly, a WGS study performed in an ICU in Brighton in 2012 that had implemented hand hygiene campaign, barrier nursing and decolonization did not find evidence of significant transmission of MRSA or MSSA over a 6-month period (Price et al. 2014b). This contrasts with a study performed in a Thai ICU, where adherence to infection control interventions including hand hygiene was poor, and there was significant number of MRSA transmissions linked to a small number of long-stay patients with prolonged MRSA colonization (Tong et al. 2015).

There are a number of risks to sustainable control of MRSA both in organizations and in countries that have seen impressive recent reductions and those that have maintained low levels for a long period. New HA-MRSA strains may emerge to spread despite current infection control interventions. For example, some strains can acquire clinically significant resistance to antiseptics such as chlorhexidine (Batra et al. 2010), which have become a major component of infection control practice in many countries (Edgeworth 2011; Huang et al. 2013). Some HA-MRSA strains may be intrinsically more transmissible (Cooper et al. 2012), and outbreak still occurs in settings where endemic transmission has been controlled (Harris et al. 2013).

Alternatively, new MRSA strains may emerge from the community and become imported into hospitals. Livestock-associated MRSA (LA-MRSA) clones such as ST-398 have emerged in a number of countries, including Denmark and the Netherlands that hitherto had low rates of MRSA (Verkade and Kluytmans 2014). LA-MRSA have been imported into hospitals, although there is evidence these strains are less transmissible in hospitals than HA-MRSA strains (Hetem et al. 2013; Verkade and Kluytmans 2014). Of perhaps more concern is that successful human CA-MRSA clones known to spread well in the community are transported into hospitals to become endemic and a common cause of nosocomial infection (Seybold et al. 2006; Otter and French 2011). The recent success with control of HA-MRSA was dependent on the hospital being the main reservoir. If the community were to become the main reservoir, sustained control of nosocomial MRSA infections would be much more challenging (Tosas Auguet et al. 2016).

3.3 MRSA Transmission in the Community

Although HA-MRSA-colonized patients return to the community where infection control practice is minimal, there has been little evidence that such strains undergo

sustained transmission outside a healthcare facility (Tosas Auguet et al. 2016). Since the 1980s, MRSA outbreaks have been increasingly described in the community with individuals and groups that had no epidemiological exposure with hospitals (Fridkin et al. 2005; David and Daum 2010). These strains were also genotypically distinct from the known HA-MRSA strains in that area. They were frequently found to carry the panton valentine leucocydin (PVL) gene and were characterized by clusters of severe skin and soft tissues infection and sporadic severe necrotizing pneumonia with concomitant influenza infection in children and young adults that had a high mortality (Gillet et al. 2002). In the USA, a dominant CA-MRSA strain, USA300, has spread rapidly to become a leading cause of skin and soft tissue disease in the community (King et al. 2006) and as a cause of abscesses presenting to emergency departments across the USA (Moran et al. 2006). USA300 is the dominant clone in some other countries (Reyes et al. 2009; Deleo et al. 2010), but in most countries different clones have emerged often with no one clone dominating (e.g. ST80/81, SWP, ST22) (Deleo et al. 2010; Otter and French 2010; Chuang and Huang 2013). Outbreaks of skin and soft tissue infection have common risk factors of overcrowding, frequent skin abrasion or limited personal hygiene, such as with contact sports, in prisons, amongst intravenous drug users and indigenous communities (Campbell et al. 2004; Kazakova et al. 2005).

Studies have identified the home as the main setting for amplification of successful clones in a community, with links from there to schools, the work place, sports clubs and other places where there is frequent human contact (Davis et al. 2012; Knox et al. 2015). Therefore, although the literature is dominated by reports of outbreaks in community facilities (Campbell et al. 2004; Kazakova et al. 2005), it is proposed that most transmission actually takes place in the home (Macal et al. 2014; Knox et al. 2015). WGS analysis is being applied to CA-MRSA transmission studies in the community and households where environmental contamination is thought to play an important role in transmission and infections (Knox et al. 2012; Eells et al. 2014). Attempts have been made to apply infection control interventions to prevent transmission and infection in homes, but decolonization and household cleaning has had only limited success (Fritz et al. 2012; Miller et al. 2012).

It is unclear what underpins the dominance of clones such as USA300 or of successful international HA-MRSA clones such as ST239 and ST22. Recent evidence that MRSA clones can differ in their transmissibility implies there is a bacterial genetic basis for emergence of dominant clones (Cooper et al. 2012; Hetem et al. 2013). Use of WGS for surveillance and analysis of emerging endemic and outbreak clones (Holden et al. 2010; Harris et al. 2013; Miller et al. 2014) may help identify genetic markers of increased transmissibility that can help rapidly target interventions. Indeed, although recent control of endemic HA-MRSA was achieved largely without molecular diagnostics and WGS, such technologies may prove vital in the future for identifying new clones that have overcome current preventative strategies and help us keep ahead of this highly versatile and virulent pathogen.

4 Summary

This chapter has provided an overview of where *S. aureus* is carried on the human body, traditional and emerging molecular technologies for identification and genetic analysis of sampled isolates, and how that information is used to prevent and treat infection due to particularly MRSA but also MSSA strains. A particular priority focus of infection prevention and control teams on MRSA over the last 10 years drove development and introduction of rapid molecular techniques. However, in many countries MRSA prevalence has now fallen dramatically and other emerging nosocomial bacteria, particularly multi-drug resistant GNB, are gaining more attention. MRSA molecular diagnostics introduced at the height of the epidemic have often been de-commissioned, returning to slower but usually cheaper methods, such as chromogenic agar sometimes supported by culture automation platforms to reduce laboratory costs. Similarly, landmark WGS MRSA transmission studies that pointed towards their imminent introduction into routine service, now seems a more distant proposition in most settings. These developments illustrate the pragmatic nature of service laboratories that constantly adapt to changing clinical need and laboratory cost pressures. Indeed, looking ahead to when new potentially more virulent and transmissible MRSA or MSSA clones emerge, experience already gained with rapid molecular and WGS techniques will facilitate rapid re-deployment to play an important role in guiding infection control and treatment decisions.

References

Altun O, Almuhayawi M, Ullberg M, Ozenci V (2013) Clinical evaluation of the Filmarray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol 51:4130–4136

Barrett SP, Mummery RV, Chattopadhya B (1998) Trying to control MRSA causes more problems than it solves. J Hosp Infect 39:85–93

Batra R, Eziefula AC, Wyncoll D, Edgeworth J (2008) Throat and rectal swabs may have an important role in MRSA screening of critically ill patients. Intensive Care Med 34:1703–1706

Batra R, Cooper BS, Whiteley C et al (2010) Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant *Staphylococcus aureus* in an intensive care unit. Clin Infect Dis 50:210–217

Beal SG, Ciurca J, Smith G et al (2013) Evaluation of the nanosphere verigene gram-positive blood culture assay with the versaTREK blood culture system and assessment of possible impact on selected patients. J Clin Microbiol 51:3988–3992

Bernard L, Kereveur A, Durand D et al (1999) Bacterial contamination of hospital physicians' stethoscopes. Infect Control Hosp Epidemiol 20:626–628

Bhalla A, Pultz NJ, Gries DM et al (2004) Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. Infect Control Hosp Epidemiol 25:164–167

Binnicker M, Espy M, Irish C, Vetter E (2015) Direct detection of influenza A and B viruses in less than 20 minutes using a commercially available rapid PCR assay. J Clin Microbiol 53:2353–2354

Boyce JM, Havill NL, Otter JA, Adams NM (2007) Widespread environmental contamination associated with patients with diarrhoea and methicillin-resistant *Staphylococcus aureus* colonization of the gastrointestinal tract. Infect Control Hosp Epidemiol 28:1142–1147

Brown DF, Walpole E (2001) Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media. J Antimicrob Chemother 47:187–189

Brumfitt W, Hamilton-Miller J (1989) Methicillin-resistant *Staphylococcus aureus*. N Engl J Med 320:1188–1196

Campbell KM, Vaughn AF, Russell KL et al (2004) Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infections in an outbreak of disease among military trainees in San Diego, California, in 2002. J Clin Microbiol 42:4050–4053

Cercenado E, Marín M, Burillo A et al (2012) Rapid detection of *Staphylococcus aureus* in lower respiratory tract secretions from patients with suspected ventilator-associated pneumonia: Evaluation of the Cepheid Xpert MRSA/SA SSTI assay. J Clin Microbiol 50:4095–4097

Chapin KC, Musgnug MC (2004) Evaluation of penicillin binding protein 2a latex agglutination assay for identification of methicillin-resistant *Staphylococcus aureus* directly from blood cultures. J Clin Microbiol 42:1283–1284

Chuang YY, Huang YC (2013) Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. Lancet Infect Dis 13:698–708

Coia JE, Duckworth GJ, Edwards DI et al (2006) Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. J Hosp Infect 63 (Suppl 1):S1–S44

Cooper BS, Kypraios T, Batra R, et al (2012) Quantifying type-specific reproduction numbers for nosocomial pathogens: evidence for heightened transmission of an Asian sequence type 239 MRSA clone. PLoS Comput Biol 8:e1002454

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23:616–687

Davis MF, Iverson SA, Baron P et al (2012) Household transmission of meticillin-resistant *Staphylococcus aureus* and other staphylococci. Lancet Infect Dis 12:703–716

Deleo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375:1557–1568

Denys GA, Renzi PB, Koch KM, Wissel CM (2013) Three-way comparison of BBL CHROMagar MRSA II, MRSASelect, and spectra MRSA for detection of methicillin-resistant *Staphylococcus aureus* isolates in nasal surveillance cultures. J Clin Microbiol 51:202–205

Edgeworth JD (2011) Has decolonization played a central role in the decline in UK methicillin-resistant *Staphylococcus aureus* transmission? A focus on evidence from intensive care. J Antimicrob Chemother 66 Suppl 2:ii41–47

Edgeworth JD, Yadegarfar G, Pathak S et al (2007) An outbreak in an intensive care unit of a strain of methicillin-resistant *Staphylococcus aureus* sequence type 239 associated with an increased rate of vascular access device-related bacteremia. Clin Infect Dis 44:493–501

Eells S, David M, Taylor A et al (2014) Persistent environmental contamination with USA300 methicillin-resistant *Staphylococcus aureus* and other pathogenic strain types in households with *S. aureus* skin infections. Infect Control Hosp Epidemiol 35:1373–1382

Emonts M, Uitterlinden AG, Nouwen JL et al (2008) Host polymorphisms in interleukin 4, complement factor H, and C-reactive protein associated with nasal carriage of *Staphylococcus aureus* and occurrence of boils. J Infect Dis 197:1244–1253

Enright MC, Enright MC, Robinson DA et al (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A 99:7687–7692

Fridkin SK, Hageman JC, Morrison M et al (2005) Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 352:1436–1444

Fritz SA, Hogan PG, Hayek G et al (2012) Household versus individual approaches to eradication of community-associated *Staphylococcus aureus* in children: a randomized trial. Clin Infect Dis 54:743–751

Gillet Y, Issartel B, Vanhems P et al (2002) Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359:753–759

Goldenberg S, Edgeworth J (2015) The Enigma ML FluAB-RSV assay: a fully automated molecular test for the rapid detection of influenza A, B and respiratory syncytial viruses in respiratory specimens. Expert Rev Mol Diagn 15:23–32

Golubchik T, Batty EM, Miller RR et al (2013) Within-host evolution of *Staphylococcus aureus* during asymptomatic carriage. PLoS ONE 8:e61319

González C, Rubio M, Romero-Vivas J et al (1999) Bacteremic pneumonia due to *Staphylococcus aureus*: a comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms. Clin Infect Dis 29:1171–1177

Hamdan-Partida A, Sainz-Espuñes T, Bustos-Martínez J (2010) Characterization and persistence of *Staphylococcus aureus* strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. J Clin Microbiol 48:1701–1705

Harris SR, Feil EJ, Holden MTG et al (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327:469–474

Harris SR, Cartwright EJ, Torok ME et al (2013) Whole-Genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. Lancet Infect Dis 13:130–136

Hetem DJ, Bootsma MCJ, Troelstra A, Bonten MJM (2013) Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 19:1797–1802

Holden MTG, Feil EJ, Lindsay JA et al (2004) Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci U S A 101:9786–9791

Holden MTG, Lindsay JA, Corton C et al (2010) Genome sequence of a recently emerged, highly transmissible, multi-antibiotic- and antiseptic-resistant variant of methicillin-resistant *Staphylococcus aureus*, sequence type 239 (TW). J Bacteriol 192:888–892

Huang SS, Septimus E, Kleinman K et al (2013) Targeted versus universal decolonization to prevent ICU infection. N Engl J Med 368:2255–2265

Jamal W, Al Roomi E, AbdulAziz LR, Rotimi VO (2014) Evaluation of curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. J Clin Microbiol 52:2487–2492

Jarlier V, Trystram D, Brun-Buisson C et al (2010) Curbing methicillin-resistant *Staphylococcus aureus* in 38 French hospitals through a 15-year institutional control program. Arch Intern Med 170:552–559

Jeyaratnam D, Whitty CJM, Phillips K et al (2008) Impact of rapid screening tests on acquisition of meticillin resistant *Staphylococcus aureus*: cluster randomised crossover trial. BMJ 336:927–930

Johnson AP, Davies J, Guy R et al (2012) Mandatory surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) bacteremia in England: the first 10 years. J Antimicrob Chemother 67:802–809

Kazakova SV, Hageman JC, Matava M et al (2005) A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N Engl J Med 352:468–475

Khatib R, Riederer K, Saeed S et al (2005) Time to positivity in *Staphylococcus aureus* bacteremia: possible correlation with the source and outcome of infection. Clin Infect Dis 41:594–598

King MD, Humphrey BJ, Wang YF et al (2006) Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med 144:309–317

Kluytmans J, Van Belkum A, Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10:505–520

Knox J, Uhlemann AC, Miller M et al (2012) Environmental contamination as a risk factor for intra-household *Staphylococcus aureus* transmission. PLoS ONE 7:e49900

Knox J, Uhlemann A, Lowy F (2015) *Staphylococcus aureus* infections: transmission within households and the community. Trends Microbiol 23:437–444

Lebon A, Labout JAM, Verbrugh HA et al (2008) Dynamics and determinants of *Staphylococcus aureus* carriage in infancy: the generation R study. J Clin Microbiol 46:3517–3521

Lee S, Park YJ, Park KG et al (2013) Comparative evaluation of three chromogenic media combined with broth enrichment and the real-time PCR-based Xpert MRSA assay for screening of methicillin-resistant *staphylococcus aureus* in nasal swabs. Ann Lab Med 33:255–260

Leone M, Malavieille F, Papazian L et al (2013) Routine use of *Staphylococcus aureus* rapid diagnostic test in patients with suspected ventilator-associated pneumonia. Crit Care 17:R170

Lestari ES, Severin JA, Filius PMG et al (2008) Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. Eur J Clin Microbiol Infect Dis 27:45–51

Lim SH, Mix S, Xu Z et al (2014) Colorimetric sensor array allows fast detection and simultaneous identification of sepsis-causing bacteria in spiked blood culture. J Clin Microbiol 52:592–598

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532

Macal CM, North MJ, Collier N et al (2014) Modeling the transmission of community-associated methicillin-resistant *Staphylococcus aureus*: a dynamic agent-based simulation. J Transl Med 12:124

Mestas J, Felsenstein S, Dien Bard J (2014) Direct identification of bacteria from positive BacT/ALERT blood culture bottles using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry. Diagn Microbiol Infect Dis 80:193–196

Metso L, Maki M, Tissari P et al (2014) Efficacy of a novel PCR- and microarray-based method in diagnosis of a prosthetic joint infection. Acta Orthop 85:165–170

Meurman O, Routamaa M, Poltonen R (2005) Screening for methicillin resistant *Staphylococcus aureus*: which anatomical sites to culture? J Hops Infect 61:351–353

Miller LG, Tan J, Eells SJ et al (2012) Prospective investigation of nasal mupirocin, hexachlorophene body wash, and systemic antibiotics for prevention of recurrent community-associated methicillin-resistant *Staphylococcus aureus* infections. Antimicrob Agents Chemother 56:1084–1086

Miller RM, Price JR, Batty EM et al (2014) Healthcare-associated outbreak of meticillin-resistant *Staphylococcus aureus* bacteraemia: Role of a cryptic variant of an epidemic clone. J Hosp Infect 86:83–89

Moran GJ, Krishnadasan A, Gorwitz RJ et al (2006) Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 355:666–674

Muthukrishnan G, Lamers RP, Ellis A et al (2013) Longitudinal genetic analyses of *Staphylococcus aureus* nasal carriage dynamics in a diverse population. BMC Infect Dis 13:221

Nahimana I, Francioli P, Blanc DS (2006) Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12:1168–1174

Nouwen J, Boelens H, Van Belkum A, Verbrugh H (2004) Human factor in *Staphylococcus aureus* nasal carriage. Infect Immun 72:6685–6688

Opota O, Croxatto A, Prod'hom G, Greub G (2015) Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect 21:313–322

Otter JA, French GL (2010) Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. Lancet Infect Dis 10:227–239

Otter JA, French GL (2011) Community-associated meticillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated infection. J Hosp Infect 79:189–193

Otter JA, Yezli S, French GL (2011) The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol 32:687–699

Parcell BJ, Phillips G (2014) Use of Xpert® MRSA PCR point-of-care testing beyond the laboratory. J Hosp Infect 87:119–121

Peterson LR, Liesenfeld O, Woods CW et al (2010) Multicenter evaluation of the lightcycler methicillin-resistant *Staphylococcus aureus* (MRSA) advanced test as a rapid method for detection of MRSA in nasal surveillance swabs. J Clin Microbiol 48:1661–1666

Pittet D, Allegrani B, Sax H et al (2006) Evidence-based model for hand transmission during patient care and the role of improved practices. Lancet Infect Dis 6:641–652

Price J, Gordon NC, Crook D et al (2013) The usefulness of whole genome sequencing in the management of *Staphylococcus aureus* infections. Clin Microbiol Infect 19:784–789

Price C, Kon S, Metzger S (2014) Rapid antibiotic susceptibility phenotypic characterization of *Staphylococcus aureus* using automated microscopy of small numbers of cells. J Microbiol Methods 50–58

Price JR, Golubchik T, Cole K et al (2014b) Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of *Staphylococcus aureus* in an intensive care unit. Clin Infect Dis 58:609–618

Ratnayake L, Olver W (2011) Rapid PCR detection of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *S. aureus* samples from charcoal-containing blood culture bottles. J Clin Microbiol 49:2382

Reyes J, Rincón S, Díaz L et al (2009) Dissemination of methicillin-resistant *Staphylococcus aureus* USA300 sequence type 8 lineage in Latin America. Clin Infect Dis 49:1861–1867

Rosdahl VT, Knudsen AM (1991) The decline of methicillin resistance among Danish *Staphylococcus aureus* strains. Infect Control Hosp Epidemiol 12:83–88

Rossney AS, Herra CM, Brennan GI et al (2008) Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. J Clin Microbiol 46:3285–3290

Ruimy R, Angebault C, Djossou F et al (2010) Are host genetics the predominant determinant of persistent nasal *Staphylococcus aureus* carriage in humans? J Infect Dis 202:924–934

Sexton T, Clarke P, O'Neill E et al (2006) Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. J Hosp Infect 62:187–194

Seybold U, Kourbatova EV, Johnson JG et al (2006) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis 42:647–656

Skov R, Smyth R, Larsen AR et al (2006) Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and etest on Mueller-Hinton agar. J Clin Microbiol 44:4395–4399

Soriano A, Martínez JA, Mensa J et al (2000) Pathogenic significance of methicillin resistance for patients with *Staphylococcus aureus* bacteremia. Clin Infect Dis 30:368–373

Thompson RL, Cabezudo I, Wenzel RP (1982) Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann Intern Med 97:309–317

Tosas Auguet O, Betley JR, Stabler RA et al (2016) Evidence for community transmission of community-associated but not health-care-associated methicillin-resistant *Staphylococcus aureus* strains linked to social and material deprivation: spatial analysis of cross sectional data 13:e1001944

Tong S, Holden M, Nickerson E et al (2015) Genome sequencing defines phylogeny and spread of methicillin-resistant *Staphylococcus aureus* in a high transmission setting. Genome Res 25:111–125

Van Belkum A, Melles DC, Nouwen J et al (2009) Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect Genet Evol 9:32–47

Van den Akker ELT, Nouwen JL, Melles DC et al (2006) *Staphylococcus aureus* nasal carriage is associated with glucocorticoid receptor gene polymorphisms. J Infect Dis 194:814–818

Van den Kieboom C, Ferwerda G, de Baere I, et al (2015) Assessment of a molecular diagnostic platform for integrated isolation and quantification of mRNA in whole blood. Eur J Clin Microbiol Infect Dis [Epub ahead of print]

Van Der Zee A, Hendriks WDH, Roorda L et al (2013) Review of a major epidemic of methicillin-resistant *Staphylococcus aureus*: the costs of screening and consequences of outbreak management. Am J Infect Control 41:204–209

VandenBergh MFQ, Yzerman EPF, Van Belkum A et al (1999) Follow-up of *Staphylococcus aureus* nasal carriage after 8 years: redefining the persistent carrier state. J Clin Microbiol 37:3133–3140

Vandenbroucke-Grauls CM (1996) Methicillin-resistant *Staphylococcus aureus* control in hospitals: the Dutch experience. Infect Control Hosp Epidemiol 17:512–513

Vandenesch F, Bes M, Lebeau C et al (1993) Coagulase-negative *Staphylococcus aureus*. Lancet 342:995–996

Verkade E, Kluytmans J (2014) Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. Infect Genet Evol 21:523–530

Von Eiff C, Becker K, Machka K et al (2001) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med 344:11–16

Wassenberg MWM, Kluytmans JAJW, Bosboom RW et al (2011) Rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* carriage at different anatomical sites: costs and benefits of less extensive screening regimens. Clin Microbiol Infect 17:1704–1710

Wertheim HFL, Vos MC, Boelens HAM et al (2004a) Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. J Hosp Infect 56:321–325

Wertheim HFL, Vos MC, Ott A et al (2004b) Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364:703–705

Wertheim HFL, Melles DC, Vos MC et al (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762

Wertheim HFL, Walsh E, Choudhurry R et al (2008) Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. PLoS Med 5:0104–0112

Widen R, Healer V, Silbert S (2014) Laboratory evaluation of the BD MAX MRSA assay. J Clin Microbiol 52:2686–2688

Wieser A, Schneider L, Jung J, Schubert S (2012) MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl Microbiol Biotechnol 93:965–974

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

Wolk D, Struelens M, Pancholi P et al (2009) Rapid detection of *Staphylococcus aureus* and methicillin-resistant S. aureus (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. J Clin Microbiol 47:823–826

Yokoe DS, Mermel LA, Anderson DJ et al (2008) A compendium of strategies to prevent healthcare-associated infections in acute care hospitals. Infect Control Hosp Epidemiol 29 (Suppl 1):S12–S21

Young B, Golubchic T, Batty E et al (2012) Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. Proc Natl Acad Sci U S A 109:4550–4555

Worldwide Epidemiology and Antibiotic Resistance of *Staphylococcus aureus*

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Abstract *Staphylococcus aureus* is an important human pathogen, responsible for infections in the community and the healthcare setting. Although much of the attention is focused on the methicillin-resistant "variant" MRSA, the methicillin-susceptible counterpart (MSSA) remains a prime species in infections. The epidemiology of *S. aureus*, especially of MRSA, showed a rapid evolution in the last years. After representing a typical nosocomial multidrug-resistant pathogen, MRSA has recently emerged in the community and among farmed animals thanks to its ability to evolve and adapt to different settings. Global surveillance has shown that MRSA represents a problem in all continents and countries where studies have been carried out, determining an increase in mortality and the need to use last-resource expensive antibiotics. *S. aureus* can easily acquire resistance to antibiotics and MRSA is characteristically multidrug resistant. Resistance to vancomycin, the principal anti-MRSA antibiotic is rare, although isolates with decreased susceptibility are recovered in many areas. Resistance to the more recently introduced antibiotics, linezolid and daptomycin, has emerged; however, they remain substantially active against the large majority of MSSA and MRSA. Newer antistaphylococcal drugs have been developed, but since their clinical use has been very limited so far, little is known about the emergence of resistance. Molecular typing techniques have allowed to identify the major successful clones and lineages of MSSA and MRSA, including high-risk clones, and to trace their diffusion. In the face of a continuously evolving scenario, this review depicts the most common clones circulating in different geographical areas and in different settings at present. Since the evolution of *S. aureus* will continue, it is important to maintain the attention on the epidemiology of *S. aureus* in the future with a global view.

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Abbreviations

ACME	Arginine catabolic mobile element
CA-MRSA	Community-associated MRSA
CC	Clonal complex
ccr	Cassette chromosome recombinase
CLSI	Clinical and Laboratory Standard Institute
ECDC	European Centre for Disease Prevention and Control
EARS-Net	European Antibiotic Resistance Surveillance Network
EUCAST	European Commission for Antimicrobial Susceptibility Testing
GISA	Glycopeptide-intermediate *Staphylococcus aureus*
GRSA	Glycopeptide-resistant *Staphylococcus aureus*
HA-MRSA	Healthcare-associated MRSA
HGT	Horizontal gene transfer
h-VISA	Heterogeneous vancomycin-intermediate *Staphylococcus aureus*
LA-MRSA	Livestock-associated MRSA
mec	Methicillin-resistant gene
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
PBP	Penicillin-binding protein
PFGE	Pulsed-field gel electrophoresis
PVL	Panton–Valentine leukocidin
SCC*mec*	Staphylococcal chromosome cassette *mec*
spa	Staphylococcal protein A
ST	Sequence type
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRE	Vancomycin-resistant enterococci
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WHO	World Health Organization
WGS	Whole genome sequencing

Contents

1 Introduction

Staphylococcus aureus is a common colonizer of the skin and mucosa surfaces of humans and approximately 30 % of the individuals carry *S. aureus* in the anterior nares (Wertheim et al. 2005). Since the beginning of the microbiological era, *S. aureus* has been recognized as an important pathogen responsible for infections in the healthcare setting and in the community. *S. aureus* infections are initiated by the entrance of the microorganism through a breach of the skin or mucosa and can involve local structures or spread to distant organs to generate life-threatening invasive infections such as bacteremia, pneumonia, and osteomyelitis.

The success of *Staphylococcus aureus* as both a colonizer and a pathogen is largely due to its ability to adapt to different environments thanks to the acquisition of new DNA by horizontal gene transfer (HGT) and to spread clonally. Through HGT *S. aureus* can use an ample and flexible repertoire of colonization determinants, immune evasion factors, and toxins (enterotoxins, exfoliative toxins, leukocidins, etc.) (Lindsay 2014) and can evolve rapidly in response to the greatest challenge to the microbial world in the last 70 years: the introduction of antibiotics. Although *S. aureus* is a species naturally susceptible to antibiotics, over the years it has become resistant to virtually every antibiotic that has entered clinical use. In the span of 10 years after penicillin was available at the middle of the last century, a large proportion of nosocomial *S. aureus* strains became resistant to penicillin by acquisition of a plasmid carrying the penicillinase gene (*penZ*, now *blaZ*) complex (Novick and Bouanchaud 1971; Pantosti et al. 2007) and two decades later 80 % of *S. aureus* isolates were resistant to penicillin (Chambers 2001).

The penicillinase-resistant antistaphylococcal penicillins, whose prototype is methicillin, appeared an adequate response to penicillin-resistant *S. aureus*. However, resistance emerged soon and in 1960 the first MRSA was identified in a London hospital (Jevons 1961). Methicillin resistance is due to the acquisition of a new gene, *mecA,* that codes for a novel penicillin-binding protein (PBP), designated PBP2a that makes the strain resistant to all beta-lactam antibiotics, including antistaphylococcal penicillins, cephalosporins, and carbapenems (Pantosti et al. 2007). The *mecA* gene is contained in a mobile genetic element designated Staphylococcal chromosome cassette (SCC) *mec* that is chromosomally integrated (Katayama et al. 2000).

Acquisition of *mecA* initiated the successful spread of methicillin-resistant *S. aureus* (MRSA), one of the most important multidrug-resistant (MDR) nosocomial pathogens.

2 General Epidemiology of *S. aureus*

The recent epidemiology of *S. aureus* is especially focused on the increase and spread of MRSA in healthcare setting and the community. However, in the past century methicillin-susceptible *S. aureus* (MSSA) was a prominent cause of outbreaks and global spread in healthcare settings and today remains one of the principal pathogens in hospital infection. An example from the past is represented by the MSSA strain called phage type 80/81 that was rampant at the middle of the last century in hospitals causing infections and death in newborn units, in patients and hospital staff in the UK, USA, and Canada (Uhlemann et al. 2014), becoming the first pandemic *S. aureus* clone to be identified (Chambers and Deleo 2009). This strain was resistant to penicillin, highly transmissible, and hypervirulent. Interestingly, it contained the genes for the Panton–Valentine leukocidin (PVL), a leukotoxin that later became a marker for community-associated (CA)-MRSA.

Although this clone disappeared in hospitals with the introduction of methicillin (Chambers and Deleo 2009), the success of *S. aureus* continued into the last decades of the nineteenth century, when *S. aureus* prevalence in healthcare-associated infections, especially bacteremia, increased. This event was ascribed to the increase in the number of immunocompromised individuals, in the use of intravascular devices and, finally, in the multidrug resistance of a portion of the isolates that were MRSA (Lowy 1998). MRSA increased importantly in the mid-1970s in Europe, in the next decade in the USA and later at a global level (Chambers and Deleo 2009). Indeed, MRSA did not replace MSSA infections but actually added to them (Johnson et al. 2005).

At present, *S. aureus* maintains a leading role as a nosocomial pathogen in different countries. In the USA, *S. aureus* was number one among the pathogens isolated from infections according to the National Healthcare Safety Network that collected data from approximately 2.000 hospitals. In particular, *S. aureus* was the most prevalent pathogen in ventilator-associated pneumonia and in surgical site infections. A variable portion of the isolates, from 43 to 58 % according to the type of infections or the hospital ward, was MRSA (Sievert et al. 2013).

In Europe, a recent point-prevalence survey, carried out in acute care hospitals of 33 countries and coordinated by the European Center for Disease Control and Prevention (ECDC), revealed that *S. aureus* is the second most commonly isolated microorganism after *E. coli*, and it remains the first cause of surgical site infections, while MRSA proportion greatly varies according to the country (ECDC 2013). Data collected by the European Antibiotic Resistance Surveillance Network (EARS-Net) has clearly shown important differences among countries in the proportions of MRSA from bacteremia, showing a distinct North–South trend. In 2013, in the face of a European population-weighted mean percentage of 18 %, Iceland, the Scandinavian countries, and the Netherlands reported an MRSA proportion below 2 %, while some East European and South European countries reported a proportion from 32 to 64 % (ECDC 2014). Interestingly, EARS-Net documented a downward trend for MRSA in France, UK, Germany, and Ireland likely due to the

implementation of strategies to control the spread and transmission of MRSA in the healthcare settings (Pearson et al. 2009; Jarlier et al. 2010).

A worldwide picture of MRSA spread is shown in the global report on antimicrobial resistance surveillance, issued by the World Health Organization in 2014 (WHO 2014). Although comprehensive antibiotic resistance data were available only for Europe, America, and Australia, MRSA was reported in all the continents. Most countries reported a proportion of MRSA exceeding 20 % and, occasionally, up to 80 %. This implies that second-line (or "reserve") antibiotics are required for the treatment or the prophylaxis of *S. aureus* infections in most countries worldwide. Noteworthy, MRSA infections are associated with an increase in mortality and in length of hospital stay, leading to a high economic burden with respect to MSSA infections (WHO 2014).

3 Molecular Epidemiology

3.1 Molecular Typing Methods

The ability of *S. aureus* to cause a wide range of infections, to spread in both hospital and the community and to cause outbreaks, has required the development of tools able to distinguish isolates and to outline *S. aureus* epidemiology. Phenotypic methods, including phage typing (Blair and Williams 1961), have been commonly used since the 1960s but in the last decades they have been replaced by molecular typing methods (Deurenberg and Stobberingh 2008). Today, sequence-based methods are the most used to monitor the spread and circulation of the diverse *S. aureus* lineages and to study evolutionary events (Nubel et al. 2011).

A description of the main molecular methods currently used to characterize *S. aureus* is given below.

3.1.1 Pulsed-Field Gel Electrophoresis (PFGE)

Before the introduction of the sequence-based methods, PFGE was considered the gold standard for typing many bacterial species, including *S. aureus*. PFGE is a fingerprinting method based on macrorestriction of genomic DNA by using rare-cutting restriction enzymes, such as SmaI for *S. aureus* (Bannerman et al. 1995). The resulting banding patterns can be resolved in an electric field applying an alternative voltage gradient and analyzed by visual inspection (Tenover et al. 1995) or by using specialized software (Reed et al. 2007). PFGE is a useful tool to study the local epidemiology, such as in the occurrence of an outbreak (Tenover et al. 1995) showing a higher discriminatory power than other typing methods. In USA, the major MRSA clones are defined based on the national PFGE database (e.g., USA100 and USA 300) (McDougal et al. 2003). PFGE limitations include

cost, a rather labor-intensive procedure and the need for technical expertise (Deurenberg and Stobberingh 2008). In addition, protocols and nomenclature are scarcely harmonized (Stefani et al. 2012).

3.1.2 Multilocus Sequence Typing (MLST)

MLST represents the most widely used method to classify *S. aureus* isolates into clones. The method is based on sequencing the internal fragments of 7 housekeeping genes; sequences are then analyzed with the help of the software and the database at the MLST Web site (http://saureus.mlst.net) to obtain an allele number for each gene. The succession of the alleles of the seven genes originates an allelic profile defined sequence type (ST) (Enright et al. 2002). Using the algorithm eBURST (www.eburst.mlst.net), related STs can be grouped into clusters designated clonal complexes (CC)s. Advantages of MLST are its reproducibility, portability, and its universal nomenclature, so that ST data can be easily compared (Deurenberg and Stobberingh 2008). MLST can also provide basic insights of the *S. aureus* population structure in terms of clonal relatedness (Nubel et al. 2011).

3.1.3 Staphylococcal Protein A (*spa*) Typing

This technique is based on the sequence of a single gene, the staphylococcal protein A gene, and in particular of the highly polymorphic X-region which contains different short tandem repeats whose combination originates different *spa* types (Harmsen et al. 2003). The method is supported by a central *spa* server (http://www.seqnet.org/) that at the moment hosts more than 15,000 *spa* types. The discriminative power of *spa* typing is lower than that of PFGE but higher than that of MLST with which it is mostly concordant in terms of CC definition (Cookson et al. 2007; Strommenger et al. 2008). However, the high mutation rate of the *spa* locus may lead to an evolutionary convergence (homoplasy) and, in turn, to problems with the distinction of clones (Nubel et al. 2011). Nevertheless, *spa* typing represents a rapid and easy tool to investigate the epidemiology of *S. aureus* infections, especially at the local level.

3.1.4 SCC*mec* Typing

This method is based on the identification of the structurally different SCC*mec* elements; thus, it can be used to classify MRSA only. SCC*mec* typing is performed by targeting its key elements, the *mec* complex class and the cassette chromosome recombinase (*ccr*) complex type (Kondo et al. 2007). The Web site of the International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) (http://www.sccmec.org/Pages/SCC_HomeEN.html; accessed on June 26, 2015) currently reports 11 SCC*mec* types differing in size from 20 to

60 kb. The larger SCC*mec* elements (types I–III), which are characteristic of the "classical" nosocomial lineages, can also contain transposons and integrated plasmids that carry resistance to other antibiotics and heavy-metal resistance operons (Pantosti et al. 2007). SCC*mec* type IV encodes methicillin resistance only and being smaller than other SCC*mec* is probably more easily transferable (Ma et al. 2002). It can be further distinguished into 8 subtypes (named from a to h) on the basis of differences in the J1 (accessory or junkyard) region (de Lencastre et al. 2007; Milheirico et al. 2007). SCC*mec* types IV and V are typically found in community-associated MRSA (CA-MRSA) and in livestock-associated (LA)-MRSA. The type of SCC*mec* can be useful to trace the evolutionary origin of MRSA clones and, therefore, it is often part of the designation of a specific clone.

3.1.5 Whole Genome Sequencing (WGS)

Whole genome sequencing (WGS) has the potential to become a primary typing technique in microbiology laboratories, replacing all the other typing methods (Price et al. 2013) also due to the decreased costs of equipment and materials. It offers the best possible resolution for measuring inter-strain similarity and for phylogenetic analysis and can produce information on antigenic array, virulence and antibiotic resistance, predicting phenotypes of interest (Sabat et al. 2013). Several platforms for next-generation sequencing (NGS) are now available (Price et al. 2013; Metzker 2010). Sequencing results consist of thousands of reads corresponding to genomic DNA fragments, generally smaller than 400 base pair (Price et al. 2013). In order to rebuild the genome sequence, the reads have to be assembled. Two methods can be used: A method called *mapping-based assembly* consisting in the comparison of the sequences with those of a reference strain or by de novo *assembly* where the reads are assembled in larger regions named contigs that need to be further assembled (Schatz et al. 2010) but that often do not generate a complete coverage (Nielsen et al. 2011). The typing strategies that can be obtained by WGS are based on allelic variations of genes that are part of the core genome (extended MLST or cgMLST) (Maiden et al. 2013) or on the analysis of the single-nucleotide mutations (SNPs) in the genome as compared to a reference sequence. These types of analyses might be the most readily implementable for typing, although other types (e.g., K-mer) have been proposed (Maiden et al. 2013; Koser et al. 2012a).

The major open problems with WGS rely on the reproducibility of the results obtained with different platforms, the availability of rapid and easy bioinformatics tools, the harmonization of bioinformatic analyses, and the development of a common nomenclature and an open-access database (Sabat et al. 2013). Regarding *S. aureus*, WGS can show differences among strains that are indistinguishable by the PFGE, the most discriminative method used so far (Salipante et al. 2015). WGS has been successfully used to investigate hospital outbreaks, such as MRSA outbreaks in neonatal intensive care units where SNP analysis allowed to clearly discriminate outbreak from non-outbreak strains (Koser et al. 2012b; Harris et al. 2013).

3.2 *Worldwide Distribution of the Principal Clones and Lineages*

The development and extensive use of molecular typing techniques has allowed the identification of different MSSA and MRSA clones and their worldwide distribution. The vast majority of *S. aureus* isolates collected during 1960–2004 have been found to belong to 11 CCs, the most abundant being CC30 (Chambers and Deleo 2009). With respect to MSSA, the MRSA lineages are less numerous since introduction of SCC*mec* must occur into MSSA lineages that are "permissive" for this element, that has to be acquired and maintained (Enright et al. 2002; Robinson and Enright 2003). This event has occurred a limited number of times, although according to recent findings MRSA emergence is probably more common than previously thought (Nubel et al. 2008).

Most of the recent molecular epidemiology data concerning *S. aureus* are focused on MRSA, while the molecular epidemiology of MSSA is quite scarce. Therefore, the following paragraphs deal with the distribution of MRSA clones in different settings and geographical areas and only a short part is dedicated to MSSA.

Recent studies have demonstrated that clones are in continuous evolution: old clones wane and sometimes re-emerge (Chambers and Deleo 2009); exchange and spreading of clones and lineages between different settings and countries are occurring at a rapid rate due to globalization. Therefore, the following description must be intended as an epidemiological snapshot that is due to change with time.

3.2.1 Healthcare-Associated MRSA

For a couple of decades after the emergence of MRSA, these strains were confined to the healthcare setting in Europe and later also in the USA (Chambers and Deleo 2009). The majority of MRSA infections were caused by *S. aureus* phage type 83A (now classified as ST250, CC8) designated as the "archaic clone," to which also the very first MRSA isolate belonged.

The archaic clone gradually disappeared in the 1980s to be replaced by new pandemic clones (Enright et al. 2002; Chambers and Deleo 2009). One successful lineage was ST239-SCC*mec* III also designated the Brazilian/Hungarian clone. ST239 is a hybrid clone originating by the introduction of a large chromosomal fragment from ST30 (CC30) into the CC8 background (Deurenberg and Stobberingh 2008; Smyth et al. 2010). ST239 became prevalent in UK, Australia, and USA between the 1970s and the early 1980s, in Europe and South America in the following decade (1980–1990) and subsequently in Asia and Middle East (1990–2000).

The original nomenclature of the HA-MRSA clones included the geographical area where the clones were first recovered or more widespread (e.g., the New York clone, the Brazilian clone) (Murchan et al. 2003) and their classification was based on phage typing and other phenotypic traits (Kerr et al. 1990) and later on PFGE (Oliveira et al. 2002).

Although for the major clones the original nomenclature or the local designation are often maintained (e.g., EMRSA-15 for ST22 in UK or USA100 for ST5 in USA) (Chambers and Deleo 2009), today, the most accepted nomenclature of the circulating clones is based on the ST-SCC*mec* type and additionally the corresponding CC. Indeed, a single CC can include MRSA clones with different geographical distribution that can be associated with different SCC*mec* elements and possibly other characteristics such as antibiotic resistance determinants or virulence factors (Monecke et al. 2011; Nubel et al. 2011). CC5, for example, encompasses clones belonging to ST5-SCC*mec* II (USA100), which is the most common HA-MRSA in USA (Tenover and Goering 2009) as well as ST5-SCC*mec* IV (USA800), also known as the "Pediatric clone" (Monecke et al. 2011).

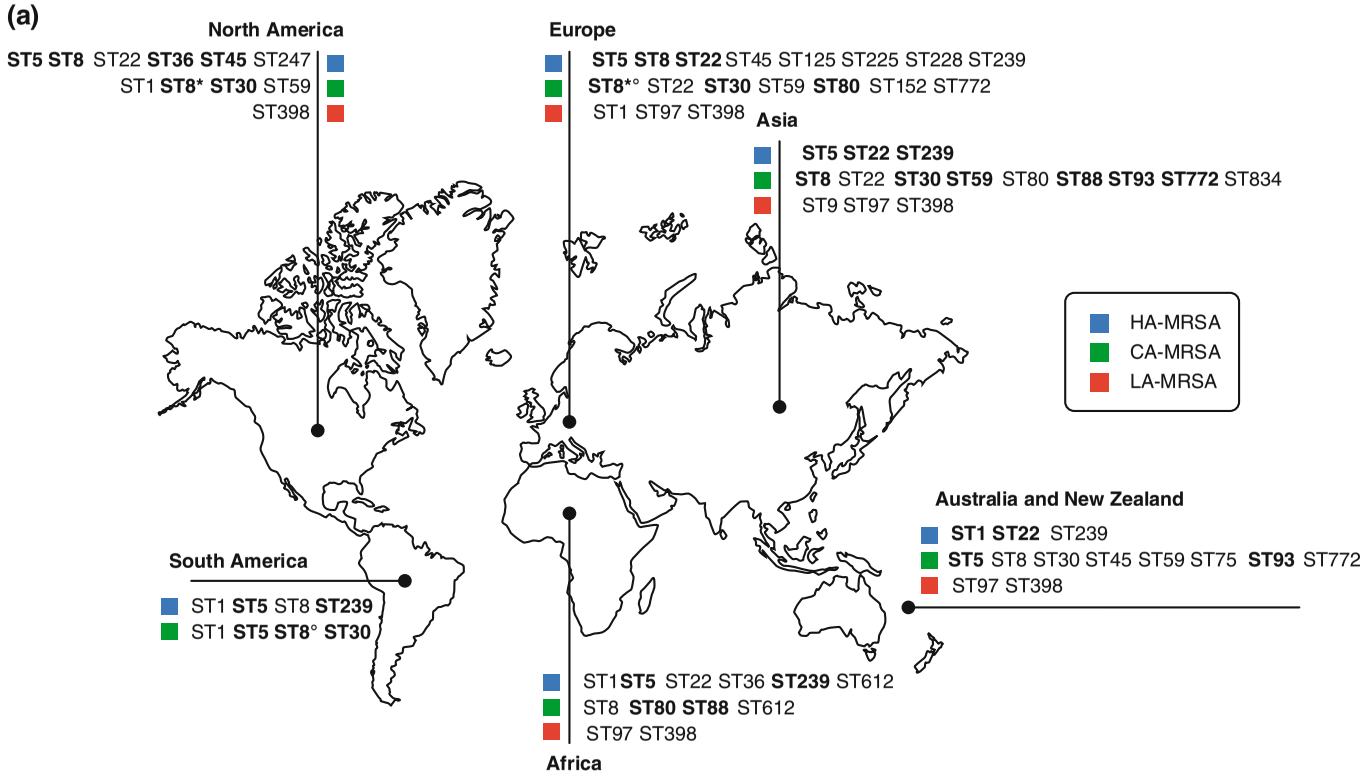
The geographical distribution of the most common HA-MRSA clones is shown in Fig. 1.

In North America, ST5-SCC*mec* II (USA100), ST8-SCC*mec* IVh (USA500), ST36-SCC*mec* IV (USA200), and ST45-SCC*mec* IV are the most common HA-MRSA clones. Other clones, such as ST22-SCC*mec* IV and ST247-SCC*mec* I, are also present, although at lower frequency (Chatterjee and Otto 2013; Stefani et al. 2012; Nichol et al. 2013).

In South America, MRSA belonging to ST5 and ST239 are the most frequent HA-MRSA isolates. In particular, in Brazil the predominant nosocomial lineages are ST239-SCC*mec* III (the Brazilian clone), ST5, and ST1 (Silva-Carvalho et al. 2009; Caboclo et al. 2013), while in Argentina the majority of HA-MRSA isolates are related to ST5-SCC*mec* I, which is also locally called the Cordobes–Chilean clone (Becker et al. 2012; Egea et al. 2014). The latter clone is also present in Colombia together with ST8-SCC*mec* IVc representing the most prevalent lineages; the Chilean clone has recently displaced the Pediatric clone that was disseminated in Colombia at the end of 1990s (Jimenez et al. 2012).

In Europe, ST5 (CC5), ST8 (CC8), and ST22 (CC22) are predominant in most countries. In addition, specific clones display a preferential geographical distribution at country level; for instance, the northern Balcan-Adriatic clone, ST228-SCC*mec* I, is typically detected in Italy, Germany, Austria, Croatia, Hungary and Slovenia (Grundmann et al. 2010a; Monaco et al. 2010); ST125-SCC*mec* IV has been identified in Spain with *spa* type t067 (Perez-Vazquez et al. 2009); the Berlin epidemic clone, ST45-SCC*mec* IV is common in Germany and Belgium, but it also circulates in the Netherlands, Switzerland, and Croatia; in France, ST8-SCC*mec* IV, named "Lyon clone," is the most abundant HA-MRSA followed by ST5-SCC*mec* I, which is also known as the "Geraldine clone" (Dauwalder et al. 2008). In the recent years, ST22-SCC*mec* IV (EMRSA-15), the most common clone in the UK since the 1990s, has spread into several countries including Germany, Hungary, Portugal, and Italy becoming the major European HA-MRSA clone (Grundmann et al. 2014; Holden et al. 2013). The rapid evolution of the HA-MRSA clones and the expansion of ST22-SCC*mec* IV in Europe have been documented by two surveys involving isolates from surveys involving isolates from bacteremia from 25 European countries (Grundmann et al. 2010a, 2014) (Fig. 2).

In Africa, genotyping data of HA-MRSA isolates are still limited; nevertheless, recent studies suggest the predominance of the clones ST5 carrying different



* USA300
° USA300-LV
The most common clones are indicated in bold

Fig. 1 (continued)

(b)

MRSA	CC	MLST	SCC*mec*	Geographic distribution	References
HA	CC1	ST1	IV, V	South America, Africa, Australia and New Zealand	Monecke et al. 2011; Silva-Carvalho et al. 2009; Caboclo et al. 2012; Ben JomaaJemili et al. 2013; Raji et al. 2013; Ritchie et al. 2014; Williamson et al. 2014
	CC5	ST5	I, II, IV	North America, South America, Europe, Africa, Asia	Nichol et al. 2013; Egea et al. 2014; Chatterjee et al. 2013; Stefani et al. 2012; Abdulgader et al. 2015; Schaumburg et al. 2014; Chen et al. 2014
		ST228	I	Europe	Grundmann et al. 2010; Monacoet al. 2010
	CC8	ST8	IV	North America, Europe, Australia, New Zealand	Nichol et al. 2013; Nimmo et al. 2014; Dauwalderet al. 2008; Grundmann et al. 2010; Williamson et al. 2014
		ST239	III	South America, Europe, Africa, Australia, New Zealand	Egea et al. 2014; Smyth et al. 2010; Grundmann et al. 2010; Schaumburg et al. 2014; Abdulgader et al. 2015; Chenet al. 2014; Williamson et al. 2014
		ST247	I	North America, Europe	Nichol et al. 2013; Grundmann et al. 2010
		ST612	IV	Africa, Australia, New Zealand	Abdulgader et al. 2015; Schaumburg et al. 2014; Williamson et al. 2014
	CC22	ST22	IV	North America, Europe, Africa, Asia, Australia, New Zealand	Holden et al. 2013; Nicholet al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015; Schaumburg et al. 2014; Chenet al. 2014; Williamson et al. 2014
	CC30	ST36	II, IV	North America, Europe, Africa	Nichol et al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015
	CC45	ST45	IV	North America, Europe	Nichol et al. 2013; Stefani et al. 2012
CA	CC1	ST1	IV	North America, South America, Australia, New Zealand	Nichol et al. 2013; Egeaet al. 2014; Gelatti et al. 2013; Williamson et al. 2014
		ST772	IV, V	Asia, Europe	Chen et al. 2014; Williamson et al. 2014; Ellington et al. 2010; Sanchini et al. 2011
	CC5	ST5	IV	South America, Europe, Africa, Australia, New Zealand	Egea et al. 2014; Gelattiet al. 2013; Grundmann et al. 2010; Abdulgader et al. 2015; Schaumburg et al. 2014; Williamson et al. 2014
	CC8	ST8	IV	North America, South America, Europe, Africa, Asia, Australia, New Zealand	Nichol et al. 2013; Nimmo et al. 2014; David et al. 2010; Egeaet al. 2014; Jimenezet al. 2012; Reyes et al. 2009; Abdulgader et al. 2015; Schaumburg et al. 2014; Sanchini et al. 2011; Williamson et al. 2014
	CC22	ST22	IV	Europe, Asia	Ellington et al. 2010; Moneckeet al. 2011
	CC30	ST30	IV, V	North America, South America, Europe, Asia, Australia, New Zealand	Nichol et al. 2013; Egeaet al. 2014; Gelattiet al. 2013; David et al. 2010; Mediavilla et al. 2012; Chen et al. 2014; Williamson et al. 2014
	CC45	ST45	IV, V, VI	North America, Australia, New Zealand	Monecke et al. 2011; Williamson et al. 2014
	CC59	ST59	IV, V	North America, Europe, Asia, Australia, New Zealand	David et al. 2010; Mediavilla et al. 2012; Sowashet al. 2014; Monecke et al. 2011; Chenet al. 2014; Williamson et al. 2014
	CC80	ST80	IV	Europe, Africa	David et al. 2010; Moneckeet al. 2011; Grundmann et al. 2010; Sanchiniet al. 2011; Abdulgader et al. 2015; Schaumburg et al. 2014
	CC88	ST88	IV	Europe, Africa, Australia	Monecke et al. 2011; Sanchiniet al. 2011; Abdulgader et al. 2015; Schaumburg et al. 2014
	Singleton	ST93	IV	Asia, Australia, New Zealand	Williamson et al. 2014; Moneckeet al. 2011; Chenet al. 2014
	CC152	ST152	V	Europe, Africa	Monecke et al. 2011; Mediavilla et al. 2012; Abdulgader et al. 2015; Schaumburg et al. 2014
LA	CC1	ST 1	IV	Europe	Cuny et al. 2013; Franco et al. 2011
	CC9	ST 9	IV, V	Europe, Asia	Monecke et al. 2011; Kocket al. 2013; Chenet al. 2014
	CC97	ST97	IV, V	Europe, Africa, Australia	Pantosti et al. 2012; Kock et al. 2013; Monecke et al. 2011
	CC398	ST 398	IV, V	North America, South America, Europe, Africa, Asia, Australia, New Zealand	Casey et al. 2014; Verkadeet al. 2014; Cunyet al. 2013; Van Cleefet al. 2011; Monacoet al. 2013; Ehlani et al. 2015; Moneckeet al. 2011; Williamson et al. 2014

CC: clonal complex; MLST: multilocus sequence typing; SCC*mec*: Staphylococcal chromosome cassette *mec*

Fig. 1 Geographical distribution of the prevalent MRSA clones

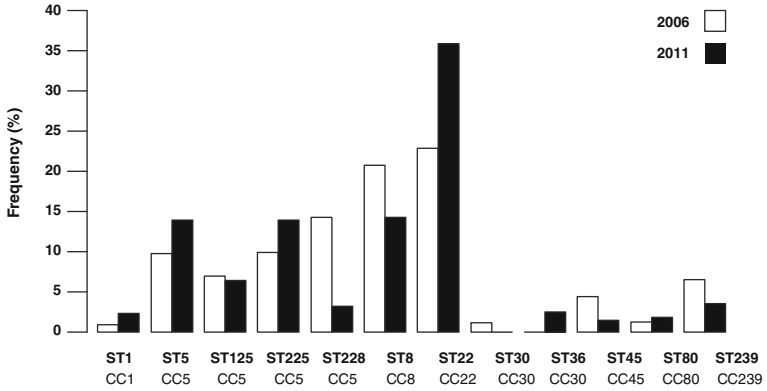


Fig. 2 Evolution of HA-MRSA clones in Europe (2006 and 2011) according sequence type (ST) and clonal complex (CC) distribution

SCC*mec* types (I, II or IV) and ST239-SCC*mec* III. Other lineages, such as ST22-SCC*mec* IV, ST36-SCC*mec* II, and ST612-SCC*mec* IV, a double-locus variant of ST8, are reported mainly from South Africa (Abdulgader et al. 2015) while ST1 has been reported from hospitals in Tunisia and Nigeria (Raji et al. 2013; Mariem et al. 2013).

In Asia, about 90 % of hospital infections are due to ST239-SCC*mec* III (Smyth et al. 2010). This clone is the predominant clone in all countries where molecular typing of HA-MRSA has been carried out including China, Korea (Moon et al. 2010), Indonesia, Philippines, Thailand, Vietnam, India, and Pakistan (Shabir et al. 2010; Chen and Huang 2014). The only exception is represented by Japan where ST5-SCC*mec* II is the predominant clone since 1990s (Chen and Huang 2014). ST5-SCC*mec* II is also largely disseminated in China, Korea, and Taiwan (Tsao et al. 2014). In Southeast Asian countries, ST241, a single-locus variant of ST239, is also present (Chen and Huang 2014).

In Australia, the major circulating HA-MRSA clone was ST239-SCC*mec* III till the end of 1990s when ST22-SCC*mec* IV became the most common clone (Williamson et al. 2014). In New Zealand, ST1 is the prevalent clone detected among strains isolated from patients with bacteremia (Ritchie et al. 2014); other clones belonging to CC1, CC30, CC59, and CC101 are also found in hospitals in the Southwest Pacific area (Williamson et al. 2014).

3.2.2 CA-MRSA

In the 1990s, MRSA epidemiology dramatically changed due to the emergence of new MRSA lineages in the community, later called CA-MRSA.

CA-MRSA infections were first identified in remote areas of Western Australia in the late 1980s; the causative strain was subsequently identified as ST8-SCC*mec* IV (Nimmo and Coombs 2008). At the beginning of the 1990s, in the USA, MRSA

infections started to emerge in the community among children without predisposing risk factors (Herold et al. 1998). During 1997–99, in the mid-western region of USA, an MRSA strain, designated as USA400 by PFGE, was responsible for a small outbreak of sepsis and necrotizing pneumonia among healthy children (DeLeo et al. 2010; David et al. 2015). USA400 was the prevalent CA-MRSA clone in USA until 2001 when the new unrelated clone USA300 replaced it to become one of the most successful clone ever. CA-MRSA were responsible for skin and soft tissues infections (SSTIs) in young healthy individuals without predisposing risk factors for MRSA acquisition and with no association with the healthcare system (Stryjewski and Chambers 2008), causing outbreaks among prisoners, athletes, military population, homosexual men, and newborns. Sporadically, CA-MRSA were responsible for serious infections, such as necrotizing pneumonia, necrotizing fasciitis, sepsis, and osteomyelitis (Crum et al. 2006; Bukharie 2010).

Molecular typing of CA-MRSA revealed that these isolates generally carry SCC*mec* elements type IV or V and display resistance to fewer non-beta-lactam antibiotics with respect to "classical" HA-MRSA that harbor SCC*mec* type I, II, or III and are usually multiresistant (Benoit et al. 2008; David and Daum 2010). In addition, CA-MRSA carry genes for PVL, a prophage-encoded, bicomponent pore-forming cytotoxin that specifically targets human neutrophils, causing their destruction and the consequent tissue damage (Boyle-Vavra and Daum 2007; Spaan et al. 2015). SCC*mec* IV and PVL genes have represented molecular markers used to trace the emergence of CA-MRSA worldwide (Vandenesch et al. 2003). Sixty to 100 % of CA-MRSA strains carry PVL genes (Rossney et al. 2007; Shallcross et al. 2013); the prevalence of PVL-positive isolates is dependent on lineages and geographical areas (Munckhof et al. 2003). According to recent studies, PVL is an important contributor, in association with other factors, to the virulence of CA-MRSA and to its ability to disseminate (Chambers and Deleo 2009; Zhang et al. 2008). Recently, the role of PVL in the pathogenesis of CA-MRSA necrotizing pneumonia has been clearly established using a rabbit model (Diep et al. 2010; Chi et al. 2014).

In recent years, the epidemiological distinction between CA-MRSA and HA-MRSA infections has become blurred due to the introduction of CA-MRSA into the healthcare system, especially in USA (David and Daum 2010; Pantosti and Venditti 2009). Therefore, the distinction between CA-MRSA and HA-MRSA requires more than one criteria including molecular typing (David and Daum 2010; Otter and French 2012).

The burden of CA-MRSA infections is geographically diversified. Although data from many areas are sparse and difficult to compare, evidence shows that the prevalence of CA-MRSA infections is higher in the USA than in other areas (Mediavilla et al. 2012; Witte 2009). PVL-positive CA-MRSA were reported as responsible for the majority of acute SSTIs in patients presenting at the US emergency departments (Moran et al. 2006). In Europe, PVL-positive CA-MRSA infections were relatively rare in England and Ireland but common in Greece (Shallcross et al. 2013). In Australia, CA-MRSA represented 7.8 % of the isolates obtained from outpatient infections (Nimmo and Coombs 2008).

At present, CA-MRSA are associated with more than 20 distinct world-wide-spread genetic lineages. The most common CA-MRSA lineages are displayed in Fig. 1.

In USA, the major CA-MRSA clone is USA300 (ST8-SCC*mec* IVa), which contains the PVL genes and the arginine catabolic mobile element (ACME) that has been shown to enhance the ability of the strain to colonize the skin (David and Daum 2010). Besides USA 300, USA400 (ST1-SCC*mec* IV), ST30-SCC*mec* IV, and ST59-SCC*mec* IV represent other less common CA-MRSA clones that can be found throughout the country (Mediavilla et al. 2012; Monecke et al. 2011). In Canada, USA300 and USA400 are the most common CA-MRSA clones (Nichol et al. 2013).

In South America, one of the most frequent clones is represented by the USA300 variant named USA300-LV (ST8-SCC*mec* IVc) that contains PVL but lacks ACME. USA300-LV, first reported in Colombia in 2006 (Reyes et al. 2009), is now present in several other Latin American countries, including Argentina, Venezuela, Peru, Ecuador, and Brazil. More recently, USA300-LV has also been described as cause of HA infections (Jimenez et al. 2012; Egea et al. 2014). In Argentina, the most common CA-MRSA clones are ST5-SCC*mec* IV and ST30-SCC*mec* IV, while in Uruguay and Brazil ST30-SCC*mec* IV is prevalent (Egea et al. 2014; Gelatti et al. 2013; Jimenez et al. 2012).

In Europe, circulating CA-MRSA strains belong to a variety of clones; the majority of the infections are due to the European clone ST80-SCC*mec* IV (Vandenesch et al. 2003), which typically shows resistance to fusidic acid (Monecke et al. 2011). USA300 was reported from Denmark in 2000 (AR Larsen et al. 2007) and subsequently from other countries (Austria, England, France, Ireland, Netherlands, Spain, and Italy), as cause of sporadic infections or small outbreaks (Nimmo 2012; Sanchini et al. 2013). USA300-LV has also been reported from Spain and Italy (Cercenado and Ruiz de Gopegui 2008; Sanchini et al. 2011; David and Daum 2010). ST30-SCC*mec* IV is also largely spread in different geographical areas (David and Daum 2010; Sanchini et al. 2011), while a variety of other CA-MRSA clones have been identified with variable frequency including ST22, ST59, ST152, and ST772 (Monecke et al. 2011; Ellington et al. 2010; Mediavilla et al. 2012).

In Africa, few predominant CA-MRSA clones are spread in different regions. In North Africa, the European clone ST80-SCC*mec* IV predominates, likely due to the geographical proximity to Europe. ST88-SCC*mec* IV is predominant in West, Central, and East Africa, while ST8-SCC*mec* IV, including isolates closely related to USA300, has been recently reported in Gabon and Ghana (Schaumburg et al. 2014). ST612-SCC*mec* IV, PVL-positive, typically circulates in South Africa (Abdulgader et al. 2015).

In Asia, there is great heterogeneity in the circulating CA-MRSA clones and their prevalence varies considerably among countries. ST59, carrying SCC*mec* type IV, V, or a variant named V_T (V_{Taiwan}), is spread in Taiwan, China, Vietnam, and Japan (David and Daum 2010; Sowash and Uhlemann 2014; Chen and Huang 2014); ST30-SCC*mec* IV is ubiquitously detected but prevails in Singapore, Hong Kong, Philippines, and Japan. ST772-SCC*mec* V, named the Bengal Bay clone, is

predominant in India, where ST22-SCC*mec* IV is also present. ST8, ST88, and ST93 are spread in Japan, while ST834, belonging to CC9, is characteristically present in Cambodia (Chen and Huang 2014; David and Daum 2010; Monecke et al. 2011; Sowash and Uhlemann 2014; Williamson et al. 2014).

In Australia, the most common CA-MRSA clone is ST93-SCC*mec* IV (the Queensland clone). However, a large diversity of CA-MRSA clones has also been documented in this geographical area, including PVL-negative clones (Nimmo and Coombs 2008), such as ST75, which is a genetically divergent *S. aureus* strain and should probably be allocated to a separate species (Williamson et al. 2014). In New Zealand, ST30-SCC*mec* IV has been the prevalent clone up to 2005 when ST5-SCC*mec* IV, an emerging clone resistant to fusidic acid, has displaced it. Other CA-MRSA lineages, typical of other geographical areas, are present both in Australia and New Zealand, such as USA300, ST59-SCC*mec* V_T, and ST772-SCC*mec* V (Williamson et al. 2014).

3.2.3 LA-MRSA

It has been recently established that livestock represents a reservoir of MRSA (Pantosti 2012; van Cleef et al. 2011). Several cases of human colonization and infections caused by MRSA of animal origin (designated LA-MRSA), mainly from pigs or cattle, have been reported (Smith and Pearson 2011). The first LA-MRSA was identified in Europe in 2005 (Voss 2005). It belonged to a new MRSA lineage (ST398, CC398) and showed features different from those of other MRSA clones such as non-typeability by PFGE (Bens et al. 2006), presence of SCC*mec* type IV or V, and resistance to tetracycline and trimethoprim–sulfamethoxazole, antibiotics commonly used in animal production (Argudin et al. 2011). PVL and enterotoxins genes were generally not present (Hallin et al. 2011). Colonization and infection with ST398 have been documented mainly in countries where animal production is intensive, occurring primarily in farm workers, veterinarians, and other people exposed to livestock (Monaco et al. 2013; Van Cleef et al. 2010; Casey et al. 2014). However, cases have also been reported in subjects with no known contact with animals and in hospitalized patients (Wulf et al. 2008; Kock et al. 2009).

Although LA-MRSA ST398 was found to be globally spread, other MRSA non-CC398 have emerged in farm animals in different geographical areas: ST9 (CC9) detected in pigs in Asia and Europe; ST97 (CC97) isolated from bovine mastitis and chickens in different areas of the Americas and Europe; ST1 (CC1) frequently found in bovine mastitis and in pigs in Europe (Franco et al. 2011); ST22 (CC22) and ST5 (CC5) isolated from pigs in Ireland and Canada, respectively (Cuny et al. 2013). Another clone, ST130 (CC130), recovered in cattle, horses, and sheep, was found to harbor *mecC*, a novel *mec* homologue that is not detectable by conventional diagnostic assays (Garcia-Alvarez et al. 2011; Ito et al. 2012). All these non-CC398 LA-MRSA lineages were found to colonize or infect humans with different prevalence, being ST9 and ST97 the less frequently recovered (Kock et al. 2013).

3.2.4 Molecular Epidemiology of MSSA

Although MSSA is a leading cause of infections, both in the community and in the healthcare setting, only few studies have been published describing the molecular epidemiology of MSSA. Since MSSA represents the reservoir for the emergence of MRSA through SCC*mec* introduction, it is important to recognize MSSA clones endowed with capacity to cause serious infections and to spread globally. For instance, the PVL-positive MSSA clone phage 80/81 that was spread in hospitals in the middle of the past century has been recognized by modern typing techniques as belonging to CC30. After acquiring *mecA,* the clone has disseminated globally as MRSA CC30, one of the principal CA-MRSA clones (Robinson et al. 2005).

In general, all the studies have highlighted that the MSSA population is more heterogeneous than the MRSA population, since the MSSA isolates belong to a larger number of different clones and lineages. This depends, at least in part, on the fact that MSSA are carried by approximately one-third of the human population and that their circulation is much antecedent to MRSA emergence (Deurenberg and Stobberingh 2008; Grundmann et al. 2010b).

Approximately 40–50 % of MSSA isolates in different geographical areas have a genetic background shared with the major MRSA CCs, namely CC5, CC8, CC22, CC30, and CC45, while the rest belongs to lineages that contain predominantly MSSA, such as CC7, CC9, CC12, CC15, CC25, CC51, and CC101 (Deurenberg and Stobberingh 2008). Each of these lineages includes different *spa* types and/or PFGE types (Deurenberg and Stobberingh 2008; Goering et al. 2008). The presence of successful MSSA lineages with a wide geographical distribution suggests that they possess factors favoring the ability to cause and to transmit disease among humans.

In a recent study, MSSA isolates from uncomplicated SSTIs in the community setting obtained in global clinical trials were characterized by using PFGE and other molecular typing techniques. The most common clones, accounting for approximately 36 % of the isolates, were ST30, ST45, ST1 (USA400), and ST8 (USA300) that were recovered in USA, South America, South Africa, and Europe (Goering et al. 2008).

In a large study performed in USA, MSSA collected from a variety of sources, including blood, urine, the respiratory tract, and the skin, representing both community- and healthcare-acquired infections were typed by *spa* typing (Miko et al. 2013): 274 *spa* types were identified among 708 isolates, obtaining 15 genetic clusters. The most common genetic clusters corresponded to USA100, USA800 (CC5) and to USA300 (CC8), the same lineages found among MRSA in the healthcare setting or in the community.

In the previously cited European survey on *S. aureus* from invasive infections conducted in 2006–2007, the diversity index based on *spa* typing was higher (0.985) for MSSA than for MRSA (0.940). In this study, the most frequent MSSA clones, isolated from bacteremia in hospitalized patients, were (in ranking order): ST7, ST15, ST5, ST45, ST8, ST30, ST1, and ST22. Moreover, MSSA showed a lower degree of geographical clustering than MRSA (Grundmann et al. 2010).

Although MSSA is more rarely associated with PVL than MRSA, based on the epidemiology in USA and some European countries (Shallcross et al. 2013) the global scenario is very diversified. Several MSSA lineages are found to carry PVL genes; ST1, ST5, ST25, and ST152 have a pandemic spread (Rasigade et al. 2010), while other lineages appear to be more restricted to some geographical areas; for instance, ST8 MSSA, related to MRSA USA300, is frequent in USA, while ST80 is present in Europe and Africa as the MRSA counterparts, and ST188 is found in France, New Caledonia, and Polynesia. In Europe, in the community setting, the most prevalent PVL-positive MSSA lineages are CC30 and CC121 (Rasigade et al. 2010; Sanchini et al. 2014).

Several studies found a high rate of carriage and infections due to PVL-positive MSSA in Africa, with isolates belonging to a variety of different clones including ST15, ST30, ST121, and ST152 (Schaumburg et al. 2011). ST152 is a divergent MSSA clone that was first identified in Mali. The reason for this occurrence is unknown, although the humid environment of tropical Africa and host factors, such as altered C5a receptor, which has been identified as PVL target (Spaan et al. 2013), could contribute to this peculiar epidemiological picture (Schaumburg et al. 2014).

Another MSSA clone has emerged recently becoming a source of concern: MSSA ST398 (CC398) mainly with *spa* type 571. This strain that contains the phage-encoded immune evasion cluster genes (Chroboczek et al. 2013) and is characteristically resistant to erythromycin, due to presence of *ermT,* and susceptible to tetracycline, seems to represent the basal human clade from which the animal-adapted ST398 MRSA clone emerged (Valentin-Domelier et al. 2011). MSSA ST398 is responsible for serious human infections in different geographical regions including North America, Europe, China, and the Caribbean (Verkade and Kluytmans 2014). In France, MSSA ST398 accounts for 7.5 % of all MSSA endocarditis cases (Chroboczek et al. 2013).

The molecular epidemiology of MSSA shows a large clonal heterogeneity across geographical areas. The prevalence of MSSA clones with the same genetic background of pandemic MRSA clones suggests that factors, other than methicillin resistance, contribute to the success of a specific clone.

4 *S. aureus* and Antibiotic Resistance

As already mentioned, *S. aureus* has a unique ability to rapidly acquire antibiotic resistance to virtually any antimicrobial molecules that has been developed. Resistance is often acquired by HGT from other species or genera, although chromosomal mutations also contribute to resistance to some antibiotics. HGT allows acquisition of preconstituted clusters of genes that concur to a resistance trait (e.g., the *mec* complex or the *vanA* complex for methicillin or vancomycin resistance, respectively), while mutations can provide resistance to novel or synthetic antibiotics that do not have natural analogues and for which resistance determinants are not available in nature (e.g., for linezolid).

The evolution of the different MRSA lineages has involved the acquisition of antibiotic resistance determinants. Therefore, certain MRSA clones can be associated with characteristic resistance traits or patterns. For instance, CA-MRSA lineages retain susceptibility to most non-beta-lactam antibiotics, but USA300 is characteristically resistant to erythromycin and ciprofloxacin (David and Daum 2010) and the European CA-MRSA ST80 clone is resistant to fusidic acid and tetracycline (Monecke et al. 2011). LA-MRSA is commonly resistant to tetracycline, the most used antibiotic in the farming industry (Pantosti 2012). HA-MRSA lineages tend to be resistant to a broad range of antibiotic agents including the aminoglycosides although the most recent emerging clones are resistant to a narrower spectrum of antibiotics. ST22 (EMRSA-15) is characteristically resistant to fluoroquinolones and macrolides, but it is susceptible to gentamycin (Ellington et al. 2010; Johnson et al. 2005); the Lyon clone (ST8) is resistant to fluoroquinolones, susceptible to gentamycin and variably susceptible to other aminoglycosides and macrolides (Dauwalder et al. 2008).

The mechanisms and the genetic determinants leading to resistance to the most common agents used to treat staphylococcal infections have been extensively reviewed (Lowy 2003; Pantosti et al. 2007). Today, there are a number of newly developed antibiotics that display good anti-MRSA activity, such as lipoglycopeptides (derivatives of vancomycin or teicoplanin such as telavancin and dalbavancin) and new antistaphylococcal cephalosporins, such as ceftobiprole and ceftaroline (Morata et al. 2015). These two last molecules, as all beta-lactam antibiotics, are substrate analogues of PBPs resulting in their block, impaired cell wall synthesis and cell death. But, unlike other beta-lactams, both ceftobiprole and ceftaroline have high affinity also for PBP2a, that mediates methicillin resistance in *S. aureus*, thus are active also against MRSA (Moisan et al. 2010; Davies et al. 2007). Little is known about resistance development with these molecules since their clinical use has been very limited so far. Here, we will briefly summarize resistance to the last-line antibiotics for MRSA treatment: vancomycin, linezolid, and daptomycin.

4.1 Vancomycin

Vancomycin and the other glycopeptide antibiotic teicoplanin have been the mainstay of MRSA treatment for 30 years (Srinivasan et al. 2002). Isolates with decreased susceptibility to vancomycin were described for the first time in Japan in 1997 (Hiramatsu et al. 1997b) and thereafter in several other countries. These isolates, mostly MRSA, showed a spectrum of vancomycin minimal inhibitory concentrations (MICs) ranging from borderline susceptibility to full resistance (Gardete et al. 2012). In between these extremes, isolates with intermediate susceptibility to vancomycin (VISA) and those still susceptible but containing a minority population with intermediate susceptibility (heterogeneous VISA or hVISA) were present (Liu and Chambers 2003).

The recognition of VISA and hVISA is complicated by problems with laboratory methods and with different breakpoints; the reference method is MIC determination by broth microdilution (CLSI 2014; EUCAST 2015), and a labor-intensive test, such as the population analysis profile, is required to detect hVISA (Howden et al. 2010). Both the Clinical and Laboratory Standard Institute (CLSI) and the European Commission for Antimicrobial Susceptibility Testing (EUCAST) have established the vancomycin breakpoint for susceptibility at MIC <=1 ug/ml (CLSI 2014; EUCAST 2015), thus indicating that vancomycin is poorly or not effective against isolates with higher MIC. However, CLSI has retained the intermediate category (MIC 4-8 ug/ml) to define VISA, clearly differentiating them from vancomycin-resistant *Staphylococcus aureus* (VRSA) (MIC => 16 mg/ml) since completely different resistance mechanisms are implicated in these strains. This difference is recognized in terms of nomenclature also by EUCAST that has designated glycopeptide-intermediate *S. aureus* (GISA) and glycopeptide-resistant *S. aureus* (GRSA) isolates with low-level and high-level resistance, respectively (EUCAST 2015).

VISA is associated with a thickened cell wall that traps vancomycin before it reaches the molecular target that is the nascent peptidoglycan at the inner side of the cell wall. Different mutations or expression of genes that are related to cell wall synthesis, is associated with the emergence of VISA from susceptible parental strains in vitro or in vivo (Howden et al. 2010). In particular, type I and type II polymorphisms of the accessory gene regulator locus (*agr*) or alterations of its function have been associated with the development of VISA and hVISA (Howden et al. 2010; Sakoulas et al. 2002). True homogeneous VISA isolates remain a small number in the published reports (Gardete et al. 2012) and have not been found in susceptibility studies in large series of isolates (Mendes et al. 2014b, c). On the contrary, hVISA have been detected in most institutions where they have been searched for; thus, their prevalence may be underestimated (Howden et al. 2010). In 2003, the hVISA prevalence for was reported to be 2 % in MRSA and 0.05 % in MSSA (Liu and Chambers 2003), although strong inter-institutional differences were noted (Hiramatsu et al. 1997a). Previous vancomycin treatment and the genetic background of *S. aureus* are predisposing factors for VISA or hVISA development (Howden et al. 2014). Although VISA and hVISA have emerged in every principal MRSA lineage, including CA-MRSA USA300 (Gardete et al. 2012) and also in MSSA (Pillai et al. 2009), they have been found especially in CC5 and CC8 background (Howe et al. 2004; Monaco et al. 2010).

The occurrence of VRSA is quite rare but has always raised fear of bleak scenarios (Conly and Johnston 2002). Only 17 VRSA that have been confirmed by molecular methods are reported in the indexed literature so far (Table 1).The first VRSA was isolated in Michigan in 2002 (Weigel et al. 2003) and the vast majority of the other VRSA strains (13 out of 17) were reported from the USA, in particular from Michigan. The isolates from other countries originated 1 each from India, Iran, Brazil, and Portugal, this last being to date the only VRSA from Europe (Finks et al. 2009; Friaes et al. 2015; Limbago et al. 2014; Rossi et al. 2014; Saha et al. 2008; Sievert et al. 2008; Azimian et al. 2012).

Table 1 VRSA isolated from 2002 to 2013 reported in indexed journals

Country or state or city	Date	Source	Vancomycin MIC (μg/ml)	SCC*mec*	*spa* type	MLST	CC	References
USA/MI	2002	Plantar ulcers	1024	II	t062	ST371	CC5	Weigel et al. 2003, Limbago et al. 2014
USA/PA	2002	Plantar ulcers	32	II	t002	ST5	CC5	Tenover et al. 2004, Sievert et al. 2008
USA/NY	2004	Urine	64	IV	t002	ST5	CC5	Sievert et al. 2008
USA/MI	2005	Toe wound	256	II	t002	ST5	CC5	Zhu et al. 2008; Sievert et al. 2008
USA/MI	2005	Surgical site wound	512	II	t002	ST231	CC5	Zhu et al. 2008; Sievert et al. 2008
USA/MI	2005	Plantar ulcers	1024	NT	t002	ST85	CC5	Zhu et al. 2008, Sievert et al. 2008
USA/MI	2006	Triceps wound	512	II	t062	ST231	CC5	Zhu et al. 2008, Sievert et al. 2008
USA/MI	2007	Toe wound	1024	ND	t002	ST5	CC5	Finks et al. 2009
USA/MI	2007	Surgical wound	1024	ND	t002	ST5	CC5	Finks et al. 2009
India/West Bengal	2007	Pus	64	ND	-	-	-	Saha et al. 2008
USA/MI	2009	Plantar wound	NR	ND	t002	ST5	CC5	Limbago et al. 2014
USA/DE	2010	Wound drainage	NR	ND	t002	ST5	CC5	Limbago et al. 2014
USA/DE	2010	Vaginal swab	NR	ND	t045	ST5	CC5	Limbago et al. 2014
USA/DE	2012	Foot wound	256	ND	t019	-	CC30	Limbago et al. 2014
Iran/Mashhad	2011	Bronchial aspirate	512	III	t037	ST239[a]	CC8	Azimian et al. 2012
Brazil/Sao Paolo	2012	Blood culture	32	IV	t292	ST85	CC8	Rossi et al. 2014
Portugal/Lisbon	2013	Toe wound	1024	II	t002	ST105	CC5	Friaes et al. 2015

MIC: Minimal inhibitory concentration; SCC*mec* Staphylococcal chromosome cassette *mec*; *spa:* Staphylococcal protein A; MLST: multilocus sequence typing; CC: clonal complex

[a]ST updated according to Larsen J et al. 2012

Vancomycin resistance in *S. aureus* is a clear example of HGT from another bacterial species, as resistance is conferred by the acquisition of the *vanA* cluster, an operon consisting of 5 genes, carried by the transposon Tn1546, the resistance hallmark of vancomycin-resistant enterococci (VRE) (Courvalin 2006). The genes composing the *vanA* cluster act synergistically to modify the cell wall peptidoglycan making it resistant to the vancomycin action. Tn1546 acquisition by *S. aureus* generally occurs from a VRE species (more commonly *Enterococcus faecalis* or *Enterococcus faecium*) by means of a plasmid, such as a promiscuous plasmid of the Inc18 family (Zhu et al. 2010). This may explain why VRSA generally emerge in chronic infections with mixed flora, such as ulcers of the extremities, where MRSA and VRE may coexist, in chronically ill patients under long-term vancomycin therapy (Sievert et al. 2008). Most VRSA belong to CC5, suggesting that only some genetic backgrounds, are permissive to the introduction of the *van*A cluster. The burden carried by this group of resistance genes on the overall fitness of *S. aureus* could also explain the apparent low propensity of VRSA to transmit to other patients or cause outbreaks (Howden et al. 2010). It is noteworthy that the VRSA from Brazil is related to USA300 and PVL-positive (Rossi et al. 2014); this emergence is worrisome due to the intrinsic high transmissibility of the USA300 clone.

Sources different from the peer-reviewed journals suggest that VRSA may indeed be more common. For instance, the MLST Web site (http://saureus.mlst.net/ accessed on June 4, 2015) includes 23 VRSA although the molecular evidence for the presence of Tn1546 is not provided. This list includes isolates from the USA (other than those already published) and isolates from Pakistan, Japan, South Chorea, Iraq, India, China, and Brazil. A number of isolates from Pakistan appears to be genetically heterogeneous, belonging to at least 5 different lineages. An apparent high number of VRSA is also reported from India (Askari Ea et al. 2013). The VRSA status of these isolates has never been confirmed by independent investigators; therefore, some skepticism about the circulation of VRSA in these countries must be maintained.

4.2 Linezolid

Linezolid belongs to a new antibiotic class, the oxazolidinones, introduced into medical practice in 2000. Linezolid exerts its antibacterial action by binding to the 23S subunit of the bacteria ribosome at domain V, thus inhibiting protein synthesis (Leach et al. 2007). Although linezolid is a synthetic drug and no natural reservoir of resistance genes would be expected, in 2001 the first linezolid-resistant *S. aureus* was reported in the USA in a patient who had received 1-month linezolid treatment (Tsiodras et al. 2001).

Two different mechanisms are known to confer linezolid resistance to *S. aureus*. The first is due to mutations occurring in the linezolid binding site (23S rRNA), the most common being the G2576T mutation, or in the ribosomal proteins L3 and L4

(Mendes et al. 2014a). The second mechanism is due to the presence of the plasmid-born chloramphenicol-florfenicol resistance (*cfr*) gene that encodes a 23S rRNA methyl transferase (Schwarz et al. 2000), conferring resistance to different antibiotics, including linezolid.

cfr carrying MRSA have caused intra- and inter-hospital outbreaks; a large outbreak that occurred in an ICU in Madrid, was due both to clonal expansion of the linezolid-resistant MRSA as well as to transmission of the *cfr* plasmid to other MRSA clones (Bonilla et al. 2010; Ikeda-Dantsuji et al. 2011; Morales et al. 2010; Sanchez Garcia et al. 2010). The prevalence of linezolid resistance among clinical *S. aureus* isolates remains very low: a study including isolates only from the USA reported resistance rates below 0.2 % from 2004 to 2012 (Mendes et al. 2014b). In addition, a surveillance program conducted across the same period on 25,000 *S. aureus* isolates mostly from blood, wound and lower respiratory tract, the percentage of the linezolid-resistant strains remained below 0.1 % in countries from 5 continents (Mendes et al. 2014c). Linezolid resistance generally develops in patients who had been receiving long linezolid treatments; therefore, it can be higher in selected groups of patients, such as cystic fibrosis patients (Endimiani et al. 2011).

4.3 Daptomycin

Daptomycin is a natural lipopeptide antibiotic introduced in 2003 in the USA and in 2005 in Europe (Sakoulas 2009) for treatment of skin and soft tissue infections and bacteremia (Bayer et al. 2013). Its mechanism of action is probably multifaceted and not completely understood. Daptomycin is an anionic molecule that requires the presence of calcium ions to be active (Straus and Hancock 2006): the daptomycin–calcium complex inserts itself in the bacterial cell membrane causing depolymerization and permeabilization with leakage of small ions and cell death (Humphries et al. 2013). Cell wall probably represents another target of daptomycin, since resistant isolates exhibit a thickened cell wall (Bertsche et al. 2011). Also the genetic determinants of daptomycin resistance have not been fully identified. Resistance seems to be associated with a progressive accumulation of mutations in a few *S. aureus* genes. The most common mutations occur in *mpr*F, coding for a bifunctional enzyme involved in the metabolism of the cell membrane. The *mpr*F mutations are associated with gain-in-function determining increase in the relative positive charge of the cell membrane leading to a decreased insertion of the calcium–daptomycin complex (Jones et al. 2008). Resistance has been associated also with mutations in the *yyc* cluster and in *rpoB* and *rpoC* (Bayer et al. 2013) and with enhanced expression of the regulatory systems *vra*SR (Mehta et al. 2012).

Soon after the introduction of daptomycin in clinical use, treatment failures due to the emergence of daptomycin-resistant *S. aureus* were reported, especially in patients treated for endocarditis where the bacterial load is presumably high (Hayden et al. 2005; Julian et al. 2007). However, in spite of the increasing

daptomycin use in clinical practice in recent years, reports about daptomycin resistance emergence remain sporadic. Data collected in a surveillance study including over 97,000 *S. aureus* from the years 2005–2012 obtained from 400 clinical centers in the Americas, Europe, and the Asia-Pacific Region, showed that the prevalence of daptomycin-resistant *S. aureus* was extremely low (0.05 %) in all geographical regions with no trend toward increased resistance over the years. Daptomycin MICs were similar between MRSA and MSSA isolates, yielding a similar prevalence of resistance in both groups (Sader et al. 2014).

Several studies documented that *S. aureus* isolates from patients, who previously received vancomycin therapy, were more prone to develop daptomycin resistance (Julian et al. 2007) and showed a relationship between decreased susceptibility to vancomycin and resistance to daptomycin (Bayer et al. 2013). In one of the first study, out of 70 VISA isolates, 80 % were found resistant to daptomycin (Patel et al. 2006). The link between VISA and daptomycin-resistant phenotypes consists probably in the thickened cell wall that may influence the penetration of both vancomycin and daptomycin, thus preventing the interaction with their respective targets (Cui et al. 2006).

5 Conclusions

The recent evolution in *S. aureus* epidemiology has led to the emergence of lineages that are endowed with characteristics of adaptation to different environments or hosts, and with antibiotic resistance traits. In this scenario, the characterization of *S. aureus* isolates by molecular typing is of utmost importance to better understand *S. aureus* micro and macroevolution. The dynamic evolution of *S. aureus* facilitated by travel, migration, and globalization has enabled geographically restricted clones to spread and become pandemic. The early recognition of high-risk clones that are particularly able to adapt and spread in the clinical environment is important for their control, since effective antibiotics are limited. Antimicrobial agents such as glycopeptides, linezolid, daptomycin, and other newer antibiotics are still active against the majority of isolates, but their efficacy will be jeopardized by increased use.

Given the affordable costs, the advent of WGS in routine use will represent a major breakthrough in the study of *S. aureus* epidemiology. Molecular typing should be applied on a larger scale to improve the understanding of *S. aureus* circulation, especially in low-income countries where the burden of *S. aureus* infections is probably higher than in industrialized countries, but it is not well acknowledged so far.

Economic and political efforts should aim at strengthening surveillance systems based on molecular typing at local and global level and at introducing appropriate control measures both in healthcare and in community settings.

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References

Abdulgader SM, Shittu AO, Nicol MP, Kaba M (2015) Molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. Front Microbiol 6:348. doi:10.3389/fmicb.2015.00348

AR Larsen MS, Goering RV, Sørum M, Skov R (2007) Emergence and dissemination of the methicillin resistant *Staphylococcus aureus* USA300 clone in Denmark (2000–2005) Eurosurveillance 12(2)

Argudin MA, Tenhagen BA, Fetsch A, Sachsenroder J, Kasbohrer A, Schroeter A, Hammerl JA, Hertwig S, Helmuth R, Braunig J, Mendoza MC, Appel B, Rodicio MR, Guerra B (2011) Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. Appl Environ Microbiol 77(9):3052–3060. doi:10.1128/AEM.02260-10

Askari Ea, Tabatabai, SMa, Arianpoor Aa, Nasab MN (2013) *VanA*-positive vancomycin-resistant *Staphylococcus aureus*: systematic search and review of reported cases. Infect Dis Clin Pract 21(2):91–93

Azimian A, Asghar Havaei S, Fazeli H, Naderi M, Ghazvini K, Mirab Samiee S, Soleimani M, Najar Peerayeh S (2012) Genetic analysis of a vancomycin-resistant *Staphylococcus aureus* strain isolated in Iran. MBio 3(6). doi:mBio.00442-12 [pii] 10.1128/mBio.00442-12

Bannerman TL, Hancock GA, Tenover FC, Miller JM (1995) Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J Clin Microbiol 33(3): 551–555

Bayer AS, Schneider T, Sahl HG (2013) Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. Ann N Y Acad Sci 1277:139–158. doi:10.1111/j.1749-6632.2012.06819.x

Becker AP, Santos O, Castrucci FM, Dias C, D'Azevedo PA (2012) First report of methicillin-resistant *Staphylococcus aureus* Cordobes/Chilean clone involved in nosocomial infections in Brazil. Epidemiol Infect 140(8):1372–1375. doi:10.1017/S095026881100210X

Benoit SR, Estivariz C, Mogdasy C, Pedreira W, Galiana A, Bagnulo H, Gorwitz R, Fosheim GE, McDougal LK, Jernigan D (2008) Community strains of methicillin-resistant *Staphylococcus aureus* as potential cause of healthcare-associated infections, Uruguay, 2002–2004. Emerg Infect Dis 14(8):1216–1223. doi:10.3201/eid1408.071183

Bens CC, Voss A, Klaassen CH (2006) Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. J Clin Microbiol 44 (5):1875–1876. doi:10.1128/JCM.44.5.1875-1876.2006

Bertsche U, Weidenmaier C, Kuehner D, Yang SJ, Baur S, Wanner S, Francois P, Schrenzel J, Yeaman MR, Bayer AS (2011) Correlation of daptomycin resistance in a clinical *Staphylococcus aureus* strain with increased cell wall teichoic acid production and D-alanylation. Antimicrob Agents Chemother 55(8):3922–3928. doi:AAC.01226-10 [pii] 10.1128/AAC.01226-10

Blair JE, Williams RE (1961) Phage typing of staphylococci. Bull World Health Organ 24(6):771–784

Bonilla H, Huband MD, Seidel J, Schmidt H, Lescoe M, McCurdy SP, Lemmon MM, Brennan LA, Tait-Kamradt A, Puzniak L, Quinn JP (2010) Multicity outbreak of linezolid-resistant *Staphylococcus epidermidis* associated with clonal spread of a *cfr*-containing strain. Clin Infect Dis 51(7):796–800. doi:10.1086/656281

Boyle-Vavra S, Daum RS (2007) Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. Lab Invest 87(1):3–9. doi:3700501 [pii] 10.1038/labinvest.3700501

Bukharie HA (2010) A review of community-acquired methicillin-resistant *Staphylococcus aureus* for primary care physicians. J Fam Community Med 17(3):117–120. doi:10.4103/1319-1683.74320

Caboclo RM, Cavalcante FS, Iorio NL, Schuenck RP, Olendzki AN, Felix MJ, Chamon RC, dos Santos KR (2013) Methicillin-resistant *Staphylococcus aureus* in Rio de Janeiro hospitals: dissemination of the USA400/ST1 and USA800/ST5 SCC*mec* type IV and USA100/ST5 SCC*mec* type II lineages in a public institution and polyclonal presence in a private one. Am J Infect Control 41(3):e21–26. doi:10.1016/j.ajic.2012.08.008

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance standards for antimicrobial susceptibility testing. Twenty-Fourth informational supplement. CLSI document M100-S24, Wayne, PA

Casey JA, Shopsin B, Cosgrove SE, Nachman KE, Curriero FC, Rose HR, Schwartz BS (2014) High-density livestock production and molecularly characterized MRSA infections in Pennsylvania. Environ Health Perspect 122(5):464–470. doi:10.1289/ehp.1307370

Cercenado E, Ruiz de Gopegui E (2008) Community-acquired methicillin-resistant *Staphylococcus aureus*. Enferm Infecc Microbiol Clin 26(Suppl 13):19–24 doi:13128776

Chambers HF (2001) The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis 7(2):178–182. doi:10.3201/eid0702.700178

Chambers HF, Deleo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7(9):629–641. doi:10.1038/nrmicro2200

Chatterjee SS, Otto M (2013) Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. Clin Epidemiol 5:205–217. doi:10.2147/CLEP.S37071

Chen CJ, Huang YC (2014) New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect 20(7):605–623. doi:10.1111/1469-0691.12705 S1198-743X(14)61146-0[pii]

Chi CY, Lin CC, Liao IC, Yao YC, Shen FC, Liu CC, Lin CF (2014) Panton-Valentine leukocidin facilitates the escape of *Staphylococcus aureus* from human keratinocyte endosomes and induces apoptosis. J Infect Dis 209(2):224–235. doi:10.1093/infdis/jit445

Chroboczek T, Boisset S, Rasigade JP, Tristan A, Bes M, Meugnier H, Vandenesch F, Etienne J, Laurent F (2013) Clonal complex 398 methicillin susceptible *Staphylococcus aureus*: a frequent unspecialized human pathogen with specific phenotypic and genotypic characteristics. PLoS ONE 8(11):e68462. doi:10.1371/journal.pone.0068462

Conly JM, Johnston BL (2002) VISA, hetero-VISA and VRSA: the end of the vancomycin era? Can J Infect Dis 13(5):282–284

Cookson BD, Robinson DA, Monk AB, Murchan S, Deplano A, de Ryck R, Struelens MJ, Scheel C, Fussing V, Salmenlinna S, Vuopio-Varkila J, Cuny C, Witte W, Tassios PT, Legakis NJ, van Leeuwen W, van Belkum A, Vindel A, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Muller-Premru M, Hryniewicz W, Rossney A, O'Connell B, Short BD, Thomas J, O'Hanlon S, Enright MC (2007) Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus strains*: the HARMONY collection. J Clin Microbiol 45(6):1830–1837. doi:JCM.02402-06 [pii] 10.1128/JCM.02402-06

Courvalin P (2006) Vancomycin resistance in gram-positive cocci. Clin Infect Dis 42 Suppl 1:S25–34. doi:CID36951 [pii] 10.1086/491711

Crum NF, Lee RU, Thornton SA, Stine OC, Wallace MR, Barrozo C, Keefer-Norris A, Judd S, Russell KL (2006) Fifteen-year study of the changing epidemiology of methicillin-resistant *Staphylococcus aureus*. Am J Med 119(11):943–951. doi:S0002-9343(06)00093-3 [pii] 10.1016/j.amjmed.2006.01.004

Cui L, Tominaga E, Neoh HM, Hiramatsu K (2006) Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 50(3):1079–1082. doi:50/3/1079 [pii] 10.1128/AAC.50.3.1079-1082.2006

Cuny C, Kock R, Witte W (2013) Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. Int J Med Microbiol 303(6–7):331–337. doi:10.1016/j.ijmm.2013.02.010

Dauwalder O, Lina G, Durand G, Bes M, Meugnier H, Jarlier V, Coignard B, Vandenesch F, Etienne J, Laurent F (2008) Epidemiology of invasive methicillin-resistant *Staphylococcus*

aureus clones collected in France in 2006 and 2007. J Clin Microbiol 46(10):3454–3458. doi:JCM.01050-08 [pii] 10.1128/JCM.01050-08

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23 (3):616–687. doi:23/3/616 [pii] 10.1128/CMR.00081-09

David MZ, Acree ME, Sieth JJ, Boxrud DJ, Dobbins G, Lynfield R, Boyle-Vavra S, Daum RS (2015) Pediatric *Staphylococcus aureus* isolate genotypes and infections from the dawn of the community-associated methicillin-resistant *S. aureus* epidemic era in Chicago, 1994 to 1997. J Clin Microbiol 53(8):2486–2491. doi:10.1128/JCM.00096-15

Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K (2007) Binding of ceftobiprole and comparators to the penicillin-binding proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Antimicrob Agents Chemother 51(7):2621–2624. doi:10.1128/AAC.00029-07

de Lencastre H, Oliveira D, Tomasz A (2007) Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. Curr Opin Microbiol 10(5):428–435. doi:S1369-5274(07) 00117-8 [pii] 10.1016/j.mib.2007.08.003

DeLeo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375(9725):1557–1568. doi:S0140-6736(09) 61999-1 [pii] 10.1016/S0140-6736(09)61999-1

Deurenberg RH, Stobberingh EE (2008) The evolution of *Staphylococcus aureus*. Infect Genet Evol 8(6):747–763. doi:S1567-1348(08)00141-X [pii] 10.1016/j.meegid.2008.07.007

Diep BA, Chan L, Tattevin P, Kajikawa O, Martin TR, Basuino L, Mai TT, Marbach H, Braughton KR, Whitney AR, Gardner DJ, Fan X, Tseng CW, Liu GY, Badiou C, Etienne J, Lina G, Matthay MA, DeLeo FR, Chambers HF (2010) Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci U S A 107(12):5587–5592. doi:10.1073/pnas.0912403107

European Centre for Disease Prevention and Control (ECDC) (2013) Point prevalence survey of healthcare- associated infections and antimicrobial use in European acute care hospitals. Stockholm. doi:10.2900/86011

European Centre for Disease Prevention and Control (ECDC) (2014) Antimicrobial resistance surveillance in Europe 2013. Stockholm, Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)

Egea AL, Gagetti P, Lamberghini R, Faccone D, Lucero C, Vindel A, Tosoroni D, Garnero A, Saka HA, Galas M, Bocco JL, Corso A, Sola C (2014) New patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) clones, community-associated MRSA genotypes behave like healthcare-associated MRSA genotypes within hospitals, Argentina. Int J Med Microbiol 304 (8):1086–1099. doi:S1438-4221(14)00101-5 [pii] 10.1016/j.ijmm.2014.08.002

Ellington MJ, Hope R, Livermore DM, Kearns AM, Henderson K, Cookson BD, Pearson A, Johnson AP (2010) Decline of EMRSA-16 amongst methicillin-resistant *Staphylococcus aureus* causing bacteraemias in the UK between 2001 and 2007. J Antimicrob Chemother 65(3):446–448. doi:10.1093/jac/dkp448

Endimiani A, Blackford M, Dasenbrook EC, Reed MD, Bajaksouszian S, Hujer AM, Rudin SD, Hujer KM, Perreten V, Rice LB, Jacobs MR, Konstan MW, Bonomo RA (2011) Emergence of linezolid-resistant *Staphylococcus aureus* after prolonged treatment of cystic fibrosis patients in Cleveland, Ohio. Antimicrob Agents Chemother 55(4):1684–1692. doi:AAC.01308-10 [pii] 10.1128/AAC.01308-10

Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A 99 (11):7687–7692. doi:10.1073/pnas.122108599

European Commette on Antimicrobial Susceptibility Testing (EUCAST) (2015) Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0. 2015;http://www.eucast.org

Finks J, Wells E, Dyke TL, Husain N, Plizga L, Heddurshetti R, Wilkins M, Rudrik J, Hageman J, Patel J, Miller C (2009) Vancomycin-resistant *Staphylococcus aureus*, Michigan, USA, 2007. Emerg Infect Dis 15(6):943–945. doi:10.3201/eid1506.081312

Franco A, Hasman H, Iurescia M, Lorenzetti R, Stegger M, Pantosti A, Feltrin F, Ianzano A, Porrero MC, Liapi M, Battisti A (2011) Molecular characterization of *spa* type t127, sequence type 1 methicillin-resistant *Staphylococcus aureus* from pigs. J Antimicrob Chemother 66(6):1231–1235. doi:dkr115 [pii] 10.1093/jac/dkr115

Friaes A, Resina C, Manuel V, Lito L, Ramirez M, Melo-Cristino J (2015) Epidemiological survey of the first case of vancomycin-resistant *Staphylococcus aureus* infection in Europe. Epidemiol Infect 143(4):745–748. doi:S0950268814001423 [pii] 10.1017/S0950268814001423

Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA (2011) Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11(8):595–603. doi:10.1016/S1473-3099(11)70126-8

Gardete S, Kim C, Hartmann BM, Mwangi M, Roux CM, Dunman PM, Chambers HF, Tomasz A (2012) Genetic pathway in acquisition and loss of vancomycin resistance in a methicillin resistant *Staphylococcus aureus* (MRSA) strain of clonal type USA300. PLoS Pathog 8(2): e1002505. doi:10.1371/journal.ppat.1002505 PPATHOGENS-D-11-00616 [pii]

Gelatti LC, Bonamigo RR, Inoue FM, Carmo MS, Becker AP, Castrucci FM, Pignatari AC, Da PA (2013) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying SCC*mec* type IV in southern Brazil. Rev Soc Bras Med Trop 46(1):34–38. doi:10.1371/journal.pmed.1000215 [pii]

Goering RV, Shawar RM, Scangarella NE, O'Hara FP, Amrine-Madsen H, West JM, Dalessandro M, Becker JA, Walsh SL, Miller LA, van Horn SF, Thomas ES, Twynholm ME (2008) Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. J Clin Microbiol 46(9):2842–2847. doi:10.1128/JCM.00521-08

Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, European Staphylococcal Reference Laboratory Working G (2010) Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med 7(1):e1000215. doi:10.1371/journal.pmed.1000215

Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, Glasner C, Sabat AJ, Weist K, Heuer O, Friedrich AW (2014) The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: results of a second structured survey. Euro Surveill 19(49)

Hallin M, De Mendonca R, Denis O, Lefort A, El Garch F, Butaye P, Hermans K, Struelens MJ (2011) Diversity of accessory genome of human and livestock-associated ST398 methicillin resistant *Staphylococcus aureus* strains. Infect Genet Evol 11(2):290–299. doi:10.1016/j.meegid.2010.10.021

Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41(12):5442–5448

Harris SR, Cartwright EJ, Torok ME, Holden MT, Brown NM, Ogilvy-Stuart AL, Ellington MJ, Quail MA, Bentley SD, Parkhill J, Peacock SJ (2013) Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study. Lancet Infect Dis 13(2):130–136. doi:S1473-3099(12)70268-2 [pii] 10.1016/S1473-3099(12)70268-2

Hayden MK, Rezai K, Hayes RA, Lolans K, Quinn JP, Weinstein RA (2005) Development of daptomycin resistance in vivo in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 43(10):5285–5287. doi:43/10/5285 [pii] 10.1128/JCM.43.10.5285-5287.2005

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS (1998) Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA 279(8):593–598

Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I (1997a) Dissemination in Japanese hospitals of strains of *Staphylococcus aureus*

heterogeneously resistant to vancomycin. Lancet 350(9092):1670–1673. doi:S0140-6736(97)07324-8 [pii] 10.1016/S0140-6736(97)07324-8

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997b) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40(1):135–136

Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, Harris SR, Strommenger B, Layer F, Witte W, de Lencastre H, Skov R, Westh H, Zemlickova H, Coombs G, Kearns AM, Hill RL, Edgeworth J, Gould I, Gant V, Cooke J, Edwards GF, McAdam PR, Templeton KE, McCann A, Zhou Z, Castillo-Ramirez S, Feil EJ, Hudson LO, Enright MC, Balloux F, Aanensen DM, Spratt BG, Fitzgerald JR, Parkhill J, Achtman M, Bentley SD, Nubel U (2013) A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res 23(4):653–664. doi:gr.147710.112 [pii] 10.1101/gr.147710.112

Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML (2010) Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 23(1):99–139. doi:23/1/99 [pii] 10.1128/CMR.00042-09

Howden BP, Peleg AY, Stinear TP (2014) The evolution of vancomycin intermediate *Staphylococcus aureus* (VISA) and heterogenous-VISA. Infect Genet Evol 21:575–582. doi:S1567-1348(13)00136-6 [pii] 10.1016/j.meegid.2013.03.047

Howe RA, Monk A, Wootton M, Walsh TR, Enright MC (2004) Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. Emerg Infect Dis 10(5):855–857. doi:10.3201/eid1005.030556

Humphries RM, Pollett S, Sakoulas G (2013) A current perspective on daptomycin for the clinical microbiologist. Clin Microbiol Rev 26(4):759–780. doi:26/4/759 [pii] 10.1128/CMR.00030-13

Ikeda-Dantsuji Y, Hanaki H, Sakai F, Tomono K, Takesue Y, Honda J, Nonomiya Y, Suwabe A, Nagura O, Yanagihara K, Mikamo H, Fukuchi K, Kaku M, Kohno S, Yanagisawa C, Nakae T, Yoshida K, Niki Y (2011) Linezolid-resistant *Staphylococcus aureus* isolated from 2006 through 2008 at six hospitals in Japan. J Infect Chemother 17(1):45–51. doi:10.1007/s10156-010-0085-1

Ito T, Hiramatsu K, Tomasz A, de Lencastre H, Perreten V, Holden MT, Coleman DC, Goering R, Giffard PM, Skov RL, Zhang K, Westh H, O'Brien F, Tenover FC, Oliveira DC, Boyle-Vavra S, Laurent F, Kearns AM, Kreiswirth B, Ko KS, Grundmann H, Sollid JE, John JF, Jr., Daum R, Soderquist B, Buist G (2012) Guidelines for reporting novel *mecA* gene homologues. Antimicrob Agents Chemother 56(10):4997–4999. doi:AAC.01199-12 [pii] 10.1128/AAC.01199-12

Jarlier V, Trystram D, Brun-Buisson C, Fournier S, Carbonne A, Marty L, Andremont A, Arlet G, Buu-Hoi A, Carlet J, Decre D, Gottot S, Gutmann L, Joly-Guillou ML, Legrand P, Nicolas-Chanoine MH, Soussy CJ, Wolf M, Lucet JC, Aggoune M, Brucker G, Regnier B (2010) Curbing methicillin-resistant *Staphylococcus aureus* in 38 French hospitals through a 15-year institutional control program. Arch Intern Med 170(6):552–559. doi:170/6/552 [pii] 10.1001/archinternmed.2010.32

Jevons MP (1961) "Celbenin"—resistant Staphylococci. Br Med J 1:124–125

Jimenez JN, Ocampo AM, Vanegas JM, Rodriguez EA, Mediavilla JR, Chen L, Muskus CE, Velez LA, Rojas C, Restrepo AV, Ospina S, Garces C, Franco L, Bifani P, Kreiswirth BN, Correa MM (2012) CC8 MRSA strains harboring SCC*mec* type IVc are predominant in Colombian hospitals. PLoS One 7(6):e38576. doi:10.1371/journal.pone.0038576 PONE-D-12-02320 [pii]

Johnson AP, Pearson A, Duckworth G (2005) Surveillance and epidemiology of MRSA bacteraemia in the UK. J Antimicrob Chemother 56(3):455–462. doi:10.1093/jac/dki266

Jones T, Yeaman MR, Sakoulas G, Yang SJ, Proctor RA, Sahl HG, Schrenzel J, Xiong YQ, Bayer AS (2008) Failures in clinical treatment of *Staphylococcus aureus* Infection with daptomycin are associated with alterations in surface charge, membrane phospholipid

asymmetry, and drug binding. Antimicrob Agents Chemother 52(1):269–278. doi:AAC.00719-07 [pii] 10.1128/AAC.00719-07

Julian K, Kosowska-Shick K, Whitener C, Roos M, Labischinski H, Rubio A, Parent L, Ednie L, Koeth L, Bogdanovich T, Appelbaum PC (2007) Characterization of a daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* strain in a patient with endocarditis. Antimicrob Agents Chemother 51(9):3445–3448. doi:AAC.00559-07 [pii] 10.1128/AAC.00559-07

Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 44(6):1549–1555

Kerr S, Kerr GE, Mackintosh CA, Marples RR (1990) A survey of methicillin-resistant *Staphylococcus aureus* affecting patients in England and Wales. J Hosp Infect 16(1):35–48

Kock R, Harlizius J, Bressan N, Laerberg R, Wieler LH, Witte W, Deurenberg RH, Voss A, Becker K, Friedrich AW (2009) Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. Eur J Clin Microbiol Infect Dis 28(11):1375–1382. doi:10.1007/s10096-009-0795-4

Kock R, Schaumburg F, Mellmann A, Koksal M, Jurke A, Becker K, Friedrich AW (2013) Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. PLoS ONE 8(2):e55040. doi:10.1371/journal.pone.0055040

Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51(1):264–274. doi:AAC.00165-06 [pii] 10.1128/AAC.00165-06

Koser CU, Ellington MJ, Cartwright EJ, Gillespie SH, Brown NM, Farrington M, Holden MT, Dougan G, Bentley SD, Parkhill J, Peacock SJ (2012a) Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog 8(8):e1002824. doi:10.1371/journal.ppat.1002824

Koser CU, Holden MT, Ellington MJ, Cartwright EJ, Brown NM, Ogilvy-Stuart AL, Hsu LY, Chewapreecha C, Croucher NJ, Harris SR, Sanders M, Enright MC, Dougan G, Bentley SD, Parkhill J, Fraser LJ, Betley JR, Schulz-Trieglaff OB, Smith GP, Peacock SJ (2012b) Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. N Engl J Med 366(24):2267–2275. doi:10.1056/NEJMoa1109910

Leach KL, Swaney SM, Colca JR, McDonald WG, Blinn JR, Thomasco LM, Gadwood RC, Shinabarger D, Xiong L, Mankin AS (2007) The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. Mol Cell 26(3):393–402. doi:S1097-2765(07)00221-3 [pii] 10.1016/j.molcel.2007.04.005

Limbago BM, Kallen AJ, Zhu W, Eggers P, McDougal LK, Albrecht VS (2014) Report of the 13th vancomycin-resistant *Staphylococcus aureus* isolate from the United States. J Clin Microbiol 52(3):998–1002. doi:JCM.02187-13 [pii] 10.1128/JCM.02187-13

Lindsay JA (2014) *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. Int J Med Microbiol 304(2):103–109. doi:10.1016/j.ijmm.2013.11.010

Liu C, Chambers HF (2003) *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 47(10):3040–3045

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339(8):520–532. doi:10.1056/NEJM199808203390806

Lowy FD (2003) Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest 111(9):1265–1273. doi:10.1172/JCI18535

Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, Daum RS, Hiramatsu K (2002) Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother 46(4):1147–1152

Maiden MC, Jansen van Rensburg MJ, Bray JE, Earle SG, Ford SA, Jolley KA, McCarthy ND (2013) MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol 11(10):728–736. doi:10.1038/nrmicro3093

Mariem BJ, Ito T, Zhang M, Jin J, Li S, Ilhem BB, Adnan H, Han X, Hiramatsu K (2013) Molecular characterization of methicillin-resistant Panton-Valentine leukocidin positive *Staphylococcus aureus* clones disseminating in Tunisian hospitals and in the community. BMC Microbiol 13:2. doi:1471-2180-13-2 [pii] 10.1186/1471-2180-13-2

McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol 41(11):5113–5120

Mediavilla JR, Chen L, Mathema B, Kreiswirth BN (2012) Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol 15(5):588–595. doi:S1369-5274(12)00118-X [pii] 10.1016/j.mib.2012.08.003

Mehta S, Cuirolo AX, Plata KB, Riosa S, Silverman JA, Rubio A, Rosato RR, Rosato AE (2012) VraSR two-component regulatory system contributes to *mprF*-mediated decreased susceptibility to daptomycin in in vivo-selected clinical strains of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 56(1):92–102. doi:AAC.00432-10 [pii] 10.1128/AAC.00432-10

Mendes RE, Deshpande LM, Jones RN (2014a) Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat 17(1–2):1-12. doi:10.1016/j.drup.2014.04.002

Mendes RE, Flamm RK, Hogan PA, Ross JE, Jones RN (2014b) Summary of linezolid activity and resistance mechanisms detected during the 2012 LEADER surveillance program for the United States. Antimicrob Agents Chemother 58(2):1243–1247. doi:AAC.02112-13 [pii] 10.1128/AAC.02112-13

Mendes RE, Hogan PA, Streit JM, Jones RN, Flamm RK (2014c) Zyvox(R) Annual Appraisal of Potency and Spectrum (ZAAPS) program: report of linezolid activity over 9 years (2004–12). J Antimicrob Chemother 69(6):1582–1588. doi:dkt541 [pii] 10.1093/jac/dkt541

Metzker ML (2010) Sequencing technologies—the next generation. Nat Rev Genet 11(1):31–46. doi:nrg2626 [pii] 10.1038/nrg2626

Miko BA, Hafer CA, Lee CJ, Sullivan SB, Hackel MA, Johnson BM, Whittier S, Della-Latta P, Uhlemann AC, Lowy FD (2013) Molecular characterization of methicillin-susceptible *Staphylococcus aureus* clinical isolates in the United States, 2004 to 2010. J Clin Microbiol 51(3):874–879. doi:10.1128/JCM.00923-12

Milheirico C, Oliveira DC, de Lencastre H (2007) Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCCmec IV multiplex'. J Antimicrob Chemother 60(1):42–48. doi:dkm112 [pii] 10.1093/jac/dkm112

Moisan H, Pruneau M, Malouin F (2010) Binding of ceftaroline to penicillin-binding proteins of *Staphylococcus aureus* and *Streptococcus pneumoniae*. J Antimicrob Chemother 65(4):713–716. doi:10.1093/jac/dkp503

Monaco M, Sanchini A, Grundmann H, Pantosti A (2010) Vancomycin-heteroresistant phenotype in invasive methicillin-resistant *Staphylococcus aureus* isolates belonging to *spa* type 041. Eur J Clin Microbiol Infect Dis 29(7):771–777. doi:10.1007/s10096-010-0922-2

Monaco M, Pedroni P, Sanchini A, Bonomini A, Indelicato A, Pantosti A (2013) Livestock-associated methicillin-resistant *Staphylococcus aureus* responsible for human colonization and infection in an area of Italy with high density of pig farming. BMC Infect Dis 13:258. doi:1471-2334-13-258 [pii] 10.1186/1471-2334-13-258

Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S, Ehricht R (2011) A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS ONE 6(4):e17936. doi:10.1371/journal.pone.0017936

Moon SY, Lee HJ, Lee MS (2010) Molecular characteristics of methicillin-resistant Staphylococcus aureus blood isolates: clonal spread of staphylococcal cassette chromosome *mec* type IVA between the community and the hospital. Microb Drug Resist 16(3):217–222. doi:10.1089/mdr.2010.0010

Morales G, Picazo JJ, Baos E, Candel FJ, Arribi A, Pelaez B, Andrade R, de la Torre MA, Fereres J, Sanchez-Garcia M (2010) Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. Clin Infect Dis 50(6):821–825. doi:10.1086/650574

Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA, Group EMINS (2006) Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 355(7):666–674. doi:10.1056/NEJMoa055356

Morata L, Mensa J, Soriano A (2015) New antibiotics against gram-positives: present and future indications. Curr Opin Pharmacol 24:45–51. doi:10.1016/j.coph.2015.07.004

Munckhof WJ, Schooneveldt J, Coombs GW, Hoare J, Nimmo GR (2003) Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infection in Queensland, Australia. Int J Infect Dis 7(4):259–264. doi:S1201971203901044 [pii]

Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE, Fussing V, Salmenlinna S, Vuopio-Varkila J, El Solh N, Cuny C, Witte W, Tassios PT, Legakis N, van Leeuwen W, van Belkum A, Vindel A, Laconcha I, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Coombes G, Cookson B (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J Clin Microbiol 41(4):1574–1585

Nichol KA, Adam HJ, Roscoe DL, Golding GR, Lagace-Wiens PR, Hoban DJ, Zhanel GG (2013) Changing epidemiology of methicillin-resistant *Staphylococcus aureus* in Canada. J Antimicrob Chemother 68 Suppl 1:i47–55. doi:dkt026 [pii] 10.1093/jac/dkt026

Nielsen R, Paul JS, Albrechtsen A, Song YS (2011) Genotype and SNP calling from next-generation sequencing data. Nat Rev Genet 12(6):443–451. doi:nrg2986 [pii] 10.1038/nrg2986

Nimmo GR (2012) USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 18(8):725–734. doi:10.1111/j.1469-0691.2012.03822.x S1198-743X(14)63431-5 [pii]

Nimmo GR, Coombs GW (2008) Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) in Australia. Int J Antimicrob Agents 31(5):401–410. doi:S0924-8579(07)00429-3 [pii] 10.1016/j.ijantimicag.2007.08.011

Novick RP, Bouanchaud D (1971) The problems of drug-resistant pathogenic bacteria. Extrachromosomal nature of drug resistance in *Staphylococcus aureus*. Ann N Y Acad Sci 182:279–294

Nubel U, Roumagnac P, Feldkamp M, Song JH, Ko KS, Huang YC, Coombs G, Ip M, Westh H, Skov R, Struelens MJ, Goering RV, Strommenger B, Weller A, Witte W, Achtman M (2008) Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A 105(37):14130–14135. doi:10.1073/pnas.0804178105

Nubel U, Strommenger B, Layer F, Witte W (2011) From types to trees: reconstructing the spatial spread of *Staphylococcus aureus* based on DNA variation. Int J Med Microbiol 301(8):614–618. doi:S1438-4221(11)00093-2 [pii] 10.1016/j.ijmm.2011.09.007

Oliveira DC, Tomasz A, de Lencastre H (2002) Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. Lancet Infect Dis 2(3):180–189

Otter JA, French GL (2012) Community-associated meticillin-resistant *Staphylococcus aureus*: the case for a genotypic definition. J Hosp Infect 81(3):143–148. doi:10.1016/j.jhin.2012.04.009

Pantosti A (2012) Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. Front Microbiol 3:127. doi:10.3389/fmicb.2012.00127

Pantosti A, Venditti M (2009) What is MRSA? Eur Respir J 34(5):1190–1196. doi:10.1183/09031936.00007709

Pantosti A, Sanchini A, Monaco M (2007) Mechanisms of antibiotic resistance in *Staphylococcus aureus*. Future Microbiol 2(3):323–334. doi:10.2217/17460913.2.3.323

Patel JB, Jevitt LA, Hageman J, McDonald LC, Tenover FC (2006) An association between reduced susceptibility to daptomycin and reduced susceptibility to vancomycin in *Staphylococcus aureus*. Clin Infect Dis 42(11):1652–1653. doi:CID39100 [pii] 10.1086/504084

Pearson A, Chronias A, Murray M (2009) Voluntary and mandatory surveillance for methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) bacteraemia in England. J Antimicrob Chemother 64(Suppl 1):i11–17. doi:dkp260 [pii] 10.1093/jac/dkp260

Perez-Vazquez M, Vindel A, Marcos C, Oteo J, Cuevas O, Trincado P, Bautista V, Grundmann H, Campos J, Group ESs-t (2009) Spread of invasive Spanish *Staphylococcus aureus spa*-type t067 associated with a high prevalence of the aminoglycoside-modifying enzyme gene *ant*(4')-Ia and the efflux pump genes *msrA/msrB*. J Antimicrob Chemother 63(1):21–31. doi:10.1093/jac/dkn430

Pillai SK, Wennersten C, Venkataraman L, Eliopoulos GM, Moellering RC, Karchmer AW (2009) Development of reduced vancomycin susceptibility in methicillin-susceptible *Staphylococcus aureus*. Clin Infect Dis 49(8):1169–1174. doi:10.1086/605636

Price JR, Didelot X, Crook DW, Llewelyn MJ, Paul J (2013) Whole genome sequencing in the prevention and control of *Staphylococcus aureus* infection. J Hosp Infect 83(1):14–21. doi:S0195-6701(12)00337-4 [pii] 10.1016/j.jhin.2012.10.003

Raji A, Ojemhen O, Umejiburu U, Ogunleye A, Blanc DS, Basset P (2013) High genetic diversity of *Staphylococcus aureus* in a tertiary care hospital in Southwest Nigeria. Diagn Microbiol Infect Dis 77(4):367–369. doi:10.1016/j.diagmicrobio.2013.08.030

Rasigade JP, Laurent F, Lina G, Meugnier H, Bes M, Vandenesch F, Etienne J, Tristan A (2010) Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981–2007. J Infect Dis 201(10):1589–1597. doi:10.1086/652008

Reed KD, Stemper ME, Shukla SK (2007) Pulsed-field gel electrophoresis of MRSA. Methods Mol Biol 391:59–69. doi:10.1007/978-1-59745-468-1_5

Reyes J, Rincon S, Diaz L, Panesso D, Contreras GA, Zurita J, Carrillo C, Rizzi A, Guzman M, Adachi J, Chowdhury S, Murray BE, Arias CA (2009) Dissemination of methicillin-resistant *Staphylococcus aureus* USA300 sequence type 8 lineage in Latin America. Clin Infect Dis 49(12):1861–1867. doi:10.1086/648426

Ritchie SR, Thomas MG, Rainey PB (2014) The genetic structure of *Staphylococcus aureus* populations from the Southwest Pacific. PLoS ONE 9(7):e100300. doi:10.1371/journal.pone.0100300

Robinson DA, Enright MC (2003) Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 47(12):3926–3934

Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC (2005) Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired meticillin-resistant clone. Lancet 365(9466):1256–1258. doi:S0140-6736(05)74814-5 [pii] 10.1016/S0140-6736(05)74814-5

Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, Rincon S, Narechania A, Xing G, Di Gioia TS, Doi A, Tran TT, Reyes J, Munita JM, Carvajal LP, Hernandez-Roldan A, Brandao D, van der Heijden IM, Murray BE, Planet PJ, Weinstock GM, Arias CA (2014) Transferable vancomycin resistance in a community-associated MRSA lineage. N Engl J Med 370(16):1524–1531. doi:10.1056/NEJMoa1303359

Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC (2007) The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. J Clin Microbiol 45(8):2554–2563. doi:JCM.00245-07 [pii] 10.1128/JCM.00245-07

Sabat AJ, Budimir A, Nashev D, Sa-Leao R, van Dijl J, Laurent F, Grundmann H, Friedrich AW, Markers ESGoE (2013) Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill 18(4):20380

Sader HS, Farrell DJ, Flamm RK, Jones RN (2014) Daptomycin activity tested against 164457 bacterial isolates from hospitalised patients: summary of 8 years of a Worldwide Surveillance Programme (2005–2012). Int J Antimicrob Agents 43(5):465-469. doi:S0924-8579(14)00032-6 [pii] 10.1016/j.ijantimicag.2014.01.018

Saha B, Singh AK, Ghosh A, Bal M (2008) Identification and characterization of a vancomycin-resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). J Med Microbiol 57(Pt 1):72–79. doi:57/1/72 [pii] 10.1099/jmm.0.47144-0

Sakoulas G (2009) Clinical outcomes with daptomycin: a post-marketing, real-world evaluation. Clin Microbiol Infect 15(Suppl 6):11–16. doi:CLM3054 [pii] 10.1111/j.1469-0691.2009.03054.x

Sakoulas G, Eliopoulos GM, Moellering RC Jr, Wennersten C, Venkataraman L, Novick RP, Gold HS (2002) Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. Antimicrob Agents Chemother 46(5):1492–1502

Salipante SJ, SenGupta DJ, Cummings LA, Land TA, Hoogestraat DR, Cookson BT (2015) Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. J Clin Microbiol 53(4):1072–1079. doi:JCM.03385-14 [pii] 10.1128/JCM.03385-14

Sanchez Garcia M, De la Torre MA, Morales G, Pelaez B, Tolon MJ, Domingo S, Candel FJ, Andrade R, Arribi A, Garcia N, Martinez Sagasti F, Fereres J, Picazo J (2010) Clinical outbreak of linezolid-resistant *Staphylococcus aureus* in an intensive care unit. JAMA 303(22):2260–2264. doi:303/22/2260 [pii] 10.1001/jama.2010.757

Sanchini A, Campanile F, Monaco M, Cafiso V, Rasigade JP, Laurent F, Etienne J, Stefani S, Pantosti A (2011) DNA microarray-based characterisation of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* from Italy. Eur J Clin Microbiol Infect Dis 30(11):1399–1408. doi:10.1007/s10096-011-1234-x

Sanchini A, Spitoni MG, Monaco M, Raglio A, Grigis A, Petro W, Menchini M, Pesenti A, Goglio A, Pantosti A (2013) Outbreak of skin and soft tissue infections in a hospital newborn nursery in Italy due to community-acquired meticillin-resistant *Staphylococcus aureus* USA300 clone. J Hosp Infect 83(1):36–40. doi:10.1016/j.jhin.2012.09.017

Sanchini A, Del Grosso M, Villa L, Ammendolia MG, Superti F, Monaco M, Pantosti A (2014) Typing of Panton-Valentine leukocidin-encoding phages carried by methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* from Italy. Clin Microbiol Infect 20(11):O840–846. doi:10.1111/1469-0691.12679

Schatz MC, Delcher AL, Salzberg SL (2010) Assembly of large genomes using second-generation sequencing. Genome Res 20(9):1165–1173. doi:gr.101360.109 [pii] 10.1101/gr.101360.109

Schaumburg F, Kock R, Friedrich AW, Soulanoudjingar S, Ngoa UA, von Eiff C, Issifou S, Kremsner PG, Herrmann M, Peters G, Becker K (2011) Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. PLoS Negl Trop Dis 5(5):e1150. doi:10.1371/journal.pntd.0001150

Schaumburg F, Alabi AS, Peters G, Becker K (2014) New epidemiology of *Staphylococcus aureus* infection in Africa. Clin Microbiol Infect 20(7):589–596. doi:10.1111/1469-0691.12690 S1198-743X(14)61144-7 [pii]

Schwarz S, Werckenthin C, Kehrenberg C (2000) Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. Antimicrob Agents Chemother 44(9):2530–2533

Shabir S, Hardy KJ, Abbasi WS, McMurray CL, Malik SA, Wattal C, Hawkey PM (2010) Epidemiological typing of meticillin-resistant *Staphylococcus aureus* isolates from Pakistan and India. J Med Microbiol 59(Pt 3):330–337. doi:10.1099/jmm.0.014910-0

Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC (2013) The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect Dis 13(1):43–54. doi:10.1016/S1473-3099(12)70238-4

Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC (2008) Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002–2006. Clin Infect Dis 46(5):668–674. doi:10.1086/527392

Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B, Fridkin S (2013) Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2009-2010. Infect Control Hosp Epidemiol 34(1):1–14. doi:10.1086/668770

Silva-Carvalho MC, Bonelli RR, Souza RR, Moreira S, dos Santos LC, de Souza Conceicao M, de Mello Junior SJ, Carballido JM, Rito PN, Vieira VV, Teixeira LA, Sa Figueiredo AM (2009) Emergence of multiresistant variants of the community-acquired methicillin-resistant *Staphylococcus aureus* lineage ST1-SCC*mec*IV in 2 hospitals in Rio de Janeiro, Brazil. Diagn Microbiol Infect Dis 65(3):300–305. doi:10.1016/j.diagmicrobio.2009.07.023

Smith TC, Pearson N (2011) The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis 11(4):327–339. doi:10.1089/vbz.2010.0072

Smyth DS, McDougal LK, Gran FW, Manoharan A, Enright MC, Song JH, de Lencastre H, Robinson DA (2010) Population structure of a hybrid clonal group of methicillin-resistant *Staphylococcus aureus*, ST239-MRSA-III. PLoS ONE 5(1):e8582. doi:10.1371/journal.pone.0008582

Sowash MG, Uhlemann AC (2014) Community-associated methicillin-resistant *Staphylococcus aureus* case studies. Methods Mol Biol 1085:25–69. doi:10.1007/978-1-62703-664-1_2

Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, Kemmink J, de Haas CJ, van Kessel KP, Vandenesch F, Lina G, van Strijp JA (2013) The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. Cell Host Microbe 13(5):584–594. doi:S1931-3128(13)00148-0 [pii] 10.1016/j.chom.2013.04.006

Spaan AN, Schiepers A, de Haas CJ, van Hooijdonk DD, Badiou C, Contamin H, Vandenesch F, Lina G, Gerard NP, Gerard C, van Kessel KP, Henry T, van Strijp JA (2015) Differential interaction of the staphylococcal toxins Panton-Valentine leukocidin and gamma-Hemolysin CB with Human C5a Receptors. J Immunol. doi:jimmunol.1500604 [pii] 10.4049/jimmunol.1500604

Srinivasan A, Dick JD, Perl TM (2002) Vancomycin resistance in staphylococci. Clin Microbiol Rev 15(3):430–438

Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM (2012) Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 39(4):273–282. doi:S0924-8579(11)00468-7 [pii] 10.1016/j.ijantimicag.2011.09.030

Straus SK, Hancock RE (2006) Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: comparison with cationic antimicrobial peptides and lipopeptides. Biochim Biophys Acta 1758(9):1215–1223. doi:S0005-2736(06)00043-5 [pii] 10.1016/j.bbamem.2006.02.009

Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, Nubel U, Witte W (2008) *spa* Typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. J Clin Microbiol 46(2):574–581. doi:JCM.01599-07 [pii] 10.1128/JCM.01599-07

Stryjewski ME, Chambers HF (2008) Skin and soft-tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 46(Suppl 5):S368–377. doi:10.1086/533593

Tenover FC, Weigel LM, Appelbaum PC, McDougal LK, Chaitram J, McAllister S, Clark N, Killgore G, O'Hara CM, Jevitt L, Patel JB, Bozdogan B (2004) Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrob Agents Chemother 48(1):275–280

Tenover FC, Goering RV (2009) Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J Antimicrob Chemother 64(3):441–446. doi:dkp241 [pii] 10.1093/jac/dkp241

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33(9):2233–2239

Tsao SM, Wang WY, Ko WC, Huang CH, Lu CT, Chuang YC, Liu CY, Liao CH, Chen YS, Liu YC, Chen WY, Jang TN, Lin HC, Chen CM, Shi ZY, Pan SC, Yang JL, Kung HC, Liu CE, Cheng YJ, Liu JW, Sun W, Wang LS, Yu KW, Chiang PC, Lee MH, Lee CM, Hsu GJ, Chen YH, Lu PL, Thomas CY, Hsueh PR (2014) Trend in vancomycin susceptibility and correlation with molecular characteristics of methicillin-resistant *Staphylococcus aureus* causing invasive infections in Taiwan: results from the Tigecycline in vitro Surveillance in Taiwan (TIST) study, 2006–2010. Diagn Microbiol Infect Dis 80(2):162–167. doi:10.1016/j.diagmicrobio.2014.06.007

Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, Moellering RC, Ferraro MJ (2001) Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet 358(9277):207–208. doi:S0140-6736(01)05410-1 [pii] 10.1016/S0140-6736(01)05410-1

Uhlemann AC, Otto M, Lowy FD, DeLeo FR (2014) Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. Infect Genet Evol 21:563–574. doi:S1567-1348(13)00179-2 [pii] 10.1016/j.meegid.2013.04.030

Valentin-Domelier AS, Girard M, Bertrand X, Violette J, Francois P, Donnio PY, Talon D, Quentin R, Schrenzel J, van der Mee-Marquet N, Bloodstream Infection Study Group of the Reseau des Hygienistes du C (2011) Methicillin-susceptible ST398 *Staphylococcus aureus* responsible for bloodstream infections: an emerging human-adapted subclone? PLoS One 6(12):e28369. doi:10.1371/journal.pone.0028369

Van Cleef BA, Verkade EJ, Wulf MW, Buiting AG, Voss A, Huijsdens XW, van Pelt W, Mulders MN, Kluytmans JA (2010) Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. PLoS ONE 5(2):e9385. doi:10.1371/journal.pone.0009385

van Cleef BA, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, Zemlickova H, Skov RL, Vuopio-Varkila J, Cuny C, Friedrich AW, Spiliopoulou I, Paszti J, Hardardottir H, Rossney A, Pan A, Pantosti A, Borg M, Grundmann H, Mueller-Premru M, Olsson-Liljequist B, Widmer A, Harbarth S, Schweiger A, Unal S, Kluytmans JA (2011) Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg Infect Dis 17(3):502–505. doi:10.3201/eid1703.101036

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9(8):978–984. doi:10.3201/eid0908.030089

Verkade E, Kluytmans J (2014) Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. Infect Genet Evol 21:523–530. doi:10.1016/j.meegid.2013.02.013

Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC (2003) Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. Science 302(5650):1569–1571. doi:10.1126/science.1090956 302/5650/1569 [pii]

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5(12):751–762. doi:S1473-3099(05)70295-4 [pii] 10.1016/S1473-3099(05)70295-4

WHO (2014) Antimicrobial resistance: global report on surveillance 2014. World Health Organization, Geneva

Williamson DA, Coombs GW, Nimmo GR (2014) *Staphylococcus aureus* 'Down Under': contemporary epidemiology of *S. aureus* in Australia, New Zealand, and the South West Pacific. Clin Microbiol Infect 20(7):597–604. doi:10.1111/1469-0691.12702 S1198-743X(14)61145-9 [pii]

Witte W (2009) Community-acquired methicillin-resistant *Staphylococcus aureus*: what do we need to know? Clin Microbiol Infect 15(Suppl 7):17–25. doi:10.1111/j.1469-0691.2009.03097.x

Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C, Verduin CM (2008) First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, June 2007. Euro Surveill 13 (9)

Zhang K, McClure JA, Elsayed S, Tan J, Conly JM (2008) Coexistence of Panton-Valentine leukocidin-positive and -negative community-associated methicillin-resistant *Staphylococcus aureus* USA400 sibling strains in a large Canadian health-care region. J Infect Dis 197(2):195–204. doi:10.1086/523763

Zhu W, Clark NC, McDougal LK, Hageman J, McDonald LC, Patel JB (2008) Vancomycin-resistant *Staphylococcus aureus* isolates associated with Inc18-like *vanA* plasmids in Michigan. Antimicrob Agents Chemother 52(2):452–457. AAC.00908-07 [pii] doi:10.1128/AAC.00908-07

Zhu W, Murray PR, Huskins WC, Jernigan JA, McDonald LC, Clark NC, Anderson KF, McDougal LK, Hageman JC, Olsen-Rasmussen M, Frace M, Alangaden GJ, Chenoweth C, Zervos MJ, Robinson-Dunn B, Schreckenberger PC, Reller LB, Rudrik JT, Patel JB (2010) Dissemination of an Enterococcus Inc18-Like *vanA* plasmid associated with vancomycin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 54(10):4314–4320. doi:AAC.00185-10 [pii] 10.1128/AAC.00185-10

Structure and Function of Surface Polysaccharides of *Staphylococcus aureus*

Christopher Weidenmaier and Jean C. Lee

Abstract The major surface polysaccharides of *Staphylococcus aureus* include the capsular polysaccharide (CP), cell wall teichoic acid (WTA), and polysaccharide intercellular adhesin/poly-β(1-6)-N-acetylglucosamine (PIA/PNAG). These glycopolymers are important components of the staphylococcal cell envelope, but none of them is essential to *S. aureus* viability and growth in vitro. The overall biosynthetic pathways of CP, WTA, and PIA/PNAG have been elucidated, and the functions of most of the biosynthetic enzymes have been demonstrated. Because *S. aureus* CP and WTA (but not PIA/PNAG) utilize a common cell membrane lipid carrier (undecaprenyl-phosphate) that is shared by the peptidoglycan biosynthesis pathway, there is evidence that these processes are highly integrated and temporally regulated. Regulatory elements that control glycopolymer biosynthesis have been described, but the cross talk that orchestrates the biosynthetic pathways of these three polysaccharides remains largely elusive. CP, WTA, and PIA/PNAG each play distinct roles in *S. aureus* colonization and the pathogenesis of staphylococcal infection. However, they each promote bacterial evasion of the host immune defences, and WTA is being explored as a target for antimicrobial therapeutics. All the three glycopolymers are viable targets for immunotherapy, and each (conjugated to a carrier protein) is under evaluation for inclusion in a multivalent *S. aureus* vaccine. Future research findings that increase our understanding of these surface polysaccharides, how the bacterial cell regulates their expression, and their biological functions will likely reveal new approaches to controlling this important bacterial pathogen.

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Abbreviations

BY-kinases	Bacterial tyrosine kinases
CP	Capsular polysaccharide
D-FucNAc	D-*N*-acetyl fucosamine
D-GlcNAc	D-*N*-acetyl glucosamine
D-ManNAc	D-*N*-acetyl mannosamine
D-ManNAcA	D-*N*-acetyl mannosaminuronic acid
GroP	Glycerol phosphate
HGT	Horizontal gene transfer
L-FucNAc	L-*N*-acetyl fucosamine
MBL	Mannose-binding lectin
MurNAc	*N*-acetyl muramic acid
NT	Nontypeable
PIA	Polysaccharide intercellular adhesin
PNAG	Poly-β(1-6)-*N*-acetylglucosamine
PG	Peptidoglycan
RboP	Ribitol phosphate
UDP	Undecaprenyl-phosphate
WTA	Wall teichoic acid

Contents

1 Introduction

The cell envelope of *Staphylococcus aureus* and other Gram-positive microbes is a structurally complex surface organelle that exerts important protective functions for the bacterium. The cell envelope is composed of peptidoglycan, other cell wall glycopolymers (often termed secondary cell wall glycopolymers), and proteins. Besides its role in staphylococcal physiology, the cell envelope also plays a major role in staphylococcal virulence. We focus here on the cell surface polysaccharides of *S. aureus* that are major structural determinants of the cell envelope, play key roles in host cell interactions, and are therefore promising candidates as targets for anti-infective therapies and vaccines: capsular polysaccharides (CPs), cell wall teichoic acid (WTA), and polysaccharide intercellular adhesin/poly-β(1-6)-*N*-acetylglucosamine (PIA/PNAG).

2 Capsular Polysaccharides (CPs)

Most bacteria causing invasive diseases produce CPs that serve as essential virulence factors, impeding phagocytosis and enabling bacterial persistence in the bloodstream of nonimmune infected hosts (Horwitz 1980; Wilkinson 1958; Roberts et al. 1989; Bittersuermann 1993). In the case of *S. aureus*, an important opportunistic pathogen that plays a major role in nosocomial and community-acquired infections, CPs are produced by the majority of clinical isolates. Although as many as 13 serotypes were described early on, capsular serotypes 5 (CP5) and 8 (CP8) predominate among isolates from humans, accounting for ∼25 and 50 % of strains examined (Hochkeppel et al. 1987; Roghmann et al. 2005; Verdier et al. 2007). CP serotypes 1 and 2 are authentic, but they have not been detected among collections of clinical isolates. This observation is consistent with a report from Luong et al. (2002b) who discovered that the genes encoding CP1 were located on a genetic element that was defective in mobilization. Like other bacterial capsules, the *S. aureus* CPs have been shown to possess antiphagocytic properties, allowing the bacterium to persist in the blood and tissues of infected hosts (Thakker et al. 1998; Watts et al. 2005; Portoles et al. 2001).

2.1 Structures of CP5 and CP8

CP5 and CP8 share similar trisaccharide repeating units of D-*N*-acetyl mannosaminuronic acid (ManNAcA), L-*N*-acetyl fucosamine (L-FucNAc), and D-*N*-acetyl fucosamine (D-FucNAc). The trisaccharide structures are identical in monosaccharide composition and sequence, and they differ only in the glycosidic linkages between the sugars and the sites of *O*-acetylation (Fournier et al. 1984; Moreau et al. 1990; Jones 2005). The repeating unit structures of CP5 and CP8 are shown in Fig. 1.

2.2 Biosynthesis of CP

Almost all *S. aureus* strains contain either a *cap5A-P* or a *cap8A-P* gene operon that encodes enzymes for CP5 or CP8 biosynthesis, respectively. The *cap5* and *cap8* gene clusters (Fig. 2) are chromosomally allelic. The 12 flanking genes are almost

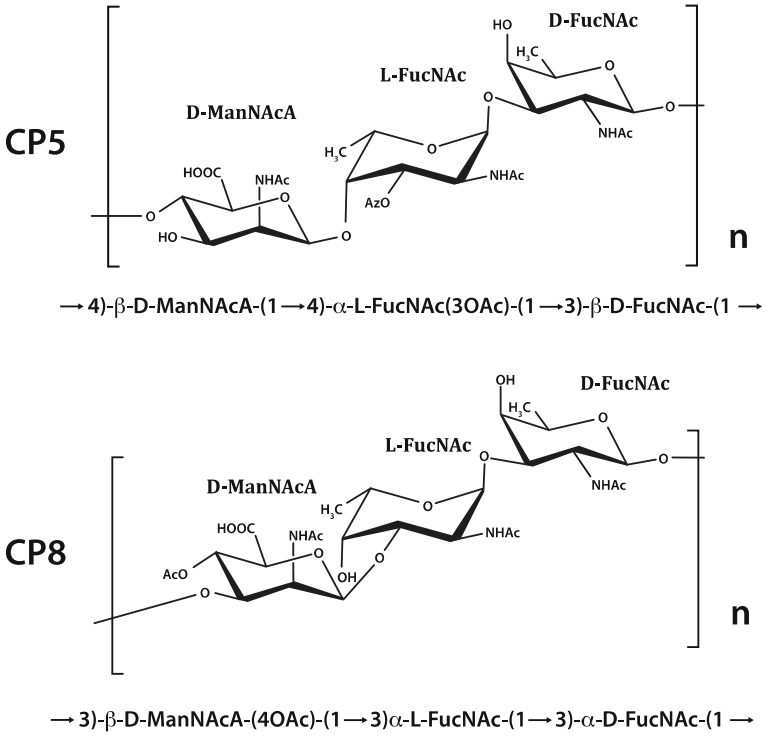

Fig. 1 Structures of the *S. aureus* serotype 5 (*CP5*) and serotype 8 (*CP8*) capsular polysaccharides. Copyright © American Society for Microbiology, Infect Immun, 82, 2014, 5049-5055, doi:10.1128/IAI.02373-14

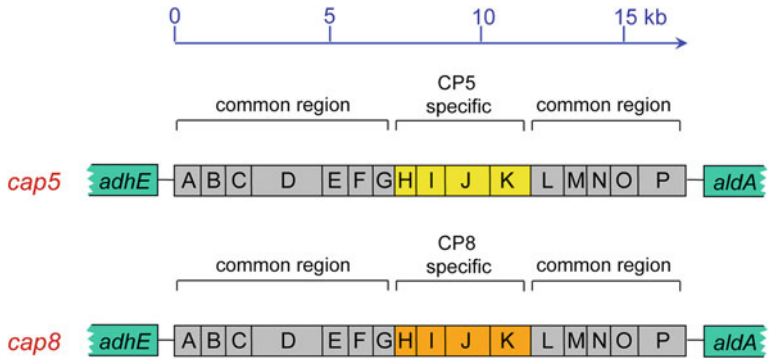

Fig. 2 *S. aureus cap5* and *cap8* gene clusters are allelic. The type-specific genes *capHIJK* are flanked on either side by biosynthetic genes that are conserved between *cap5* and *cap8*

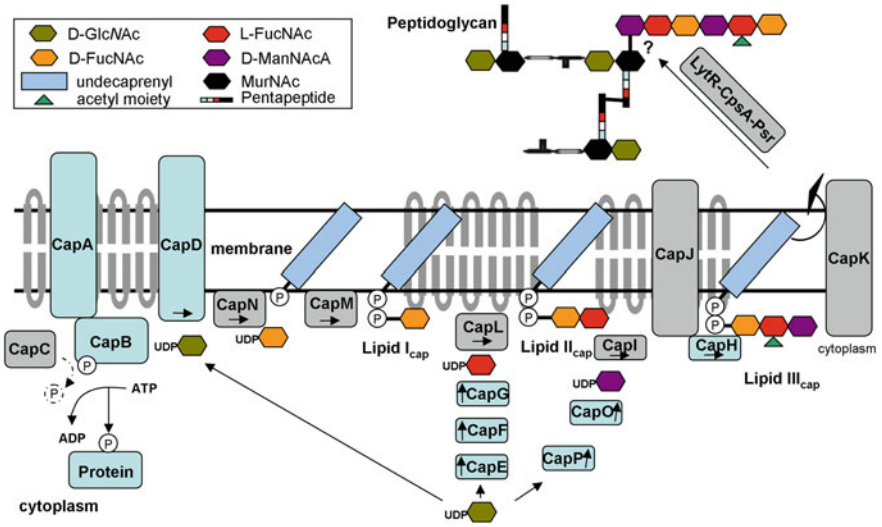

Fig. 3 Putative pathway for the biosynthesis of *S. aureus* CP. The gene products for which functions have been verified (CapABDEFGHOP) are indicated by color, and the gene products for which functions are still not confirmed are shown in *gray*

identical, whereas the central genes *capHIJK* bear little homology to each other and are type specific (Sau et al. 1997a).

The CP biosynthetic gene clusters comprise 16 genes encoding for proteins involved in capsule biosynthesis (Kiser et al. 1999b; Portoles et al. 2001; Kneidinger et al. 2003), *O*-acetylation (Bhasin et al. 1998), transport, and regulation (Soulat et al. 2006, 2007; Gruszczyk et al. 2013). Database homology searches with predicted amino acid sequences of the *cap5* operon gene products allowed for the prediction of individual enzymatic functions and the proposal of a pathway for CP5 biosynthesis in *S. aureus* (O'Riordan and Lee 2004).

Synthesis of the soluble CP5 precursors has been investigated (Fig. 3) and occurs in three distinct steps in which the universal substrate UDP-D-GlcNAc is converted into three different nucleotide-coupled sugars: UDP-D-FucNAc, UDP-L-FucNAc, and UDP-D-ManNAcA. The synthesis of UDP-D-FucNAc is accomplished by the enzyme Cap5D, which converts UDP-GlcNAc to the intermediate UDP-2-acetamido-2,6-dideoxy-α-D-xylo-hex-4-ulose (Li et al. 2014). This product can then be stereospecifically reduced at C4 to UDP-D-FucNAc by Cap5 N, although experimental evidence is still lacking to support this step. Cap5 M is predicted to transfer D-FucNAc to the membrane-anchored lipid carrier undecaprenyl-phosphate, yielding lipid I_{cap}.

The second reaction that generates the soluble precursor UDP-L-FucNAc involves the enzymes Cap5E, Cap5F, and Cap5G (Kneidinger et al. 2003). The bifunctional enzyme Cap5E catalyzes the 4,6-dehydration and 3,5-epimerization of UDP-GlcNAc, resulting in the formation of UDP-2-acetoamido-2,6-dideoxy-I-*lyxo*-4-hexulose. This intermediate is then reduced to UDP-2-acetoamido-2,6-dideoxy-I-talose by the enzyme Cap5F. The activity of the epimerase Cap5G results in the product UDP-I-FucNAc, which is most likely linked to lipid I_{cap} by the transferase Cap5L, leading to the formation of the second capsule lipid intermediate, lipid II_{cap}.

The third step involves the synthesis of UDP-ManNAcA, which is generated by the enzymes Cap5P and Cap5O (Kiser et al. 1999a; Portoles et al. 2001). Cap5P is an epimerase that converts UDP-D-GlcNAc into UDP-*N*-acetylmannosamine. This enzymatic product is oxidized to UDP-D-ManNAcA by the dehydrogenase Cap5O. The transmembrane protein Cap5I has been proposed to transfer the ManNAcA moiety to lipid II_{cap}, thereby generating the CP5 lipid precursor, lipid III_{cap}. CP5 is *O*-acetylated at position C3 of the I-FucNAc residue (Jones 2005) by the cytoplasmic enzyme Cap5H, an *O*-acetyltransferase enzyme (Bhasin et al. 1998). This modification likely occurs on lipid III_{cap}, although experimental evidence for this is lacking. Lipid III_{cap} is then translocated to the outer surface of the cell membrane, where trisaccharide polymerization takes place. These processes are likely facilitated by the putative flippase Cap5K and polymerase Cap5J, respectively (Sau et al. 1997a; O'Riordan and Lee 2004). There is evidence that the polymerized CP5 is linked to the peptidoglycan by a LytR-CpsA-Psr enzyme-mediated mechanism (Chan et al. 2014), presumably releasing the undecaprenyl-phosphate carrier to begin a new synthesis cycle. The undecaprenyl-phosphate carrier is usually found in limited amounts within the cell (Barreteau et al. 2009) and is required for the biosynthesis of peptidoglycan, WTA, and CP (Bouhss et al. 2008; Guo et al. 2008; Xia et al. 2010). Spatial and temporal regulation of these processes is poorly understood but is crucial for the maintenance of bacterial cell growth and cell division.

Modulation of the activity of biosynthetic genes within the *cap5* locus has been linked to tyrosine phosphorylation (Soulat et al. 2006, 2007). Bacterial tyrosine kinases (BY-kinases) (Grangeasse et al. 2007) belong to the family of P-loop containing kinases (Lee and Jia 2009) in which the "P-loop" designates a characteristic amino acid sequence resembling the Walker A nucleotide-binding motif. BY-kinases of Firmicutes are composed of two interacting polypeptides, a transmembrane activator protein and a cytoplasmic BY-kinase (Grangeasse et al. 2007). The cytoplasmic

kinase carries a C-terminal tyrosine cluster that undergoes autophosphorylation in the presence of ATP. Phosphate groups are then transferred to tyrosine residues of target proteins, thereby modulating their enzymatic activity. A Gram-positive protein tyrosine phosphatase has been shown to dephosphorylate the tyrosine kinase enzyme, resulting in downregulation of its activity (Morona et al. 2002).

Adjacent genes encoding a transmembrane modulator, a cytoplasmic BY-kinase, and a putative phosphatase have been identified in the genome of *S. aureus*. The *cap5ABC* genes are located at the 5′-end of the *cap5* operon, and the highly similar *cap1ABC* genes are located upstream of the *S. aureus ica* locus (Sau et al. 1997a; Soulat et al. 2006). CapA proteins are the transmembrane activators, whereas the CapB proteins are the tyrosine kinase enzymes. In vitro kinase activity could be demonstrated for Cap1B, but not for Cap5B (Soulat et al. 2006; Gruszczyk et al. 2013). Nonetheless, Soulat et al. (2007) demonstrated that the Cap5A/1B complex phosphorylates the dehydrogenase Cap5O at Tyr89 and that this modification enhanced Cap5O enzymatic activity in vitro. *S. aureus* Cap5C and Cap1C show ∼55 % amino acid similarity to *Streptococcus pneumoniae* CpsB, a manganese-dependent phosphotyrosine protein phosphatase that belongs to the polymerase and histidinol phosphatase family of phosphoesterases (Morona et al. 2002). Thus, Cap5C is predicted to dephosphorylate the tyrosine kinase activity of Cap1B to modulate CP production by *S. aureus.*

2.3 Nontypeable S. aureus Isolates

As noted above, 20–25 % of *S. aureus* clinical isolates are nontypeable (NT), i.e., they are nonreactive with antibodies to CP types 1, 2, 5, or 8. Genomic analyses of *S. aureus* isolates from humans has revealed that the *cap5(8)* locus is present in all strains, regardless of whether or not they produce CP. A molecular characterization of the *cap5(8)* locus from a limited number of isolates revealed at least three mechanisms that can account for the lack of CP production by NT isolates (Cocchiaro et al. 2006). First, random point mutations in one of the eleven essential *cap* genes yielded nonfunctional biosynthetic enzymes, resulting in the failure to synthesize CP. These mutations could be complemented by functional *cap5(8)* genes provided in trans. Secondly, mutations in regulatory loci also resulted in CP-negative phenotypes. Clinical isolates with mutations in the global regulator Agr or in the ArlRS two-component regulatory system were phenotypically NT. Some clinical isolates carried mutations in the promoter (P_{cap}) upstream of *cap5(8) A* that is critical for transcription of the *cap* locus. In light of the complex regulation of *S. aureus* CP production (discussed below), other mutations undoubtedly exist in alternative regulatory circuits that negatively impact CP expression.

The predominant community-acquired methicillin-resistant *S. aureus* (MRSA) clone in the USA is USA300. Both MRSA and methicillin-sensitive variants of USA300 (and its presumed progenitor USA500) share a CP-negative phenotype (Montgomery et al. 2008; Boyle-Vavra et al. 2015). Whole-genome sequence

analysis of 146 USA300 MRSA isolates revealed that they all carry an intact *cap5* operon. However, compared to strains that are CP5+, the USA300 lineage contains four conserved mutations. Genetic complementation experiments revealed that three of the four mutations (in the *cap5* promoter, in *cap5D*, and in *cap5E*) ablate CP production in USA300 (Boyle-Vavra et al. 2015) (Table 1).

2.4 Regulation of CP Biosynthesis

S. aureus skilfully controls CP synthesis by various regulatory mechanisms in order to adapt to its changing environments (O'Riordan and Lee 2004). CP production is maximal in the postexponential growth phase and is enhanced by growth in high-salt medium, under iron limitation, in well-aerated broth cultures, or on solid medium (O'Riordan and Lee 2004; Poutrel et al. 1995; Cunnion et al. 2001; Lee et al. 1993). In contrast, inhibition of CP production is achieved by cultivation under conditions of high glucose, CO_2, or anaerobiosis (Herbert et al. 1997). The *cap5(8)* operons are transcribed by P_{cap} upstream of *capA* (Sau et al. 1997b; Herbert et al. 2001). P_{cap} activity generally correlates with CP synthesis, suggesting that regulation occurs predominantly at the transcriptional level (Hartmann et al. 2014; Jansen et al. 2013; Meier et al. 2007; Romilly et al. 2014). Different environmental cues are signaled through the activity of a number of interactive regulatory systems composed of transcriptional factors and two-component regulatory systems. Notably, CP production is upregulated by Agr (Dassy et al. 1993; Pohlmann-Dietze et al. 2000; Luong et al. 2002a), ArlRS (Fournier et al. 2001; Cocchiaro et al. 2006; Liang et al. 2005; Luong and Lee 2006), SigB (Bischoff et al. 2004), MgrA (Gupta et al. 2013; Romilly et al. 2014; Luong et al. 2003), SpoVG (Schulthess et al. 2009), and CcpA (Seidl et al. 2006). Repression of CP production has been reported for CodY (Majerczyk et al. 2010; Pohl et al. 2009), CcpE (Hartmann et al. 2014; Ding et al. 2014), SaeRS (Steinhuber et al. 2003; Luong et al. 2011), and Air (Sun et al. 2012). Not all of these regulatory molecules act directly to influence *cap5(8)* expression, since some of them modulate CP expression through their interactions with other regulatory molecules.

A central player in the regulatory network is the quorum-sensing system Agr, which includes the *agrBDCA* operon and the divergently transcribed RNAIII molecule (Thoendel et al. 2011; Novick and Geisinger 2008). *cap5(8)* expression is highly dependent on Agr activity (Luong et al. 2002a; Pohlmann-Dietze et al. 2000; Dassy et al. 1993), and some CP-negative clinical isolates were found to lack Agr function (Fischer et al. 2014; Cocchiaro et al. 2006). The Agr-driven effector molecule RNAIII promotes *cap* expression largely via inactivation of the repressor Rot (George et al. 2015).

Within the bacterial population, there is considerable CP phenotypic variability, such that some bacterial cells are CP+ and others are CP−. Heterogeneity of CP expression in a given bacterial culture was first described by labeling staphylococci with CP antibodies and analysis by either flow cytometry (Poutrel et al. 1997) or immunofluorescence (Pohlmann-Dietze et al. 2000). More recently, George et al.

Table 1 Relationship between *S. aureus* strain type and capsule type

Clonal complex	MLST[a]	Strain name	*cap* genotype	CP phenotype	Comments
1	1	MW2	*cap8*	8	Scant CP8; *cap8A* mutant
1	1	Sanger 476	*cap8*	NT	Mutation in $cap8A_{prom}$
5	5	N315	*cap5*	NT	Mutation in *cap5B* and *arlR*
5	4	Mu3	*cap5*	Not done	
5	5	Mu50	*cap5*	5	
5	4	502A	*cap5*	5	
5	225	USA100	*cap5*	5	
8	8	NCTC8325	*cap5*	NT	*cap5E* mutant
8	8	USA300	*cap5*	NT	Mutations in $cap5A_{prom}$, *cap5D,* and *cap5E*
8	8	USA500	*cap5*	NT	Mutations in $cap5A_{prom}$ and *cap5D*
8	250	COL	*cap5*	5	
8	254	Newman	*cap5*	5	
8	239	TW20	*cap8*	Not done	
8	239	HS-522	*cap8*	8	
8	239	EMRSA 4	*cap8*	8	
8	239	EMRSA 7	*cap8*	8	
22	22	16	*cap5*	5	
30	30	MN8	*cap8*	8	
30	30	PS80	*cap8*	8	
30	30	TCH60	*cap8*	Not done	
30	30	UAMS-1	*cap8*	8	
30	30	USA1100	*cap8*	8	
30	36	Sanger 252	*cap8*	8	
45	45	Wright	*cap8*	8	
45	45	Becker	*cap8*	8	
59	59	USA1000	*cap8*	8	
59	59	IVDU33	*cap8*	8	
59	59	D535	*cap8*	8	
59	59	D551	*cap8*	8	
	25	Reynolds	*cap5*	5	
	93	HT2001634	*cap8*	8	
	151	RF122	*cap8*	8	
	398		*cap5*	Not done	

[a]MLST, *S. aureus* multilocus sequence type

used single-cell assays to measure both *cap5* expression and CP production (George et al. 2015). Both parameters exhibited heterogeneity within the population and were strongly growth-phase dependent. However, neither the temporal

expression of CP nor its heterogeneity could be attributed to the regulation by Agr, CodY, or Sae. Variation in CP production may be part of a general mechanism by which the bacterial population enhances its adaptability to its immediate surroundings to improve its overall fitness and survival. CP+ bacterial cells could thus evade uptake and killing by professional phagocytes, whereas the unencapsulated subpopulation would be better suited for adherence to matrix proteins and/or invasion of host cells.

2.5 Role of S. aureus CPs in Virulence

The majority of *S. aureus* clinical isolates worldwide expresses CP5 and CP8, and they have been shown to be critical for bacterial survival in the blood (Thakker et al. 1998; Watts et al. 2005). *S. aureus* clearance by the host is dependent upon opsonic antibodies that promote uptake and killing of this microbe by host neutrophils. CPs are important in immune evasion, allowing the bacterium to evade phagocytic uptake and killing (Thakker et al. 1998; Watts et al. 2005; Nanra et al. 2013). Antibodies to CPs neutralize the antiphagocytic nature of the capsule and effectively mediate uptake and killing of encapsulated isolates by professional phagocytes (Thakker et al. 1998; Wacker et al. 2014; Park et al. 2014). Bhasin et al. (1998) reported that CP5 *O*-acetylation rendered *S. aureus* more resistant to opsonophagocytic killing by human neutrophils than CP5 that was *O*-deacetylated. Moreover, in a mouse model of staphylococcal infection, the parental strain was able to seed the bloodstream from the peritoneal cavity and colonize the kidneys more efficiently than the *O*-deacetylated mutant (Bhasin et al. 1998).

In addition to the critical role of the CPs in bacteremia, the *S. aureus* CPs have also been shown to enhance virulence in rodent models of surgical wound infection (McLoughlin et al. 2006), septic arthritis (Nilsson et al. 1997), subcutaneous abscess formation (McLoughlin et al. 2006), and renal abscess formation (Portoles et al. 2001). Structural studies on CP8 revealed that it has a zwitterionic charge motif conferred by the negatively charged ManNAcA residue and free amino groups available on partially *N*-acetylated fucosamine residues (Tzianabos et al. 2001). Purified CP8 was shown to activate CD4+ T cells in vitro, and purified CP5 and CP8 facilitated intraabdominal abscess formation when administered to rats with an adjuvant. T cells activated in vitro with CP8 could modulate the development of abscesses in vivo.

However, CP production does not promote staphylococcal virulence in all infection models. CP5 and CP8 attenuated staphylococcal virulence in the rat model of catheter-induced endocarditis (Baddour et al. 1992; Nemeth and Lee 1995) and in murine mammary gland infections (Tuchscherr et al. 2005). Abundant CP can mask cell surface adhesins that bind to specific host target molecules (Kuypers and Proctor 1989; Moreillon et al. 1995). Additional evidence that CPs inhibit staphylococcal adherence was provided by in vitro experiments wherein only unencapsulated *S. aureus* bound to endothelial cells (Pohlmann-Dietze et al. 2000).

Likewise, CP5 or CP8 expression diminished *S. aureus* clumping factor A-mediated binding to fibrinogen and human platelets in vitro (Risley et al. 2007). Host Toll-like receptors (TLRs) are critical in innate and adaptive immune responses because they sense invading pathogens and signal the production of proinflammatory cytokine responses and prime Th1 and Th17 responses. Hilmi et al. reported that CPs mask TLR2 activity in *S. aureus* by interfering with lipoprotein recognition by TLR2 (Hilmi et al. 2014).

2.6 CP5 and CP8 as Vaccine Components

Fattom et al. (1990) at Nabi Biopharmaceuticals were the first to produce a vaccine based on the *S. aureus* CPs. They conjugated CP5 and CP8 to nontoxic recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA). CP5- and CP8-EPA were immunogenic in mice and humans, and they induced opsonic antibodies that showed efficacy in protecting rodents from lethality and reducing the bacterial burden in nonlethal staphylococcal infections (Fattom et al. 1990, 1993, 1996). More recently, active immunization with CP conjugate vaccines or passive immunization of antibodies to CP5 or CP8 has been shown to reduce bacteremia in rodent infection models (Wacker et al. 2014; Park et al. 2014; Lee et al. 1997). Likewise, capsular antibodies have shown protective efficacy in rodent models of mastitis, endocarditis, and skin abscesses (Skurnik et al. 2010; Tuchscherr et al. 2008; Lee et al. 1997).

Nabi combined the CP5 and CP8 conjugate vaccines into a bivalent vaccine called StaphVAX for immunization of humans at elevated risk for *S. aureus* infection. A phase III clinical trial of the vaccine enrolled 1804 hemodialysis patients (Shinefield et al. 2002) with the goal of preventing *S. aureus* bacteremia during the period from weeks 3 to 54 after immunization. A single dose of the vaccine was immunogenic and significantly ($P = 0.02$) reduced the incidence of *S. aureus* bacteremia by 57 % between weeks 3 and 40 after immunization. However, at the study end point (week 54), the vaccine efficacy was only 26 %, which was not statistically significant (Shinefield et al. 2002). The reduction in vaccine efficacy after week 40 correlated with a decline in CP5 and CP8 antibody levels among the vaccine recipients.

A confirmatory phase III clinical trial enrolled 3600 hemodialysis patients who were evaluated for bacteremia from 3 to 35 weeks after receiving StaphVAX (NCT00071214). To boost their antibody levels, a second dose of StaphVAX was administered, and the patients were followed for an additional six months. Results from the second trial showed that StaphVAX offered no significant protection against bacteremia over the placebo control (3–35 weeks, −23 % efficacy; 3–60 weeks, −8 % efficacy (Fattom et al. 2015). These results led Nabi to halt further development of StaphVAX.

Despite their failure in clinical trials when used alone in hemodialysis patients (Shinefield et al. 2002; Fattom et al. 2015), CP5 and CP8 conjugate vaccines are believed to be important components for a multivalent staphylococcal vaccine (Wacker et al. 2014; Nanra et al. 2013; Scully et al. 2014). GlaxoSmithKline

performed a phase I trial with a multicomponent vaccine that included CP5 and CP8 conjugated to tetanus toxoid (TT) (Levy et al. 2015) (ClinicalTrials.gov NCT01160172). Similarly, CP5 and CP8 conjugated to CRM197 are included in multicomponent vaccines that have reached early clinical trials sponsored by Pfizer (Nissen et al. 2015) (ClinicalTrials.gov NCT01018641), and CP5-CRM and CP8-CRM are included in the ongoing Pfizer phase 2b trial of the SA4Ag vaccine in subjects having elective lumbar spinal fusion procedures (NCT02388165).

Park et al. (2014) recently reported that antibodies elicited by a *S. aureus* CP8-CRM197 conjugate vaccine react with and protect against both serotype 5 and 8 isolates of *S. aureus* in a bacteremia infection model. De-*O*-acetylation of CP5 increased its reactivity with CP8 antibodies, suggesting that the shared epitope was L-FucNAc-D-FucNAc, which is *O*-acetylated in native CP5. Whether a CP8 conjugate vaccine could protect against a range of clinical serotype 5 *S. aureus* isolates remains to be determined.

3 Wall Teichoic Acid (WTA)

WTA is a major surface determinant of *S. aureus* and an integral part of its cell envelope (Brown et al. 2013; Sewell and Brown 2014). It is comprised of 30–50 ribitol phosphate subunits and is produced by all strains of *S. aureus*. It has important roles in cell wall maintenance, colonization, and susceptibility to antimicrobial agents.

3.1 Structure of WTA

A significant percentage (Neuhaus and Baddiley 2003; Bertsche et al. 2011; Weidenmaier et al. 2004) of the *S. aureus* cell wall biomass can be WTA, and due to the polymer length, WTA molecules extend beyond the peptidoglycan (PG) (Matias and Beveridge 2005, 2006). As shown in Fig. 4, the WTA polymer consists of a disaccharide linkage unit and a main chain, which is composed of phosphodiester-linked ribitol repeating units (Endl et al. 1983, 1984). The linkage unit, which connects the WTA to the peptidoglycan, is a disaccharide that contains *N*-acetylmannosamine linked β1 → 4 to *N*-acetylglucosamine-1-phosphate [ManNAc (β1 → 4) GlcNAc-1P] and two glycerol-3-phosphate (GroP) units linked to the C4 oxygen of ManNAc (Araki and Ito 1989; Kojima et al. 1985; Sewell and Brown 2014; Brown et al. 2013). The anomeric 1-P of the GlcNAc is attached via a phosphodiester bond to the C6 hydroxyl group of *N*-acetylmuramic acid (MurNAc) in the PG (Brown et al. 2013). The main chain consists of 1,5-D-ribitol phosphate (RboP) repeating units that are attached to the last GroP of the linkage unit. The RboP units of *S. aureus* WTA are modified with D-alanine and GlcNAc, which creates heterogeneity in WTA molecular composition among

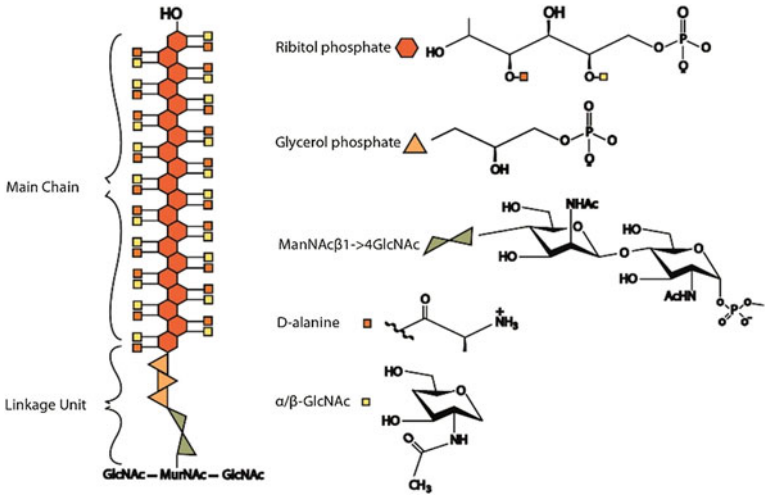

Fig. 4 WTA structure. WTA is a PG-anchored cell wall polymer that consists of phosphodiester-linked ribitol phosphate repeating units that are modified with D-alanine and GlcNAc (modified from Baur et al. (2014). A nasal epithelial receptor for *S. aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog 10: e1004089 under Creative Commons Attribution (CC BY) license)

different *S. aureus* strains. The D-alanyl residues are connected via an ester linkage to position 2 of RboP (Neuhaus and Baddiley 2003; Peschel et al. 1999, 2000). The negatively charged phosphodiesters and the positively charged D-alanine ester modifications lead to a zwitterionic charge of the repeating units (Weidenmaier et al. 2010). D-alanine ester content is regulated and variable and depends on physiological factors such as temperature, pH, and salt concentration (Jenni and Berger-Bachi 1998; Neuhaus and Baddiley 2003). The WTA GlcNAc substitution is found in position 4 of RboP, and there are indications that *S. aureus* can modulate the relative amounts of α- and β-glycosylation depending on the environmental conditions (Brown et al. 2012). A high percentage of the RboP repeating units in *S. aureus* contain *O*-GlcNAc substituents (Jenni and Berger-Bachi 1998; Weidenmaier et al. 2004). Depending on the staphylococcal strain, the anomeric configuration of the glycosidic linkage to RboP can either be exclusively α or β or a mixture of both anomers (Endl et al. 1983; Winstel et al. 2014b, 2015).

3.2 Biosynthesis of WTA

The first step in the biosynthesis of WTA is the transfer of GlcNAc-1-phosphate from UDP-GlcNAc to the undecaprenyl-phosphate lipid carrier (termed bactoprenol), catalyzed by TarO (Weidenmaier et al. 2004; Soldo et al. 2002a) (Fig. 5). RboP biosynthesis genes have been annotated as *tar* genes, whereas GroP biosynthesis

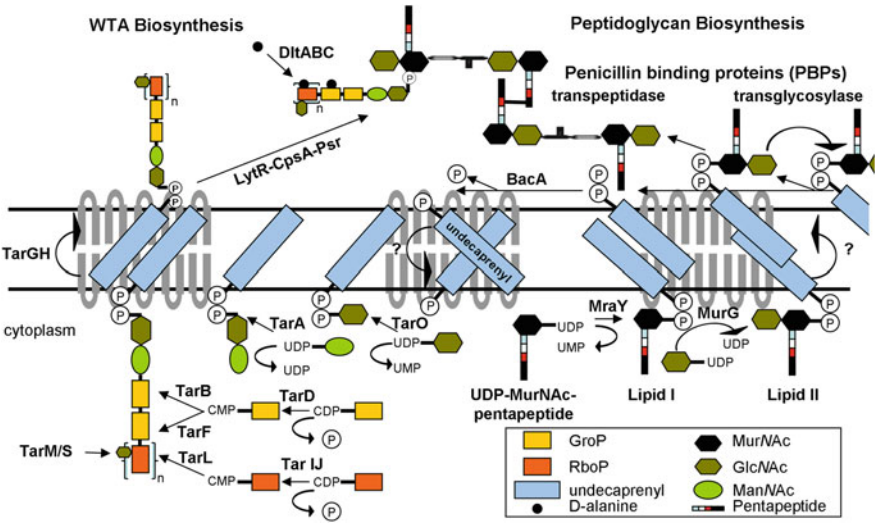

Fig. 5 Pathway for the biosynthesis of WTA in *S. aureus*. WTA biosynthesis occurs directly at the cytoplasmic membrane, starting with the addition of GlcNAc from UDP-GlcNAc to undecaprenyl-phosphate. After the addition of ManNAc, the anchor structure is completed by adding two GroP molecules. Up to 40 ribitol phosphate (*RboP*) molecules are polymerized stepwise until the WTA molecule is completed, modified with GlcNAc, and transported across the cytoplasmic membrane by TarGH. The mature polymer is linked to the C6 atom of MurNAc in the PG and modified with D-alanine. In contrast, PG biosynthesis starts with the transfer of an UDP-MurNAc-pentapeptide to undecaprenyl-phosphate. After addition of GlcNAc and transport across the cytoplasmic membrane, the multifunctional penicillin-binding proteins incorporate the new precursor into the growing peptidoglycan

genes are termed *tag* genes. Both annotations can be found for the genes responsible for the *S. aureus* linkage unit biosynthesis; we use the *tar* nomenclature throughout this chapter. The universal lipid carrier is also used in the biosynthesis of peptidoglycan as well as CPs, and therefore, the three pathways compete for the bactoprenol, which only exists in small amounts in the cytoplasmic membrane.

The next step in the WTA biosynthetic pathway is the transfer of ManNAc from UDP-ManNAc to the C4 hydroxyl group of GlcNAc. The synthesis of the ManNAc-β1,4-GlcNAc disaccharide is catalyzed by TarA (Ginsberg et al. 2006; Zhang et al. 2006). The UDP-ManNAc donor sugar is synthesized from UDP-GlcNAc by the epimerase MnaA [YvyH (Soldo et al. 2002b)]. TarB, a glycerol phosphotransferase, transfers a GroP unit from CDP-glycerol to the C4 position of ManNAc (Ginsberg et al. 2006; Bhavsar et al. 2005). The TarF enzyme then catalyzes the transfer of an additional GroP to the linkage unit (Brown et al. 2010). The GroP units are derived from CDP-glycerol, which is synthesized by TarD (Badurina et al. 2003). The >40 RboP units of the main chain are then polymerized by TarL (or a second enzyme termed TarK, see below) (Pereira et al. 2008; Ginsberg et al. 2006; Brown et al. 2008). These enzymes both prime the linkage unit and complete the main chain of the polymer (Brown et al. 2008;

Meredith et al. 2008). The CDP-ribitol substrate for the polymerization is synthesized by two enzymes: TarI, a cytidyltransferase, and TarJ, an alcohol dehydrogenase (Pereira and Brown 2004). Most *S. aureus* strains possess two full sets of *tarIJL* genes (the additional set has been termed tarI'J'K) (Qian et al. 2006), although the significance of these duplicated genes has not yet been unraveled. Still in the cytoplasm, the lipid-linked WTA polymer is modified with α-linked and/or β-linked GlcNAc by TarM and/or TarS, respectively (Brown et al. 2012; Xia et al. 2010). The WTA polymer is then transported to the extracellular surface of the cytoplasmic membrane by the TarGH ABC transporter system (Lazarevic and Karamata 1995). The lipid-linked polymer is D-alanylated by gene products from the *dlt*ABCD operon (Peschel et al. 1999; Neuhaus and Baddiley 2003; May et al. 2005) and transferred from the undecaprenyl lipid carrier to PG. The source of D-alanine is still subject to debate, and two possible mechanisms have been proposed (May et al. 2005; Wecke et al. 1996; Perego et al. 1995; Reichmann et al. 2013). Either WTA can serve directly as an acceptor of Dlt-activated D-alanine or a lipid-anchored teichoic acid with GroP backbone, termed lipoteichoic acid (LTA), gets predominantly D-alanylated by the gene products of the *dlt* operon and then serves as the source of D-alanine for WTA D-alanylation. A recent study indicated that the majority of WTA-bound D-alanine residues are transferred from LTA; however, in the absence of LTA, WTA can also serve as a direct acceptor for Dlt-activated D-alanine (Reichmann et al. 2013). Transfer of the mature WTA polymers from undecaprenyl-phosphate to the C6 hydroxyl group of MurNAc in the PG is catalyzed by three enzymes (Msr, SA0908, and SA2101, alternatively termed LytR-CpsA-Psr) whose biochemical activity has not yet been confirmed (Kawai et al. 2011; Dengler et al. 2012; Chan et al. 2013).

3.3 Regulation of WTA Biosynthesis

Regulation of WTA biosynthesis remains poorly understood. Meredith et al. (2008) reported that *S. aureus* regulates WTA polymer chain length. An analysis of extracted WTAs from strains that produce only TarL or TarK revealed that these enzymes produce electrophoretically distinct RboP WTAs, designated L-WTA and K-WTA. K-WTA was reported to be up to 50 % shorter than L-WTA, and K-WTA biosynthesis was negatively regulated by the Agr quorum-sensing system (Meredith et al. 2008). Therefore, under conditions of low Agr activity (low population density), both K-WTA and L-WTA may be produced, whereas a high population density allows predominantly for L-WTA synthesis (Swoboda et al. 2010). However, the physiological relevance of the two WTA types is not clear. Additional evidence for the regulation of WTA biosynthesis comes from studies that implicated WTA in antibiotic resistance (Bertsche et al. 2011, 2013; Mishra et al. 2014). Cell wall stress mediated by different antibiotics leads to a phenotype that is characterized by a significant increase in WTA amounts in the cell walls of antibiotic-resistant strains (see below). In addition, the stereochemistry of the

GlcNAc modifications seems to be regulated since WTA GlcNAc modifications play an important role in β-lactam resistance (Brown et al. 2012). However, when the impact of β-lactam treatment on expression of the glycosyltransferases was assessed, only *tarS* but not *tarM* expression was strongly upregulated by oxacillin (β-lactam) treatment. Another important aspect is the possible cross-regulation of WTA and CP biosynthesis. Campbell et al. (2012) reported that inhibition of late-stage WTA biosynthesis leads to a downregulation of CP biosynthesis genes. Like PG and WTAs, CP is synthesized on the universal bactoprenol carrier lipid, and the downregulation of CP biosynthetic genes may conserve the carrier lipid for use in the essential process of PG synthesis.

3.4 Role of WTA in S. aureus Physiology

WTA polymers play different roles in staphylococcal physiology that range from cell wall homeostasis to receptors for *S. aureus*-specific bacteriophages (Weidenmaier and Peschel 2008). One important aspect is the role of WTA in the regulation of PG synthesis and turnover. Functional interactions between WTA and enzymes of PG synthesis have been observed in *S. aureus* (Brown et al. 2013) since WTA targets the PG cross-linking enzymes PBP4 and FmtA to the septum (Atilano et al. 2010; Qamar and Golemi-Kotra 2012; Farha et al. 2013). These enzymes are mislocalized in *tarO* mutants lacking WTA, which leads to a decrease in PG cross-linking (Schlag et al. 2010). Several studies indicate that inhibition of early WTA biosynthesis can restore β-lactam sensitivity in MRSA strains, which could be due to simultaneous blocking of the PG cross-linking enzymes PBP2 and PBP4 (Campbell et al. 2011; Farha et al. 2013). However, β-lactam-mediated cell wall stress can lead to overexpression of WTA and therefore steric hindrance of the antibiotic. This indicates that the β-lactam resistance phenotype could be directly WTA mediated (Bertsche et al. 2011, 2013). Additional molecular studies in different mutant backgrounds are required to clearly pinpoint the role of WTA in cell wall morphogenesis.

In addition to localizing cross-linking enzymes to the septum, WTA has been implicated in the regulation of PG lytic enzymes (Schlag et al. 2010). Strains lacking WTAs exhibit increased autolysis rates (Biswas et al. 2012; Schlag et al. 2010); however, it has not been demonstrated that WTA directly regulates autolysin activity. Some reports hypothesize that autolytic enzymes with a positive net charge could interact with the polyanionic polymers of the staphylococcal cell wall (Neuhaus and Baddiley 2003; Fischer et al. 1981; Bierbaum and Sahl 1985). In line with this theory, the incorporation of D-alanine esters could modulate the recruitment of the autolytic enzymes due to electrostatic repulsion. This would allow exclusion of autolysins from sites with high amounts of D-alanylated WTA (e.g., cell poles), whereas the septum area might only contain nascent non-D-alanylated WTA, which would allow for autolytic cleavage of the PG (Frankel and Schneewind 2012; Schlag et al. 2010). However, both WTA and LTA are subject to D-alanylation, and further studies are required to elucidate the relative role and

spatial organization of WTA and LTA biosyntheses. Besides the D-alanine-mediated exclusion model, WTA or LTA could indirectly modulate enzyme activity through ion chelation (Biswas et al. 2012; Wecke et al. 1996). Some reports indicate that WTAs can scavenge extracellular metal ions due to the extension of the polymer beyond the PG layer (Kern et al. 2010; Wickham et al. 2009; Thomas and Rice 2015). WTA ion scavenging might also affect the physicochemical composition and integrity of the cell wall by minimizing repulsion between closely positioned phosphate groups. In addition, proton binding by WTA and/or LTA might create localized changes in pH, indirectly modulating the function of some enzymes, and might stabilize osmotic pressure in the cell wall (Neuhaus and Baddiley 2003; Biswas et al. 2012). Although some of these mechanisms correlated with phenotypes in WTA-negative mutants, it has not been demonstrated whether the lack of WTA affects LTA biosynthesis directly, and thus, the relative role of WTA and LTA in cell envelope homeostasis remains unclear.

3.5 *Role of WTA as Phage Receptor and Glycocode for Horizontal Gene Transfer*

Using isogenic mutants with altered WTA structures, Xia et al. (2011) first demonstrated that WTAs, but not LTAs, are required for siphovirus and myovirus infection of *S. aureus*. Whereas the siphoviruses were reported to require WTA *O*-GlcNAc modification for adsorption, the myoviruses interact directly with the backbone of WTA (Xia et al. 2011; Winstel et al. 2013).

All known *S. aureus* phages belong to the order Caudovirales (tailed phages), which are composed of an icosahedral capsid filled with double-stranded DNA and a thin filamentous tail (Xia and Wolz 2014). Based on the tail morphology, they can be further classified into three major families: Podoviridae (very short tail); Siphoviridae (long noncontractile tail); and Myoviridae (long contractile tail). Important serogroups within the siphoviridae are A, B, and F, while serogroup D is important in the myoviridae family (Xia and Wolz 2014). Φ11 and Φ80α are important phages (serogroup B) that interact with WTA GlcNAc modifications (Winstel et al. 2014b; Xia et al. 2011) and are capable of transducing DNA between *S. aureus* strains (Winstel et al. 2013, 2014b). Such phages are responsible for staphylococcal horizontal gene transfer (HGT) of mobile genetic elements like staphylococcal pathogenicity islands, where they act as helper phages (Winstel et al. 2013, 2014b). Winstel et al. (2013) demonstrated that the variable structure of glycosylated WTA constitutes a "glycocode" that is used by transducing phages and defines the routes and directions of HGT. Apparently, the presence of similar WTA structures enables DNA exchange via helper bacteriophages even across species or genera boundaries, e.g., RboP WTA containing *S. aureus* and *Listeria grayi* ATCC25401, whereas unique *S. aureus* clones producing altered WTA (e.g., PS187 GroP WTA) become separated from the *S. aureus* genetic pool and could

initiate new routes of HGT with other bacterial species and genera that share related WTA (e.g., *Listeria monocytogenes* ATCC19118 or *Staphylococcus epidermidis*, which also exhibit GroP WTA) (Winstel et al. 2013). Importantly, antibiotic stress is known to activate prophages (Goerke et al. 2006a, b), thereby contributing to phage-mediated HGT (Ubeda et al. 2005). Recently developed compounds blocking the biosynthesis of WTA (Suzuki et al. 2011b) may help to reduce the frequency of phage-dependent HGT, for example, in chronic polymicrobial infections.

3.6 Role of WTA in Antibiotic Resistance and WTA Inhibitory Compounds

WTA can modulate several aspects of *S. aureus* physiology (cell wall integrity, density, and charge) that affect antibiotic resistance. WTA plays a role in β-lactam resistance since MRSA WTA mutants or MRSA mutants that were treated with WTA biosynthesis inhibitors were found to be highly susceptible to β-lactam antibiotics, such as methicillin/oxacillin (Campbell et al. 2011; Farha et al. 2013, 2014). In contrast to MRSA mutants lacking D-alanine or α-*O*-GlcNAc substitutions on WTA, which are not affected in their resistance profiles for most β-lactam antibiotics, the lack of β-*O*-GlcNAc residues rendered MRSA susceptible to β-lactams (Brown et al. 2012). Interestingly, *tarS* but not *tarM* expression levels were significantly upregulated by oxacillin treatment, consistent with the role of the β-*O*-GlcNAc glycosyltransferase TarS in β-lactam resistance (Brown et al. 2012). However, the molecular mechanisms that confer the role of TarS in β-lactam resistance remain elusive. WTA β-*O*-GlcNAc may contribute to the scaffolding function of WTA for cell wall synthesis enzymes such as PBP2 and PBP4 or cell wall-associated proteins that directly or indirectly modulate the activity of PBPs (Brown et al. 2012; Atilano et al. 2010; Qamar and Golemi-Kotra 2012). As noted previously, WTA modulates the physicochemical properties of PG, and as such, WTA glycosylation might affect this function (Sutcliffe 2012). In addition, WTA biosynthesis is significantly upregulated under cell wall stress conditions exerted by antibiotics like daptomycin (Bertsche et al. 2011, 2013; Mishra et al. 2014). These studies provide evidence that an increase in cell wall thickness is a consequence of an elevated WTA content. Furthermore, increased WTA D-alanylation is a relatively common phenotype among daptomycin-resistant *S. aureus* strains (Bertsche et al. 2013). These phenotypic alterations are consistent with the observed changes in the positive surface charge characteristics and transcriptional enhancement of expression of genes involved in WTA biosynthesis. WTA overexpression and hyper-D-alanylation phenotypes lead to steric and electrostatic exclusion/repulsion of daptomycin in its active Ca^{2+}-complexed form (Bertsche et al. 2013). Since MRSA and daptomycin-resistant phenotypes are multifactorial, it is not clear whether daptomycin and β-lactam antibiotics affect WTA biosynthesis regulation via similar pathways.

The important roles of WTA in host colonization, infection, and drug resistance make its biosynthetic pathway a promising target for novel anti-infectives (Campbell et al. 2011; Brown et al. 2012; Suzuki et al. 2011b; Farha et al. 2013, 2014; Wang et al. 2013; Swoboda et al. 2009; Sewell and Brown 2014). The Walker laboratory developed whole-cell bioactive compound screens for substances that were growth inhibitory to wild-type *S. aureus* but not to the Δ*tarO* mutant (Lee et al. 2010; Swoboda et al. 2009). Chemicals identified in this screen were predominantly late-step inhibitors of WTA biosynthesis. One compound was further optimized through a structure–activity relationship analysis and termed targocil (Lee et al. 2010). Extensive in vitro enzymatic assays pointed to the export system of WTA as the drug target. Mapping of spontaneous suppressor mutations conferring resistance to targocil identified TarG, the translocase component of the ABC export complex, as the primary target (Boles et al. 2010). A similar screen by Merck (Wang et al. 2013) uncovered three diverse chemical classes of late-stage WTA inhibitors: tricyclic indole acids, *N*-aryl-triazoles, and C-aryl-triazoles. Again, suppressor mutations mapped to *tarG*, and TarG inhibitors were found to be effective in a mouse thigh infection model (Wang et al. 2013). However, the frequency of resistance to TarG inhibitors is high, and not only mutations in *tarG* but also loss-of-function mutations in *tarO* or *tarA* render isolates resistant. However, the latter mutations may not play a role under in vivo conditions, since loss of function in early WTA biosynthesis pathway steps leads to reduced staphylococcal virulence. The Brown laboratory recently identified ticlopidine as an inhibitor of early WTA biosynthesis. The molecular target of ticlopidine is TarO (Farha et al. 2013), and they produced chemically optimized analogs that exhibited enhanced activity against TarO (Farha et al. 2014). Another novel compound with potent bactericidal activity against *S. aureus*, termed teixobactin, simultaneously blocks the bactoprenol-bound early intermediates of both PG and WTA biosyntheses, and is likely to impact CP biosynthesis as well (Ling et al. 2015).

3.7 Role of WTA in Colonization and Virulence

An early report indicated that WTA plays a role in *S. aureus* adhesion to epithelial surfaces (Aly et al. 1980). However, more detailed molecular in vitro and in vivo studies were not reported before Weidenmaier et al. published the first characterization of a genetically defined Δ*tarO* mutant (Weidenmaier et al. 2004). Similarly, the Brown group subsequently reported that a *tarA* mutant also resulted in a WTA-negative phenotype (D'Elia et al. 2009). Interestingly, many of the genes downstream of *tarA* in the *S. aureus* WTA pathway are essential unless *tarO* or *tarA* is deleted first (D'Elia et al. 2006a, b). The conditional essential nature of WTA biosynthesis genes can be explained by toxicity of the depletion of undecaprenyl-phosphate-linked PG precursors and the resulting disruption of PG biosynthesis (Brown et al. 2013; Swoboda et al. 2010; Sewell and Brown 2014).

S. aureus nasal colonization is a major risk factor for infections with the colonizing strain, and so it is important to understand the multifactorial molecular events required for colonization (Weidenmaier et al. 2012). *S. aureus* Δ*tarO* mutants are greatly impaired in their ability to adhere to epithelial tissues and to colonize the nasal cavity and gastrointestinal tract of rodents (Weidenmaier et al. 2004; Weidenmaier and Peschel 2008; Winstel et al. 2015; Baur et al. 2014; Misawa et al. 2015). WTA has been shown to interact with a type F-scavenger receptor in the inner nasal cavity, which plays a role in creating a nasal reservoir for *S. aureus* (Baur et al. 2014). Blocking the interaction of the scavenger receptor SREC-I with WTA reduced *S. aureus* nasal colonization. The identification of a WTA receptor sheds new light on the impact of WTA as a nonprotein adhesin of *S. aureus*. WTA glycosylation was reported to be an important structural motif for *S. aureus* interaction with nasal epithelial surfaces (Winstel et al. 2015). Whether the *O*-GlcNAc modifications play a role in the interaction with SREC-I or serve as a coreceptor that recognizes the GlcNAc modifications and aids in efficient WTA-dependent adhesion to nasal epithelial surfaces remains unclear. The impact of WTA on staphylococcal virulence is not limited to colonization, since a WTA mutant was also less virulent in rabbit model of catheter-induced endocarditis (Weidenmaier et al. 2005) and a murine model of endophthalmitis (Suzuki et al. 2011a).

WTA has been shown to have important immunostimulatory activities (Weidenmaier et al. 2010). Purified WTA stimulated CD4+ T cell proliferation in vitro, which was dependent upon the zwitterionic charge of the WTA polymer (Weidenmaier et al. 2010). The data indicated a strict requirement for MHCII-dependent presentation of WTA by antigen-presenting cells. Furthermore, the authors demonstrated an impact of this WTA-dependent, T cell-mediated mechanism on *S. aureus* skin infections. Supporting data for the role of WTA as T cell epitope were recently reported by Kolata et al. (2015), who detected WTA-specific memory T cells in healthy volunteers. Recent reports indicate that humans have abundant antibodies directed against *S. aureus* WTA (Lee et al. 2015; Kurokawa et al. 2013; Hansenova Manaskova et al. 2013), and the β-*O*-GlcNAc residues of *S. aureus* WTA are immunodominant (Kurokawa et al. 2013). Human serum anti-WTA IgG that recognizes β-*O*-GlcNAc WTA induces complement factor C3 deposition and mediates opsonophagocytosis of *S. aureus* (Lee et al. 2015). Human mannose-binding lectin (MBL) binds to glycosylated WTA and activates the lectin pathway of complement activation (Kurokawa et al. 2013; Park et al. 2010). MBL-mediated complement activation is especially relevant in infants since MBL deficiency increases their susceptibility to *S. aureus* infections (Park et al. 2010; Kurokawa et al. 2013). Serum MBL in adults does not recognize WTA due to the inhibitory action of serum WTA antibodies (Kurokawa et al. 2013; Park et al. 2010).

With few exceptions, *tarS* is present in all sequenced *S. aureus* genomes, whereas *tarM* is absent in CC398 strains, as well as in several healthcare-associated MRSA strains such as N315, Mu50, Mu3, and JH1 (all clonal complex 5) (Lee et al. 2015; Winstel et al. 2013, 2014a, b). When the occurrence of α- and

β-GlcNAc transferases were analyzed by PCR in 70 *S. aureus* nasal isolates (49 different *spa* types), *tarS* (the β-GlcNAc transferase gene) was detected in all isolates, whereas *tarM* (the α-GlcNAc transferase gene) was only present in 36 % of the tested isolates (Winstel et al. 2015). The role of WTA glycosylation in staphylococcal infection and colonization is poorly understood, but dynamic regulation of WTA glycosylation may dictate host tropisms or allow *S. aureus* to react to different innate or adaptive responses in the human host.

3.8 WTA as a Vaccine Candidate

The first use of WTA as a vaccine candidate was its inclusion in a multicomponent vaccine formulation produced by Nabi, Inc. PentaStaph included a nontoxic alpha hemolysin toxoid, the S subunit of Panton–Valentine leukocidin, CP5 conjugated to recombinant exoprotein A of *Pseudomonas aeruginosa* (CP5-rEpa), and CP8-rEpa. The fifth vaccine component was 336-rEpa. 336 was described by Nabi as a surface polysaccharide composed of ribitol, phosphate, and GlcNAc, but no alanine in its structure. Moreover, the GlcNAc residue was attached to C-3 of the ribitol molecule, in contrast to the C-4 of conventional WTA (Fattom and Guidry 1999). Nabi investigators never published preclinical data supporting the inclusion of the 336 antigen in their multicomponent vaccine. PentaVax was sold to GlaxoSmithKline in 2009, and there has been no public disclosure that the vaccine has been explored further in animal or human studies.

Nonetheless, because of its multiple functions in colonization and virulence and its ubiquity among *S. aureus* isolates, WTA (coupled to a protein carrier for immunogenicity) could be a promising vaccine antigen, particularly to extend coverage to isolates lacking CP. Although healthy humans have serum antibodies to WTA (Lee et al. 2015; Kurokawa et al. 2013; Jung et al. 2012; Hansenova Manaskova et al. 2013), whether these antibodies contribute to immunity is unknown. One preclinical study showed that intradermal immunization with WTA resulted in an anti-WTA IgG response in mice that correlated with a reduction in the bacterial burden in a renal abscess model (Takahashi et al. 2013). Whether WTA elicits antibodies that mediate not only uptake but also killing by neutrophils and proves to be an effective component in multivalent vaccine formulations remains to be determined.

4 Polysaccharide Intercellular Adhesin (PIA)/Poly-*N*-Acetyl Glucosamine (PNAG)

PIA/PNAG is a glycopolymer that was first discovered as a biofilm antigen in *Staphylococcus epidermidis*, where it plays an important role in adherence to biomaterials (Mack et al. 1996b). The *ica* locus encoding PIA/PNAG is conserved

between *S. epidermidis* and *S. aureus*, although the antigen is produced in greater abundance in vitro by *S. epidermidis*. The *ica* locus is present in virtually all clinical isolates of *S. aureus*. Remarkably, PIA/PNAG is now recognized as a highly conserved polysaccharide antigen produced by many microbes (Cywes-Bentley et al. 2013).

4.1 Structure of PIA/PNAG

Elaboration of biofilms by bacteria protects them from the host immune response and from antibiotics, complicating treatment and eradication of chronic infections. *S. aureus* can produce both polysaccharide-dependent and polysaccharide-independent biofilms (Boles et al. 2010; Tu Quoc et al. 2007). The exopolysaccharide components of the biofilm received different names over the years, including capsular polysaccharide/adhesin (PS/A) (Muller et al. 1993), polysaccharide intercellular adhesin (PIA) (Mack et al. 1996a), and poly-β(1-6)-*N*-acetyl-glucosamine (PNAG) (Maira-Litran et al. 2002). Sadovskaya et al. (2005) demonstrated that PIA from *S. epidermidis* is structurally identical to PNAG from *S. aureus*. PIA and PNAG are thus synonymous terms and used preferentially by different staphylococcal researchers. In addition to its production by *S. aureus* and *S. epidermidis*, PIA/PNAG is also produced by many other microbes, including Gram-positive and Gram-negative bacteria, fungal pathogens, and protozoa (Cywes-Bentley et al. 2013). An important feature of the *S. aureus* native PIA/PNAG polymer (shown in Fig. 6) is that about 10–20 % of the GlcNAc amino groups are not N-acetylated, and this is important in retaining the polymer on the bacterial surface (Cerca et al. 2007; Vuong et al. 2004a).

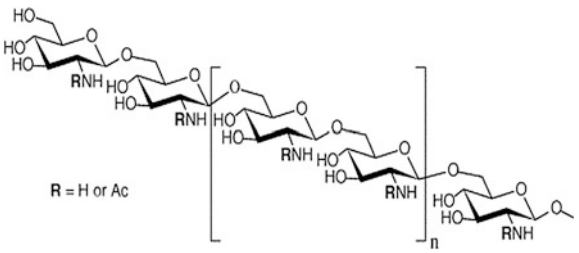

Fig. 6 Structure of *S. aureus* (PIA/PNAG). In the native polymer, 10–20 % of the GlcNAc residues have been deacetylated by IcaB, and this is important in retaining the polymer on the bacterial cell surface since it is not covalently anchored to the cell wall

4.2 Biosynthesis of PIA/PNAG

PIA/PNAG is a high molecular weight linear polymer of glucosaminylglycan that is synthesized by the gene products of the *icaADBC* locus, which is present in virtually all clinical isolates of *S. aureus*. The *icaADBC* genes appear to be translated from a single transcript (Heilmann et al. 1996). IcaA is a transmembrane glucosyltransferase that can synthesize short PNAG polymers in vitro using UDP-GlcNAc as a substrate (Gerke et al. 1998). IcaD increases the biosynthetic efficiency of IcaA, playing a predominant role in the synthesis of oligomers longer than 20 residues, whereas IcaC is an integral membrane protein that appears to be involved in linking short polymers to make longer oligomers of PNAG (Gerke et al. 1998). Consistent with the observation that the native form of PNAG is partially de-N-acetylated (Fig. 6), the product of the *icaB* gene is an extracellular N-deacetylase that removes N-linked acetate groups from the PNAG polymer (Vuong et al. 2004a). An *icaB* mutant produces only the fully acetylated PNAG, and this form of the antigen is not retained on the staphylococcal cell surface (Cerca et al. 2007; Vuong et al. 2004a).

4.3 Regulation of PIA/PNAG Biosynthesis

PNAG production in vitro is induced by high osmolarity, low levels of ethanol, iron restriction, oxygen deprivation, and growth in rich medium supplemented with glucose (Rachid et al. 2000; Cramton et al. 2001). The expression of the *icaADBC* genes is negatively regulated at the transcriptional level by the *ica* regulator (*icaR*) gene product, which is adjacent to *icaADBC* and transcribed divergently. IcaR is a DNA-binding protein that binds specifically to the *ica* promoter immediate upstream of *icaA* (Jefferson et al. 2003). In addition, CodY is a repressor (Majerczyk et al. 2008), and SarA and the stress sigma factor B (Valle et al. 2003; Cerca et al. 2008) are positive regulators of *icaADBC* transcription. Jefferson et al. isolated a PNAG-overproducing strain called MN8m. Sequence analysis of its *ica* locus revealed that MN8m carried a 5-nucleotide deletion within the promoter region upstream of *icaA*, resulting in the augmented transcription of the *ica* locus and a strong biofilm-producing phenotype (Jefferson et al. 2003).

Subsequently, Brooks and Jefferson reported that growth of PIA/PNAG-overproducing strains in vitro resulted in the rapid accumulation of nonmucoid variants. They described phase variation in PIA/PNAG expression in *S. aureus* wherein slipped-strand mispairing occurs in the *icaC* gene, and this mutation results in a truncated IcaC protein and a PIA/PNAG-negative phenotype (Scully et al. 2015). The mutation was reversible and could be detected in some clinical isolates of *S. aureus*. The authors noted that high-level production of PIA/PNAG likely carries a fitness cost, since PIA/PNAG-negative variants had a growth advantage over the parental overproducing strains.

4.4 Role of PIA/PNAG in Virulence

PIA/PNAG plays a fundamental role in intercellular adhesion of staphylococci within a biofilm, and it is also an important structural component of the biofilm matrix architecture (Cramton et al. 1999). In the host, PIA/PNAG is implicated in virulence by mediating bacterial adhesion to biomaterial surfaces and evasion from the host immune response (Vuong et al. 2004a, b). *S. aureus* Δ*ica* mutants showed decreased virulence in murine models of bacteremia, renal infection, and lethal sepsis (Kropec et al. 2005). However, PIA/PNAG was not a virulence factor in a murine model of pneumonia (Bubeck Wardenburg et al. 2007) or in animal models of foreign body infections (Fluckiger et al. 2005; Francois et al. 2003), although Fluckiger et al. (2005) did show a defect in growth for the *ica* mutant in an implant infection model when competed against the parental strain. Production of PIA/PNAG did not affect *S. aureus* colonization of the mouse nose (Schaffer et al. 2006) or the gastrointestinal tract (Misawa et al. 2015).

4.5 PIA/PNAG as a Vaccine Candidate

In addressing the vaccine potential of PIA/PNAG, McKenney et al. (1999) reported that active and passive immunization strategies protected mice against renal abscess formation in mice infected with various *S. aureus* isolates. In a follow-up study, the investigators immunized mice, rabbits, and goats with either native PNAG or chemically deacetylated PNAG (dPNAG) (∼15 % acetylation) conjugated to diphtheria toxoid. Antibodies to highly acetylated (>90 %) PNAG lacked protective efficacy and mediated significantly lower opsonic killing than antibodies raised to dPNAG (Maira-Litran et al. 2005). Mice were passively immunized IP with immune or nonimmune serum and challenged with *S. aureus* 24–48 h later. Antibodies to dPNAG reduced bacteremia and lethal peritonitis in the passively immunized animals (Maira-Litran et al. 2005). Kelly-Quintos et al. (2005) showed that *S. aureus* opsonophagocytic killing activity of human sera from cystic fibrosis patients correlated with the level of IgG specific to dPNAG to a greater extent than to native PNAG. Because dPNAG is preferentially retained on the bacterial cell surface, antibodies to dPNAG may be more effective in achieving protection than PNAG antibodies.

To circumvent the imprecise nature of chemical deacetylation, Gening et al. synthesized β(1-6)-*N*-acetylglucosamine oligosaccharides that were chemically conjugated to TT for use as vaccines (Gening et al. 2007). Synthetic $5GlcNH_2$-TT or $9GlcNH_2$-TT elicited serum antibodies in mice that were opsonic for *S. aureus* in an in vitro opsonophagocytic killing assay. In a subcutaneous abscess model, mice passively immunized with rabbit antibodies to $9GlcNH_2$-TT showed a significant reduction in bacterial burden compared to mice given normal rabbit serum.

Subsequently, the Pier group generated a fully human monoclonal antibody (mAb) to dPNAG and characterized its biological activity in vitro and in vivo (Kelly-Quintos et al. 2006). mAb F598 mediated opsonophagocytic killing of *S. aureus* strains in vitro, and it protected mice against a lethal staphylococcal infection. Alopexx Pharmaceuticals initiated a phase I trial of the F598 mAb in 20 human volunteers in May 2010. Administration of the mAb (1–20 mg/kg) was well tolerated, and no serious side effects were noted. Subsequently, Sanofi-Aventis licensed F598, which they dubbed SAR279356. In 2011, they embarked on a phase II clinical trial (NCT01389700) to evaluate the safety and efficacy of IV administration of SAR279356 in mechanically ventilated patients in intensive care units. The trial was terminated in January 2013 because of the insufficiency of enrollment. Further development of the mAb by Sanofi-Aventis will not occur.

Despite the positive data regarding PNAG as an immunogen, Skurnik et al. reported that both immunization-induced antibodies in experimental animals and natural antibodies to PNAG in health human sera interfered with the protective efficacy of immunization-induced antibody to *S. aureus* CP5 and CP8 antigens, representing potential barriers to successful use of PNAG-specific vaccines against *S. aureus* (Skurnik et al. 2010).

5 Conclusions

The major surface polysaccharides of *S. aureus* include CPs, WTA, and PIA/PNAG. Because they comprise the external surface of the staphylococcus, they mediate many host–pathogen interactions, and they play distinct roles in *S. aureus* colonization and infection. Regulation of the synthesis of these glycopolymers is complex, and how the bacterial cell orchestrates the expression of these various polysaccharides is poorly understood. CP5 and CP8 are best known for their antiphagocytic properties, which are neutralized by opsonic anticapsular antibodies. For this reason, interest in including these antigens in a multivalent vaccine persists. Among the surface polysaccharides, *S. aureus* WTA is the most consistently produced polymer. It has been shown to be an important adhesin for colonization, and it impacts cell wall integrity and homeostasis. Because it modulates the bacterial surface charge, it plays a role in resistance to cationic peptides and certain antibiotics. PIA/PNAG is a fascinating polysaccharide because it is produced not only by staphylococci, but also by other microbes, ranging from bacteria to fungi to protozoa. It promotes biofilm formation, contributes to immune evasion, and offers possibility as a target for active and passive immunotherapeutics. Further in-depth studies into all aspects of the biology of *S. aureus* surface polysaccharide will lead to a better understanding of their functions, regulation, and potential as prospective targets for novel anti-infective strategies.

References

Aly R, Shinefield HR, Litz C, Maibach HI (1980) Role of teichoic acid in the binding of *Staphylococcus aureus t*o nasal epithelial cells. J Infect Dis 141:463–465

Araki Y, Ito E (1989) Linkage units in cell walls of gram-positive bacteria. Crit Rev Microbiol 17 (2):121–135

Atilano ML, Pereira PM, Yates J, Reed P, Veiga H, Pinho MG, Filipe SR (2010) Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*. Proc Natl Acad Sci USA 107(44):18991–18996

Baddour LM, Lowrance C, Albus A, Lowrance JH, Anderson SK, Lee JC (1992) *Staphylococcus aureus* microcapsule expression attenuates bacterial virulence in a rat model of experimental endocarditis. J Infect Dis 165:749–753

Badurina DS, Zolli-Juran M, Brown ED (2003) CTP:glycerol 3-phosphate cytidylyltransferase (TarD) from *Staphylococcus aureus* catalyzes the cytidylyl transfer via an ordered Bi-Bi reaction mechanism with micromolar K(m) values. Biochim Biophys Acta 1646(1–2):196–206

Barreteau H, Magnet S, El Ghachi M, Touze T, Arthur M, Mengin-Lecreulx D, Blanot D (2009) Quantitative high-performance liquid chromatography analysis of the pool levels of undecaprenyl phosphate and its derivatives in bacterial membranes. J Chromatogr B Anal Technol Biomed Life Sci 877(3):213–220

Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, Hoffmann WH, Rudel T, Autenrieth IB, Weidenmaier C (2014) A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog 10 (5):e1004089

Bertsche U, Weidenmaier C, Kuehner D, Yang SJ, Baur S, Wanner S, Francois P, Schrenzel J, Yeaman MR, Bayer AS (2011) Correlation of daptomycin-resistance in a clinical *Staphylococcus aureus* strain with increased cell wall teichoic acid production and D-alanylation. Antimicrob Agents Chemother 55:3922

Bertsche U, Yang SJ, Kuehner D, Wanner S, Mishra NN, Roth T, Nega M, Schneider A, Mayer C, Grau T, Bayer AS, Weidenmaier C (2013) Increased cell wall teichoic acid production and D-alanylation are common phenotypes among daptomycin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates. PLoS ONE 8(6):e67398

Bhasin N, Albus A, Michon F, Livolsi PJ, Park J-S, Lee JC (1998) Identification of a gene essential for O-acetylation of the *Staphylococcus aureus* type 5 capsular polysaccharide. Mol Microbiol 27:9–21

Bhavsar AP, Truant R, Brown ED (2005) The TagB protein in *Bacillus subtilis* 168 is an intracellular peripheral membrane protein that can incorporate glycerol phosphate onto a membrane-bound acceptor in vitro. J Biol Chem 280(44):36691–36700

Bierbaum G, Sahl HG (1985) Induction of autolysis of staphylococci by the basic peptide antibiotics Pep 5 and nisin and their influence on the activity of autolytic enzymes. Arch Microbiol 141(3):249–254

Bischoff M, Dunman P, Kormanec J, Macapagal D, Murphy E, Mounts W, Berger-Bachi B, Projan S (2004) Microarray-based analysis of the *Staphylococcus aureus sigmaB* regulon. J Bacteriol 186(13):4085–4099

Biswas R, Martinez RE, Gohring N, Schlag M, Josten M, Xia G, Hegler F, Gekeler C, Gleske AK, Gotz F, Sahl HG, Kappler A, Peschel A (2012) Proton-binding capacity of *Staphylococcus aureus* wall teichoic acid and its role in controlling autolysin activity. PLoS ONE 7(7):e41415

Bittersuermann D (1993) Influence of bacterial polysialic capsules on host defense - masquerade and mimicry. Polysialic Acid. Birkhauser, Basel

Boles BR, Thoendel M, Roth AJ, Horswill AR (2010) Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation. PLoS ONE 5(4):e10146

Bouhss A, Trunkfield AE, Bugg TD, Mengin-Lecreulx D (2008) The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol Rev 32(2):208–233

Boyle-Vavra S, Li X, Alam MT, Read TD, Sieth J, Cywes-Bentley C, Dobbins G, David MZ, Kumar N, Eells SJ, Miller LG, Boxrud DJ, Chambers HF, Lynfield R, Lee JC, Daum RS (2015) USA300 and USA500 clonal lineages of *Staphylococcus aureus* do not produce a capsular polysaccharide due to conserved mutations in the *cap5* locus. mBio 6(2):e02585–02514

Brown S, Meredith T, Swoboda J, Walker S (2010) *Staphylococcus aureus* and *Bacillus subtilis* W23 make polyribitol wall teichoic acids using different enzymatic pathways. Chem Biol 17 (10):1101–1110

Brown S, Santa Maria JP Jr, Walker S (2013) Wall teichoic acids of gram-positive bacteria. Annu Rev Microbiol 67:313–336

Brown S, Xia G, Luhachack LG, Campbell J, Meredith TC, Chen C, Winstel V, Gekeler C, Irazoqui JE, Peschel A, Walker S (2012) Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. Proc Natl Acad Sci USA 109(46):18909–18914

Brown S, Zhang YH, Walker S (2008) A revised pathway proposed for *Staphylococcus aureus* wall teichoic acid biosynthesis based on in vitro reconstitution of the intracellular steps. Chem Biol 15(1):12–21

Bubeck Wardenburg J, Patel RJ, Schneewind O (2007) Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun 75(2):1040–1044

Campbell J, Singh AK, Santa Maria JP Jr, Kim Y, Brown S, Swoboda JG, Mylonakis E, Wilkinson BJ, Walker S (2011) Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in *Staphylococcus aureus*. ACS Chem Biol 6(1):106–116

Campbell J, Singh AK, Swoboda JG, Gilmore MS, Wilkinson BJ, Walker S (2012) An antibiotic that inhibits a late step in wall teichoic acid biosynthesis induces the cell wall stress stimulon in *Staphylococcus aureus*. Antimicrob Agents Chemother 56(4):1810–1820

Cerca N, Brooks JL, Jefferson KK (2008) Regulation of the intercellular adhesin locus regulator (*ica*R) by SarA, sigmaB, and IcaR in *Staphylococcus aureus*. J Bacteriol 190(19):6530–6533

Cerca N, Jefferson KK, Maira-Litran T, Pier DB, Kelly-Quintos C, Goldmann DA, Azeredo J, Pier GB (2007) Molecular basis for preferential protective efficacy of antibodies directed to the poorly-acetylated form of staphylococcal poly-N-acetyl-{beta}-(1-6)-glucosamine. Infect Immun 75(13):3406–3413

Chan YG, Frankel MB, Dengler V, Schneewind O, Missiakas D (2013) *Staphylococcus aureus* mutants lacking the LytR-CpsA-Psr family of enzymes release cell wall teichoic acids into the extracellular medium. J Bacteriol 195(20):4650–4659

Chan YG, Kim HK, Schneewind O, Missiakas D (2014) The capsular polysaccharide of *Staphylococcus aureus* is attached to peptidoglycan by the LytR-CpsA-Psr (LCP) family of enzymes. J Biol Chem 289(22):15680–15690

Cocchiaro JL, Gomez MI, Risley A, Solinga R, Sordelli DO, Lee JC (2006) Molecular characterization of the capsule locus from non-typeable *Staphylococcus aureus*. Mol Microbiol 59(3):948–960

Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F (1999) The intercellular adhesion (*ica*) locus is present in S*taphylococcus aureus* and is required for biofilm formation. Infect Immun 67(10):5427–5433

Cramton SE, Ulrich M, Gotz F, Doring G (2001) Anaerobic conditions induce expression of polysaccharide intercellular adhesin in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Infect Immun 69(6):4079–4085

Cunnion KM, Lee JC, Frank MM (2001) Capsule production and growth phase influence binding of complement to *Staphylococcus aureus*. Infect Immun 69:6796–6803

Cywes-Bentley C, Skurnik D, Zaidi T, Roux D, Deoliveira RB, Garrett WS, Lu X, O'Malley J, Kinzel K, Zaidi T, Rey A, Perrin C, Fichorova RN, Kayatani AK, Maira-Litran T, Gening ML, Tsvetkov YE, Nifantiev NE, Bakaletz LO, Pelton SI, Golenbock DT, Pier GB (2013) Antibody to a conserved antigenic target is protective against diverse prokaryotic and eukaryotic pathogens. Proc Natl Acad Sci USA 110(24):E2209–2218

D'Elia MA, Henderson JA, Beveridge TJ, Heinrichs DE, Brown ED (2009) The N-acetylmannosamine transferase catalyzes the first committed step of teichoic acid assembly in *Bacillus subtilis* and *Staphylococcus aureus*. J Bacteriol 191(12):4030–4034

D'Elia MA, Millar KE, Beveridge TJ, Brown ED (2006a) Wall teichoic acid polymers are dispensable for cell viability in *Bacillus subtilis*. J Bacteriol 188(23):8313–8316

D'Elia MA, Pereira MP, Chung YS, Zhao W, Chau A, Kenney TJ, Sulavik MC, Black TA, Brown ED (2006b) Lesions in teichoic acid biosynthesis in *Staphylococcus aureus* lead to a lethal gain of function in the otherwise dispensable pathway. J Bacteriol 188(12):4183–4189

Dassy B, Hogan T, Foster TJ, Fournier JM (1993) Involvement of the accessory gene regulator (*agr*) in expression of type-5 capsular polysaccharide by *Staphylococcus aureus*. J Gen Microbiol 139:1301–1306

Dengler V, Meier PS, Heusser R, Kupferschmied P, Fazekas J, Friebe S, Staufer SB, Majcherczyk PA, Moreillon P, Berger-Bachi B, McCallum N (2012) Deletion of hypothetical wall teichoic acid ligases in *Staphylococcus aureus* activates the cell wall stress response. FEMS Microbiol Lett 333(2):109–120

Ding Y, Liu X, Chen F, Di H, Xu B, Zhou L, Deng X, Wu M, Yang CG, Lan L (2014) Metabolic sensor governing bacterial virulence in *Staphylococcus aureus*. Proc Natl Acad Sci USA 111 (46):E4981–4990

Endl J, Seidl HP, Fiedler F, Schleifer KH (1983) Chemical composition and structure of cell wall teichoic acids of staphylococci. Arch Microbiol 135(3):215–223

Endl J, Seidl PH, Fiedler F, Schleifer KH (1984) Determination of cell wall teichoic acid structure of staphylococci by rapid chemical and serological screening methods. Arch Microbiol 137 (3):272–280

Farha MA, Koteva K, Gale RT, Sewell EW, Wright GD, Brown ED (2014) Designing analogs of ticlopidine, a wall teichoic acid inhibitor, to avoid formation of its oxidative metabolites. Bioorg Med Chem Lett 24(3):905–910

Farha MA, Leung A, Sewell EW, D'Elia MA, Allison SE, Ejim L, Pereira PM, Pinho MG, Wright GD, Brown ED (2013) Inhibition of WTA synthesis blocks the cooperative action of PBPs and sensitizes MRSA to beta-lactams. ACS Chem Biol 8(1):226–233

Fattom A, Guidry A (1999) Response to letter to the editor—questions uniqueness of surface polysaccharide. Am J Vet Res 60(5):530

Fattom A, Matalon A, Buerkert J, Taylor K, Damaso S, Boutriau D (2015) Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: phase III randomized study. Hum Vaccin Immunother 11(3):632–641

Fattom A, Schneerson R, Szu SC, Vann WF, Shiloach J, Karakawa WW, Robbins JB (1990) Synthesis and immunologic properties in mice of vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides conjugated to *Pseudomonas aeruginosa* exotoxin A. Infect Immun 58(7):2367–2374

Fattom A, Schneerson R, Watson DC, Karakawa WW, Fitzgerald D, Pastan I, Li X, Shiloach J, Bryla DA, Robbins JB (1993) Laboratory and clinical evaluation of conjugate vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides bound to *Pseudomonas aeruginosa* recombinant exoprotein A. Infect Immun 61(3):1023–1032

Fattom AI, Sarwar J, Ortiz A, Naso R (1996) A *Staphylococcus aureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. Infect Immun 64(5):1659–1665

Fischer J, Lee JC, Peters G, Kahl BC (2014) Acapsular clinical *Staphylococcus aureus* isolates lack *agr* function. Clin Microbiol Infect 20(7):O414–417

Fischer W, Rosel P, Koch HU (1981) Effect of alanine ester substitution and other structural features of lipoteichoic acids on their inhibitory activity against autolysins of *Staphylococcus aureus*. J Bacteriol 146(2):467–475

Fluckiger U, Ulrich M, Steinhuber A, Doring G, Mack D, Landmann R, Goerke C, Wolz C (2005) Biofilm formation, *icaADBC* transcription, and polysaccharide intercellular adhesin synthesis by staphylococci in a device-related infection model. Infect Immun 73(3):1811–1819

Fournier B, Klier A, Rapoport G (2001) The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. Mol Microbiol 41(1):247–261

Fournier JM, Vann WF, Karakawa WW (1984) Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. Infect Immun 45(1):87–93

Francois P, Tu Quoc PH, Bisognano C, Kelley WL, Lew DP, Schrenzel J, Cramton SE, Gotz F, Vaudaux P (2003) Lack of biofilm contribution to bacterial colonisation in an experimental model of foreign body infection by *Staphylococcus aureus* and *Staphylococcus epidermidis*. FEMS Immunol Med Microbiol 35(2):135–140

Frankel MB, Schneewind O (2012) Determinants of murein hydrolase targeting to cross-wall of *Staphylococcus aureus* peptidoglycan. J Biol Chem 287(13):10460–10471

Gening ML, Tsvetkov YE, Pier GB, Nifantiev NE (2007) Synthesis of beta-(1 → 6)-linked glucosamine oligosaccharides corresponding to fragments of the bacterial surface polysaccharide poly-N-acetylglucosamine. Carbohydr Res 342(3–4):567–575

George SE, Nguyen T, Geiger T, Weidenmaier C, Lee JC, Liese J, Wolz C (2015) Phenotypic heterogeneity and temporal expression of the capsular polysaccharide in *Staphylococcus aureus*. Mol Microbiol 98(16):1073–1088

Gerke C, Kraft A, Sussmuth R, Schweitzer O, Gotz F (1998) Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. J Biol Chem 273(29):18586–18593

Ginsberg C, Zhang YH, Yuan Y, Walker S (2006) In vitro reconstitution of two essential steps in wall teichoic acid biosynthesis. ACS Chem Biol 1(1):25–28

Goerke C, Koller J, Wolz C (2006a) Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. Antimicrob Agents Chemother 50(1):171–177

Goerke C, Wirtz C, Fluckiger U, Wolz C (2006b) Extensive phage dynamics in *Staphylococcus aureus* contributes to adaptation to the human host during infection. Mol Microbiol 61 (6):1673–1685

Grangeasse C, Cozzone AJ, Deutscher J, Mijakovic I (2007) Tyrosine phosphorylation: an emerging regulatory device of bacterial physiology. Trends Biochem Sci 32(2):86–94

Gruszczyk J, Olivares-Illana V, Nourikyan J, Fleurie A, Bechet E, Gueguen-Chaignon V, Freton C, Aumont-Nicaise M, Morera S, Grangeasse C, Nessler S (2013) Comparative analysis of the Tyr-kinases CapB1 and CapB2 fused to their cognate modulators CapA1 and CapA2 from *Staphylococcus aureus*. PLoS ONE 8(10):e75958

Guo H, Yi W, Song JK, Wang PG (2008) Current understanding on biosynthesis of microbial polysaccharides. Curr Top Med Chem 8(2):141–151

Gupta RK, Alba J, Xiong YQ, Bayer AS, Lee CY (2013) MgrA activates expression of capsule genes, but not the alpha-toxin gene in experimental *Staphylococcus aureus* endocarditis. J Infect Dis 208(11):1841–1848

Hansenova Manaskova S, Bikker FJ, Veerman EC, van Belkum A, van Wamel WJ (2013) Rapid detection and semi-quantification of IgG-accessible *Staphylococcus aureus* surface-associated antigens using a multiplex competitive Luminex assay. J Immunol Methods 397(1–2):18–27

Hartmann T, Baronian G, Nippe N, Voss M, Schulthess B, Wolz C, Eisenbeis J, Schmidt-Hohagen K, Gaupp R, Sunderkotter C, Beisswenger C, Bals R, Somerville GA, Herrmann M, Molle V, Bischoff M (2014) The catabolite control protein E (CcpE) affects virulence determinant production and pathogenesis of *Staphylococcus aureus*. J Biol Chem 289(43):29701–29711

Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F (1996) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol Microbiol 20 (5):1083–1091

Herbert S, Newell SW, Lee C, Wieland KP, Dassy B, Fournier JM, Wolz C, Doring G (2001) Regulation of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides by CO_2. J Bacteriol 183:4609–4613

Herbert S, Worlitzsch D, Dassy B, Boutonnier A, Fournier J-M, Bellon G, Dalhoff A, Doring G (1997) Regulation of *Staphylococcus aureus* capsular polysaccharide type 5: CO_2 inhibition in vitro and in vivo. J Infect Dis 176:431–438

Hilmi D, Parcina M, Stollewerk D, Ostrop J, Josten M, Meilaender A, Zaehringer U, Wichelhaus TA, Bierbaum G, Heeg K, Wolz C, Bekeredjian-Ding I (2014) Heterogeneity of host TLR2 stimulation by *Staphylocoocus aureus* isolates. PLoS ONE 9(5):e96416

Hochkeppel HK, Braun DG, Vischer W, Imm A, Sutter S, Staeubli U, Guggenheim R, Kaplan EL, Boutonnier A, Fournier JM (1987) Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J Clin Microbiol 25(3):526–530

Horwitz MA (1980) The roles of the Fc and C3 receptors in the phagocytosis and killing of bacteria by human phagocytes. J Reticuloendothel Soc 28(Suppl):17s–26s

Jansen A, Szekat C, Schroder W, Wolz C, Goerke C, Lee JC, Turck M, Bierbaum G (2013) Production of capsular polysaccharide does not influence *Staphylococcus aureus* vancomycin susceptibility. BMC Microbiol 13:65

Jefferson KK, Cramton SE, Gotz F, Pier GB (2003) Identification of a 5-nucleotide sequence that controls expression of the *ica* locus in *Staphylococcus aureus* and characterization of the DNA-binding properties of IcaR. Mol Microbiol 48(4):889–899

Jenni R, Berger-Bachi B (1998) Teichoic acid content in different lineages of *Staphylococcus aureus* NCTC8325. Arch Microbiol 170(3):171–178

Jones C (2005) Revised structures for the capsular polysaccharides from *Staphylococcus aureus* types 5 and 8, components of novel glycoconjugate vaccines. Carbohydr Res 340 (6):1097–1106

Jung DJ, An JH, Kurokawa K, Jung YC, Kim MJ, Aoyagi Y, Matsushita M, Takahashi S, Lee HS, Takahashi K, Lee BL (2012) Specific serum Ig recognizing staphylococcal wall teichoic acid induces complement-mediated opsonophagocytosis against *Staphylococcus aureus*. J Immunol 189(10):4951–4959

Kawai Y, Marles-Wright J, Cleverley RM, Emmins R, Ishikawa S, Kuwano M, Heinz N, Bui NK, Hoyland CN, Ogasawara N, Lewis RJ, Vollmer W, Daniel RA, Errington J (2011) A widespread family of bacterial cell wall assembly proteins. EMBO J 30(24):4931–4941

Kelly-Quintos C, Cavacini LA, Posner MR, Goldmann D, Pier GB (2006) Characterization of the opsonic and protective activity against *Staphylococcus aureus* of fully human monoclonal antibodies specific for the bacterial surface polysaccharide poly-N-acetylglucosamine. Infect Immun 74(5):2742–2750

Kelly-Quintos C, Kropec A, Briggs S, Ordonez C, Goldmann DA, Pier GB (2005) The role of epitope specificity in the human opsonic antibody response to the staphylococcal surface polysaccharide PNAG. J Infect Dis 192(11):2012–2019

Kern T, Giffard M, Hediger S, Amoroso A, Giustini C, Bui NK, Joris B, Bougault C, Vollmer W, Simorre JP (2010) Dynamics characterization of fully hydrated bacterial cell walls by solid-state NMR: evidence for cooperative binding of metal ions. J Am Chem Soc 132 (31):10911–10919

Kiser KB, Bhasin N, Deng L, Lee JC (1999a) *Staphylococcus aureus cap5P* encodes a UDP-N-acetylglucosamine 2-epimerase with functional redundancy. J Bacteriol 181 (16):4818–4824

Kiser KB, Cantey-Kiser JM, Lee JC (1999b) Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice. Infect Immun 67(10):5001–5006

Kneidinger B, O'Riordan K, Li J, Brisson JR, Lee JC, Lam JS (2003) Three highly conserved proteins catalyze the conversion of UDP-N-acetyl-D-glucosamine to precursors for the biosynthesis of O antigen in *Pseudomonas aeruginosa* O11 and capsule in *Staphylococcus aureus* type 5. Implications for the UDP-N-acetyl-L-fucosamine biosynthetic pathway. J Biol Chem 278(6):3615–3627

Kojima N, Araki Y, Ito E (1985) Structure of the linkage units between ribitol teichoic acids and peptidoglycan. J Bacteriol 161:299–306

Kolata JB, Kuhbandner I, Link C, Normann N, Vu CH, Steil L, Weidenmaier C, Broker BM (2015) The fall of a dogma? Unexpected high T-cell memory response to *Staphylococcus aureus* in humans. J Infect Dis 212:830

Kropec A, Maira-Litran T, Jefferson KK, Grout M, Cramton SE, Gotz F, Goldmann DA, Pier GB, Kropec A, Maira-Litran T, Jefferson KK, Grout M, Cramton SE, Gotz F, Goldmann DA, Pier GB (2005) Poly-N-acetylglucosamine production in *Staphylococcus aureus* is essential for virulence in murine models of systemic infection. Infect Immun 73(10):6868–6876

Kurokawa K, Jung DJ, An JH, Fuchs K, Jeon YJ, Kim NH, Li X, Tateishi K, Park JA, Xia G, Matsushita M, Takahashi K, Park HJ, Peschel A, Lee BL (2013) Glycoepitopes of staphylococcal wall teichoic acid govern complement-mediated opsonophagocytosis via human serum antibody and mannose-binding lectin. J Biol Chem 288(43):30956–30968

Kuypers JM, Proctor RA (1989) Reduced adherence to traumatized rat heart valves by a low-fibronectin-binding mutant of *Staphylococcus aureus*. Infect Immun 57(8):2306–2312

Lazarevic V, Karamata D (1995) The *tagGH* operon of *Bacillus subtilis* 168 encodes a two-component ABC transporter involved in the metabolism of two wall teichoic acids. Mol Microbiol 16(2):345–355

Lee DC, Jia Z (2009) Emerging structural insights into bacterial tyrosine kinases. Trends Biochem Sci 34(7):351–357

Lee JC, Park JS, Shepherd SE, Carey V, Fattom A (1997) Protective efficacy of antibodies to the *Staphylococcus aureus* type 5 capsular polysaccharide in a modified model of endocarditis in rats. Infect Immun 65(10):4146–4151

Lee JC, Takeda S, Livolsi PJ, Paoletti LC (1993) Effects of in vitro and in vivo growth conditions on expression of type 8 capsular polysaccharide by *Staphylococcus aureus*. Infect Immun 61 (5):1853–1858

Lee JH, Kim NH, Winstel V, Kurokawa K, Larsen J, An JH, Khan A, Seong MY, Lee MJ, Andersen PS, Peschel A, Lee BL (2015) Surface-glycopolymers are crucial for in vitro anti-WTA IgG-mediated complement activation and opsonophagocytosis of *Staphylococcus aureus*. Infect Immun 83:4247

Lee K, Campbell J, Swoboda JG, Cuny GD, Walker S (2010) Development of improved inhibitors of wall teichoic acid biosynthesis with potent activity against *Staphylococcus aureus*. Bioorg Med Chem Lett 20(5):1767–1770

Levy J, Licini L, Haelterman E, Moris P, Lestrate P, Damaso S, Van Belle P, Boutriau D (2015) Safety and immunogenicity of an investigational 4-component *Staphylococcus aureus* vaccine with or without AS03B adjuvant: results of a randomized phase I trial. Hum Vaccin Immunother 11(3):620–631

Li W, Ulm H, Rausch M, Li X, O'Riordan K, Lee JC, Schneider T, Muller CE (2014) Analysis of the *Staphylococcus aureus* capsule biosynthesis pathway in vitro: characterization of the UDP-GlcNAc C6 dehydratases CapD and CapE and identification of enzyme inhibitors. Int J Med Microbiol 304(8):958–969

Liang X, Zheng L, Landwehr C, Lunsford D, Holmes D, Ji Y (2005) Global regulation of gene expression by ArlRS, a two-component signal transduction regulatory system of S*taphylococcus aureus*. J Bacteriol 187(15):5486–5492

Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schaberle TF, Hughes DE, Epstein S, Jones M, Lazarides L, Steadman VA, Cohen DR, Felix CR, Fetterman KA, Millett WP, Nitti AG, Zullo AM, Chen C, Lewis K (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517 (7535):455-459

Luong T, Sau S, Gomez M, Lee JC, Lee CY (2002a) Regulation of *Staphylococcus aureus* capsular polysaccharide expression by *agr* and *sarA*. Infect Immun 70:444–450

Luong TT, Lee CY (2006) The *arl* locus positively regulates *Staphylococcus aureus* type 5 capsule via an *mgrA*-dependent pathway. Microbiology 152(Pt 10):3123–3131

Luong TT, Newell SW, Lee CY (2003) Mgr, a novel global regulator in *Staphylococcus aureus*. J Bacteriol 185(13):3703–3710

Luong TT, Ouyang S, Bush K, Lee CY (2002b) Type 1 capsule genes of *Staphylococcus aureus* are carried in a staphylococcal cassette chromosome genetic element. J Bacteriol 184 (13):3623–3629

Luong TT, Sau K, Roux C, Sau S, Dunman PM, Lee CY (2011) *Staphylococcus aureus* ClpC divergently regulates capsule via *sae* and *codY* in strain Newman but activates capsule via *codY* in strain UAMS-1 and in strain Newman with repaired *saeS*. J Bacteriol 193(3):686–694

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

Mack D, Haeder M, Siemssen N, Laufs R (1996b) Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. J Infect Dis 174(4):881–884

Maira-Litran T, Kropec A, Abeygunawardana C, Joyce J, Mark G 3rd, Goldmann DA, Pier GB (2002) Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. Infect Immun 70(8):4433–4440

Maira-Litran T, Kropec A, Goldmann DA, Pier GB (2005) Comparative opsonic and protective activities of *Staphylococcus aureus* conjugate vaccines containing native or deacetylated Staphylococcal Poly-N-acetyl-beta-(1-6)-glucosamine. Infect Immun 73(10):6752–6762

Majerczyk CD, Dunman PM, Luong TT, Lee CY, Sadykov MR, Somerville GA, Bodi K, Sonenshein AL (2010) Direct targets of CodY in *Staphylococcus aureus*. J Bacteriol 192 (11):2861–2877

Majerczyk CD, Sadykov MR, Luong TT, Lee C, Somerville GA, Sonenshein AL (2008) *Staphylococcus aureus* CodY negatively regulates virulence gene expression. J Bacteriol 190 (7):2257–2265

Matias VR, Beveridge TJ (2005) Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. Mol Microbiol 56 (1):240–251

Matias VR, Beveridge TJ (2006) Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in *Staphylococcus aureus*. J Bacteriol 188 (3):1011–1021

May JJ, Finking R, Wiegeshoff F, Weber TT, Bandur N, Koert U, Marahiel MA (2005) Inhibition of the D-alanine: D-alanyl carrier protein ligase from *Bacillus subtilis* increases the bacterium's susceptibility to antibiotics that target the cell wall. FEBS J 272(12):2993–3003

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

McLoughlin RM, Solinga RM, Rich J, Zaleski KJ, Cocchiaro JL, Risley A, Tzianabos AO, Lee JC (2006) CD4 + T cells and CXC chemokines modulate the pathogenesis of *Staphylococcus aureus* wound infections. Proc Natl Acad Sci USA 103(27):10408–10413

Meier S, Goerke C, Wolz C, Seidl K, Homerova D, Schulthess B, Kormanec J, Berger-Bachi B, Bischoff M (2007) sigmaB and the sigmaB-dependent *arlRS* and *yabJ-spoVG* loci affect capsule formation in *Staphylococcus aureus*. Infect Immun 75(9):4562–4571

Meredith TC, Swoboda JG, Walker S (2008) Late-stage polyribitol phosphate wall teichoic acid biosynthesis in *Staphylococcus aureus*. J Bacteriol 190(8):3046–3056

Misawa Y, Kelley KA, Wang X, Wang L, Park WB, Birtel J, Saslowsky D, Lee JC (2015) *Staphylococcus aureus* colonization of the mouse gastrointestinal tract is modulated by wall teichoic acid, capsule, and surface proteins. PLoS Pathog 11(7):e1005061

Mishra NN, Bayer AS, Weidenmaier C, Grau T, Wanner S, Stefani S, Cafiso V, Bertuccio T, Yeaman MR, Nast CC, Yang SJ (2014) Phenotypic and genotypic characterization of daptomycin-resistant methicillin-resistant *Staphylococcus aureus* strains: relative roles of *mprF* and *dlt* operons. PLoS ONE 9(9):e107426

Montgomery CP, Boyle-Vavra S, Adem PV, Lee JC, Husain AN, Clasen J, Daum RS (2008) Comparison of virulence in community-associated methicillin-resistant *Staphylococcus aureus* pulsotypes USA300 and USA400 in a rat model of pneumonia. J Infect Dis 198(4):561–570

Moreau M, Richards JC, Fournier JM, Byrd RA, Karakawa WW, Vann WF (1990) Structure of the type-5 capsular polysaccharide of *Staphylococcus aureus*. Carbohydrate Res 201(2):285–297

Moreillon P, Entenza JM, Francioli P, McDevitt D, Foster TJ, Francois P, Vaudaux P (1995) Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect Immun 63(12):4738–4743

Morona JK, Morona R, Miller DC, Paton JC (2002) *Streptococcus pneumoniae* capsule biosynthesis protein CpsB is a novel manganese-dependent phosphotyrosine-protein phosphatase. J Bacteriol 184(2):577–583

Muller E, Hubner J, Gutierrez N, Takeda S, Goldmann DA, Pier GB (1993) Isolation and characterization of transposon mutants of *Staphylococcus epidermidis* deficient in capsular polysaccharide/adhesin and slime. Infect Immun 61(2):551–558

Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, Scully IL, McNeil LK, Aste-Amézaga JM, Cooper D (2013) Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Hum Vaccines Immunotherapeutics 9 (3):480–487

Nemeth J, Lee JC (1995) Antibodies to capsular polysaccharides are not protective against experimental *Staphylococcus aureus* endocarditis. Infect Immun 63:375–380

Neuhaus FC, Baddiley J (2003) A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. Microbiol Mol Biol Rev 67(4):686–723

Nilsson I-M, Lee JC, Bremell T, Ryden C, Tarkowski A (1997) The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis. Infect Immun 65:4216–4221

Nissen M, Marshall H, Richmond P, Shakib S, Jiang Q, Cooper D, Rill D, Baber J, Eiden J, Gruber W, Jansen KU, Emini EA, Anderson AS, Zito ET, Girgenti D (2015) A randomized phase I study of the safety and immunogenicity of three ascending dose levels of a 3-antigen *Staphylococcus aureus* vaccine (SA3Ag) in healthy adults. Vaccine 33(15):1846–1854

Novick RP, Geisinger E (2008) Quorum sensing in staphylococci. Annu Rev Genet 42:541–564

O'Riordan K, Lee JC (2004) *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev 17(1):218–234

Park KH, Kurokawa K, Zheng L, Jung DJ, Tateishi K, Jin JO, Ha NC, Kang HJ, Matsushita M, Kwak JY, Takahashi K, Lee BL (2010) Human serum mannose-binding lectin senses wall teichoic acid glycopolymer of *Staphylococcus aureus,* which is restricted in infancy. J Biol Chem 285(35):27167–27175

Park S, Gerber S, Lee JC (2014) Antibodies to *Staphylococcus aureus* serotype 8 capsular polysaccharide react with and protect against serotype 5 and 8 isolates. Infect Immun 82 (12):5049–5055

Perego M, Glaser P, Minutello A, Strauch MA, Leopold K, Fischer W (1995) Incorporation of D-alanine into lipoteichoic acid and wall teichoic acid in *Bacillus subtilis.* Identification of genes and regulation. J Biol Chem 270(26):15598–15606

Pereira MP, Brown ED (2004) Bifunctional catalysis by CDP-ribitol synthase: convergent recruitment of reductase and cytidylyltransferase activities in *Haemophilus influenzae* and *Staphylococcus aureus*. Biochemistry 43(37):11802–11812

Pereira MP, D'Elia MA, Troczynska J, Brown ED (2008) Duplication of teichoic acid biosynthetic genes in *Staphylococcus aureus* leads to functionally redundant poly(ribitol phosphate) polymerases. J Bacteriol 190(16):5642–5649

Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F (1999) Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem 274(13):8405–8410

Peschel A, Vuong C, Otto M, Gotz F (2000) The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. Antimicrob Agents Chemother 44(10):2845–2847

Pohl K, Francois P, Stenz L, Schlink F, Geiger T, Herbert S, Goerke C, Schrenzel J, Wolz C (2009) CodY in *Staphylococcus aureus*: a regulatory link between metabolism and virulence gene expression. J Bacteriol 191(9):2953–2963

Pohlmann-Dietze P, Ulrich M, Kiser KB, Doring G, Lee JC, Fournier JM, Botzenhart K, Wolz C (2000) Adherence of *Staphylococcus aureus* to endothelial cells: influence of capsular polysaccharide, global regulator *agr,* and bacterial growth phase. Infect Immun 68(9):4865–4871

Portoles M, Kiser KB, Bhasin N, Chan KHN, Lee JC (2001) *Staphylococcus aureus* Cap5O has UDP-ManNAc dehydrogenase activity and is essential for capsule expression. Infect Immun 69:917–923

Poutrel B, Gilbert FB, Lebrun M (1995) Effects of culture conditions on production of type 5 capsular polysaccharide by human and bovine *Staphylococcus aureus* strains. Clin Diagn Lab Immunol 2:166–171

Poutrel B, Rainard P, Sarradin P (1997) Heterogeneity of cell-associated CP5 expression on *Staphylococcus aureus* strains demonstrated by flow cytometry. Clin Diagn Lab Immunol 4:275–278

Qamar A, Golemi-Kotra D (2012) Dual roles of FmtA in *Staphylococcus aureus* cell wall biosynthesis and autolysis. Antimicrob Agents Chemother 56(7):3797–3805

Qian Z, Yin Y, Zhang Y, Lu L, Li Y, Jiang Y (2006) Genomic characterization of ribitol teichoic acid synthesis in *Staphylococcus aureus*: genes, genomic organization and gene duplication. BMC Genom 7:74

Rachid S, Ohlsen K, Wallner U, Hacker J, Hecker M, Ziebuhr W (2000) Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus aureus* mucosal isolate. J Bacteriol 182(23):6824–6826

Reichmann NT, Cassona CP, Grundling A (2013) Revised mechanism of D-alanine incorporation into cell wall polymers in Gram-positive bacteria. Microbiology 159(Pt 9):1868–1877

Risley AL, Loughman A, Cywes-Bentley C, Foster TJ, Lee JC (2007) Capsular polysaccharide masks clumping factor A-mediated adherence of *Staphylococcus aureus* to fibrinogen and platelets. J Infect Dis 196(6):919–927

Roberts IS, Saunders FK, Boulnois GJ (1989) Bacterial capsules and interactions with complement and phagocytes. Biochem Soc Trans 17(3):462–464

Roghmann M, Taylor KL, Gupte A, Zhan M, Johnson JA, Cross A, Edelman R, Fattom AI (2005) Epidemiology of capsular and surface polysaccharide in *Staphylococcus aureus* infections complicated by bacteraemia. J Hosp Infect 59(1):27–32

Romilly C, Lays C, Tomasini A, Caldelari I, Benito Y, Hammann P, Geissmann T, Boisset S, Romby P, Vandenesch F (2014) A non-coding RNA promotes bacterial persistence and decreases virulence by regulating a regulator in *Staphylococcus aureus*. PLoS Pathog 10(3): e1003979

Sadovskaya I, Vinogradov E, Flahaut S, Kogan G, Jabbouri S (2005) Extracellular carbohydrate-containing polymers of a model biofilm-producing strain, *Staphylococcus epidermidis* RP62A. Infect Immun 73(5):3007–3017

Sau S, Bhasin N, Wann ER, Lee JC, Foster TJ, Lee CY (1997a) The *Staphylococcus aureus* allelic genetic loci for serotype 5 and 8 capsule expression contain the type-specific genes flanked by common genes. Microbiol 143:2395–2405

Sau S, Sun J, Lee CY (1997b) Molecular characterization and transcriptional analysis of type 8 capsule genes in *Staphylococcus aureus*. J Bacteriol 179:1614–1621

Schaffer AC, Solinga RM, Cocchiaro J, Portoles M, Kiser KB, Risley A, Randall SM, Valtulina V, Speziale P, Walsh E, Foster T, Lee JC (2006) Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect Immun 74(4):2145–2153

Schlag M, Biswas R, Krismer B, Kohler T, Zoll S, Yu W, Schwarz H, Peschel A, Gotz F (2010) Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl. Mol Microbiol 75(4):864–873

Schulthess B, Meier S, Homerova D, Goerke C, Wolz C, Kormanec J, Berger-Bachi B, Bischoff M (2009) Functional characterization of the sigmaB-dependent *yabJ-spoVG* operon in *Staphylococcus aureus*: role in methicillin and glycopeptide resistance. Antimicrob Agents Chemother 53(5):1832–1839

Scully IL, Liberator PA, Jansen KU, Anderson AS (2014) Covering all the bases: preclinical development of an effective *Staphylococcus aureus* vaccine. Front Immunol 5:109

Scully IL, Timofeyeva Y, Keeney D, Matsuka YV, Severina E, McNeil LK, Nanra J, Hu G, Liberator PA, Jansen KU, Anderson AS (2015) Demonstration of the preclinical correlate of protection for *Staphylococcus aureus* clumping factor A in a murine model of infection. Vaccine 33(41):5452–5457

Seidl K, Stucki M, Ruegg M, Goerke C, Wolz C, Harris L, Berger-Bachi B, Bischoff M (2006) *Staphylococcus aureus* CcpA affects virulence determinant production and antibiotic resistance. Antimicrob Agents Chemother 50(4):1183–1194

Sewell EW, Brown ED (2014) Taking aim at wall teichoic acid synthesis: new biology and new leads for antibiotics. J Antibiot (Tokyo) 67(1):43–51

Shinefield H, Black S, Fattom A, Horwith G, Rasgon S, Ordonez J, Yeoh H, Law D, Robbins JB, Schneerson R, Muenz L, Fuller S, Johnson J, Fireman B, Alcorn H, Naso R (2002) Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. N Engl J Med 346(7):491–496

Skurnik D, Merighi M, Grout M, Gadjeva M, Maira-Litran T, Ericsson M, Goldmann DA, Huang SS, Datta R, Lee JC, Pier GB (2010) Animal and human antibodies to distinct *Staphylococcus aureus* antigens mutually neutralize opsonic killing and protection in mice. J Clin Invest 120(9):3220–3233

Soldo B, Lazarevic V, Karamata D (2002a) *tagO* is involved in the synthesis of all anionic cell-wall polymers in *Bacillus subtilis* 168. Microbiology 148(Pt 7):2079–2087

Soldo B, Lazarevic V, Pooley HM, Karamata D (2002b) Characterization of a *Bacillus subtilis* thermosensitive teichoic acid-deficient mutant: gene *mnaA (yvyH)* encodes the UDP-N-acetylglucosamine 2-epimerase. J Bacteriol 184(15):4316–4320

Soulat D, Grangeasse C, Vaganay E, Cozzone AJ, Duclos B (2007) UDP-acetyl-mannosamine dehydrogenase is an endogenous protein substrate of *Staphylococcus aureus* protein-tyrosine kinase activity. J Mol Microbiol Biotechnol 13(1–3):45–54

Soulat D, Jault JM, Duclos B, Geourjon C, Cozzone AJ, Grangeasse C (2006) *Staphylococcus aureus* operates protein-tyrosine phosphorylation through a specific mechanism. J Biol Chem 281(20):14048–14056

Steinhuber A, Goerke C, Bayer MG, Doring G, Wolz C (2003) Molecular architecture of the regulatory locus *sae* of *Staphylococcus aureus* and its impact on expression of virulence factors. J Bacteriol 185(21):6278–6286

Sun F, Ji Q, Jones MB, Deng X, Liang H, Frank B, Telser J, Peterson SN, Bae T, He C (2012) AirSR, a [2Fe-2S] cluster-containing two-component system, mediates global oxygen sensing and redox signaling in *Staphylococcus aureus*. J Am Chem Soc 134(1):305–314

Sutcliffe IC (2012) Exposing a chink in the armor of methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci USA 109(46):18637–18638

Suzuki T, Campbell J, Swoboda JG, Walker S, Gilmore MS (2011a) Role of wall teichoic acids in *Staphylococcus aureus* endophthalmitis. Invest Ophthalmol Vis Sci 52(6):3187–3192

Suzuki T, Swoboda JG, Campbell J, Walker S, Gilmore MS (2011b) In vitro antimicrobial activity of wall teichoic acid biosynthesis inhibitors against *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 55(2):767–774

Swoboda JG, Campbell J, Meredith TC, Walker S (2010) Wall teichoic acid function, biosynthesis, and inhibition. ChemBioChem 11(1):35–45

Swoboda JG, Meredith TC, Campbell J, Brown S, Suzuki T, Bollenbach T, Malhowski AJ, Kishony R, Gilmore MS, Walker S (2009) Discovery of a small molecule that blocks wall teichoic acid biosynthesis in *Staphylococcus aureus*. ACS Chem Biol 4(10):875–883

Takahashi K, Kurokawa K, Moyo P, Jung DJ, An JH, Chigweshe L, Paul E, Lee BL (2013) Intradermal immunization with wall teichoic acid (WTA) elicits and augments an anti-WTA IgG response that protects mice from methicillin-resistant *Staphylococcus aureus* infection independent of mannose-binding lectin status. PLoS ONE 8(8):e69739

Thakker M, Park J-S, Carey V, Lee JC (1998) *Staphylococcus aureus* serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect Immun 66:5183–5189

Thoendel M, Kavanaugh JS, Flack CE, Horswill AR (2011) Peptide signaling in the staphylococci. Chem Rev 111(1):117–151

Thomas KJ, Rice CV (2015) Equilibrium binding behavior of magnesium to wall teichoic acid. Biochim Biophys Acta 1848 (10 Pt A):1981–1987

Tu Quoc PH, Genevaux P, Pajunen M, Savilahti H, Georgopoulos C, Schrenzel J, Kelley WL (2007) Isolation and characterization of biofilm formation-defective mutants of *Staphylococcus aureus*. Infect Immun 75(3):1079–1088

Tuchscherr LP, Buzzola FR, Alvarez LP, Caccuri RL, Lee JC, Sordelli DO (2005) Capsule-negative *Staphylococcus aureus* induces chronic experimental mastitis in mice. Infect Immun 73(12):7932–7937

Tuchscherr LP, Buzzola FR, Alvarez LP, Lee JC, Sordelli DO (2008) Antibodies to capsular polysaccharide and clumping factor A prevent mastitis and the emergence of unencapsulated and small-colony variants of *Staphylococcus aureus* in mice. Infect Immun 76(12):5738–5744

Tzianabos AO, Wang JY, Lee JC (2001) Structural rationale for the modulation of abscess formation by *Staphylococcus aureus* capsular polysaccharides. Proc Natl Acad Sci USA 98 (16):9365–9370

Ubeda C, Maiques E, Knecht E, Lasa I, Novick RP, Penades JR (2005) Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol Microbiol 56(3):836–844

Valle J, Toledo-Arana A, Berasain C, Ghigo JM, Amorena B, Penades JR, Lasa I (2003) SarA and not sigmaB is essential for biofilm development by *Staphylococcus aureus*. Mol Microbiol 48 (4):1075–1087

Verdier I, Durand G, Bes M, Taylor KL, Lina G, Vandenesch F, Fattom AI, Etienne J (2007) Identification of the capsular polysaccharides in *Staphylococcus aureus* clinical isolates by PCR and agglutination tests. J Clin Microbiol 45(3):725–729

Vuong C, Kocianova S, Voyich JM, Yao Y, Fischer ER, DeLeo FR, Otto M (2004a) A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. J Biol Chem 279(52):54881–54886

Vuong C, Kocianova S, Yao Y, Carmody AB, Otto M (2004b) Increased colonization of indwelling medical devices by quorum-sensing mutants of *Staphylococcus epidermidis* in vivo. J Infect Dis 190(8):1498–1505

Wacker M, Wang L, Kowarik M, Dowd M, Lipowsky G, Faridmoayer A, Shields K, Park S, Alaimo C, Kelley KA, Braun M, Quebatte J, Gambillara V, Carranza P, Steffen M, Lee JC (2014) Prevention of *Staphylococcus aureus* infections by glycoprotein vaccines synthesized in *Escherichia coli*. J Infect Dis 209(10):1551–1561

Wang H, Gill CJ, Lee SH, Mann P, Zuck P, Meredith TC, Murgolo N, She X, Kales S, Liang L, Liu J, Wu J, Santa Maria J, Su J, Pan J, Hailey J, McGuinness D, Tan CM, Flattery A, Walker S, Black T, Roemer T (2013) Discovery of wall teichoic acid inhibitors as potential anti-MRSA beta-lactam combination agents. Chem Biol 20(2):272–284

Watts A, Ke D, Wang Q, Pillay A, Nicholson-Weller A, Lee JC (2005) *Staphylococcus aureus* strains that express serotype 5 or serotype 8 capsular polysaccharides differ in virulence. Infect Immun 73(6):3502–3511

Wecke J, Perego M, Fischer W (1996) D-alanine deprivation of *Bacillus subtilis* teichoic acids is without effect on cell growth and morphology but affects the autolytic activity. Microb Drug Resist 2(1):123–129

Weidenmaier C, Goerke C, Wolz C (2012) *Staphylococcus aureus* determinants for nasal colonization. Trends Microbiol 20(5):243–250

Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, Nicholson G, Neumeister B, Mond JJ, Peschel A (2004) Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med 10(3):243–245

Weidenmaier C, McLoughlin RM, Lee JC (2010) The zwitterionic cell wall teichoic acid of *Staphylococcus aureus* provokes skin abscesses in mice by a novel CD4 + T-cell-dependent mechanism. PLoS ONE 5(10):e13227

Weidenmaier C, Peschel A (2008) Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. Nat Rev Microbiol 6(4):276–287

Weidenmaier C, Peschel A, Xiong YQ, Kristian SA, Dietz K, Yeaman MR, Bayer AS (2005) Lack of wall teichoic acids in *Staphylococcus aureus* leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis. J Infect Dis 191 (10):1771–1777

Wickham JR, Halye JL, Kashtanov S, Khandogin J, Rice CV (2009) Revisiting magnesium chelation by teichoic acid with phosphorus solid-state NMR and theoretical calculations. J Phys Chem B 113(7):2177–2183

Wilkinson J (1958) The extracellular polysaccharides of bacteria. Bacteriol Rev 22:46–73

Winstel V, Kuhner P, Salomon F, Larsen J, Skov R, Hoffmann W, Peschel A, Weidenmaier C (2015) Wall teichoic acid glycosylation governs *Staphylococcus aureus* nasal colonization. mBio 6 (4):e00632

Winstel V, Liang C, Sanchez-Carballo P, Steglich M, Munar M, Broker BM, Penades JR, Nubel U, Holst O, Dandekar T, Peschel A, Xia G (2013) Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens. Nat Commun 4:2345

Winstel V, Sanchez-Carballo P, Holst O, Xia G, Peschel A (2014a) Biosynthesis of the unique wall teichoic acid of *Staphylococcus aureus* lineage ST395. mBio 5 (2):e00869

Winstel V, Xia G, Peschel A (2014b) Pathways and roles of wall teichoic acid glycosylation in *Staphylococcus aureus*. Int J Med Microbiol 304(3-4):215-221

Xia G, Corrigan RM, Winstel V, Goerke C, Grundling A, Peschel A (2011) Wall teichoic acid-dependent adsorption of staphylococcal siphovirus and myovirus. J Bacteriol 193 (15):4006–4009

Xia G, Maier L, Sanchez-Carballo P, Li M, Otto M, Holst O, Peschel A (2010) Glycosylation of wall teichoic acid in *Staphylococcus aureus* by TarM. J Biol Chem 285(18):13405–13415

Xia G, Wolz C (2014) Phages of *Staphylococcus aureus* and their impact on host evolution. Infect Genet Evol 21:593–601

Zhang YH, Ginsberg C, Yuan Y, Walker S (2006) Acceptor substrate selectivity and kinetic mechanism of *Bacillus subtilis* TagA. Biochemistry 45(36):10895–10904

Cell Wall-Anchored Surface Proteins of *Staphylococcus aureus*: Many Proteins, Multiple Functions

Joan A. Geoghegan and Timothy J. Foster

Abstract *Staphylococcus aureus* persistently colonizes about 20 % of the population and is intermittently associated with the remainder. The organism can cause superficial skin infections and life-threatening invasive diseases. The surface of the bacterial cell displays a variety of proteins that are covalently anchored to peptidoglycan. They perform many functions including adhesion to host cells and tissues, invasion of non-phagocytic cells, and evasion of innate immune responses. The proteins have been categorized into distinct classes based on structural and functional analysis. Many surface proteins are multifunctional. Cell wall-anchored proteins perform essential functions supporting survival and proliferation during the commensal state and during invasive infections. The ability of cell wall-anchored proteins to bind to desquamated epithelial cells is important during colonization, and the binding to fibrinogen is of particular significance in pathogenesis.

Contents

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1 Introduction

Staphylococcus aureus can express up to 24 distinct cell wall-anchored (CWA) surface proteins. The precise repertoire of CWA proteins of any particular strain is variable (McCarthy and Lindsay 2010). Furthermore, expression is dependent on growth conditions and the phase of growth (McAleese et al. 2001). For example, some are only expressed under iron-restricted conditions (Hammer and Skaar 2011).

At the N-terminus, CWA proteins have a secretory signal sequence that directs the translation product to the secretory machinery in the cytoplasmic membrane. This is removed during secretion. The carboxyl termini comprise a sorting signal that facilitates covalent anchorage of the secreted protein to cell wall peptidoglycan.

In a recent review, it was proposed that CWA proteins should primarily be classified on the basis of motifs that have been defined by structural and functional analysis (Foster et al. 2014). We propose to modify this by extending the classification to include functional motifs for which there are as yet no structural details (e.g. the 5′ nucleotidase activity of adenosine synthase AdsA) (Thammavongsa et al. 2009) or where a biological function is located in an intrinsically disordered (i.e. unstructured) region (fibronectin-binding protein FnBP fibronectin-binding repeats) (Schwarz-Linek et al. 2006).

It is now clear that a single CWA protein can have more than one function (Foster et al. 2014). The repertoire of CWA proteins is limited, and as they are exposed on the cell surface, they are in direct contact with the host, and thus, they have evolved to perform crucial functions in colonization and avoiding immune responses.

The name of the protein may have been defined by the function with which it was first associated (e.g. clumping factor), or a more general name may have been ascribed (e.g. *S. aureus* surface protein Sas, iron-regulated surface determinant Isd, surface protein A Spa) which either has been retained as structural and functional details subsequently emerged or has been changed when an important structure or function was defined (SasA is now called the serine-rich surface protein SraP, and SasH is now called AdsA).

This article will review the defining features associated with CWA proteins and will provide a framework for their categorization based on the current knowledge of structure and function. The review will also describe post-translational modifications that contribute to the function of proteins once they are elaborated on the cell surface and the role of CWA proteins in colonization and virulence.

2 CWA Surface Protein Secretion and Surface Display

2.1 Secretion

An N-terminal secretory signal sequence directs CWA precursor proteins to the Sec apparatus following their translation by the ribosome in the cytoplasm. The signal sequence of the precursor protein is cleaved by signal peptidase I (SpsB) following translocation across the cytoplasmic membrane (Cregg et al. 1996). The signal sequences of many CWA proteins contain the motif YSIRK-G/S. This is responsible for directing their secretion and anchorage close to the cell division site where peptidoglycan synthesis is actively occurring (DeDent et al. 2008). Following cell division, the proteins become distributed across the cell surface. In contrast, the proteins with conventional signal sequences lacking the YSIRK/GS motif are directed to the poles where they remain.

SraP is a member of a family of large heavily glycosylated serine-rich repeat proteins found in Gram-positive cocci (Lizcano et al. 2012). The SraP signal sequence is unusually long (90 residues). These proteins cannot be translocated by the conventional Sec pathway and require an accessory Sec system for export (Siboo et al. 2008).

2.2 Sorting

CWA protein precursors contain a C-terminal sorting signal that is essential for covalent linkage to cell wall peptidoglycan (Schneewind et al. 1992, 1993). The sorting signal comprises a conserved LPXTG motif followed by a hydrophobic domain and a positively charged tail. The hydrophobic region retains the protein within the membrane where it is recognized by sortase enzymes (Fig. 1). Sortase A cleaves between the threonine and glycine residues of the LPXTG motif forming an acyl–enzyme intermediate capturing the C-terminal carboxyl group of the protein with its active site cysteine thiol (Mazmanian et al. 1999). Acyl intermediates are relieved by the nucleophilic attack of the amino group of the pentaglycine cross-bridge of lipid II (Perry et al. 2002). Sortase B processes a single substrate in *S. aureus*, IsdC, and recognizes an NPQTN motif (Marraffini and Schneewind 2005). Following transpeptidation and transglycosylation reactions, CWA proteins become incorporated into the peptidoglycan of the cell wall (Mazmanian et al. 2001).

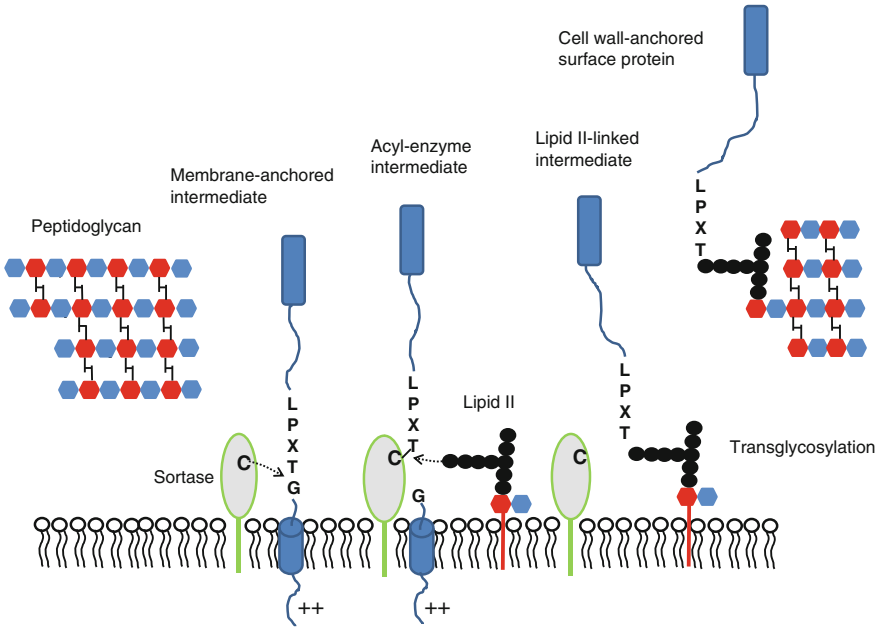


Fig. 1 Sorting and surface display of cell wall-anchored proteins. The figure depicts a single leaflet of the cytoplasmic membrane and the multilayered peptidoglycan of the cell envelope. The *red* and *blue* hexagons represent N-acetyl muramic acid and N-acetyl glucosamine, respectively. The line joining different polysaccharide chains represents the stem peptide and cross-bridges in fully cross-linked peptidoglycan. Sortase is a membrane-linked enzyme that is exposed on the outer face of the cytoplasmic membrane. The precursor of a cell wall-anchored protein is secreted through the Sec apparatus (not shown) and is transiently held by the hydrophobic transmembrane segment and the positively charged C-terminal tail region which enables sortase A to recognize the LPXTG motif. The active site cysteine (*C*) of sortase A attacks the amide bond between Thr and Gly forming a thioester acyl–enzyme intermediate. The NH2 group of the fifth glycine residue of the cross-bridge (all amino acids in lipid II are shown as *black filled circles*) in lipid II performs a nucleophilic attack on the thioester bond in the acyl-enzyme intermediate forming a cell wall protein–lipid II intermediate. Transglycosylation by penicillin-binding protein links the protein to the growing peptidoglycan. The outer surface of the cell is thereby decorated with several different cell wall-anchored proteins with their ligand-binding regions displayed

All three elements of the sorting signal are necessary for efficient anchoring of LPXTG motif-containing proteins to peptidoglycan by sortase A. Removing the charged tail results in the protein being released into the culture medium instead of becoming linked to the cell wall (Schneewind et al. 1992). Reducing the number of residues in the hydrophobic domain of the sorting signal leads to less protein becoming anchored to the cell wall (Schneewind et al. 1993).

Proteins that have become covalently linked to peptidoglycan by sortase can be released into the culture medium following cleavage of the pentaglycine cross-bridge by the glycyl–glycine endopeptidase LytM (Becker et al. 2014). In the case of protein A, a portion of the protein fails to become anchored to the cell wall

by sortase A so that a biologically active unsorted form is released into culture supernatants (O'Halloran et al. 2015).

3 Cell Wall-Anchored Protein Structure and Function

3.1 The MSCRAMM Family

3.1.1 Structure

The acronym MSCRAMM was originally applied to surface proteins that could bind to components of the extracellular matrix (ECM) such as collagen and fibronectin at a time when it was thought that *S. aureus* was primarily an extracellular pathogen that attached to glycoproteins in tissue and on the surface of cells (Patti et al. 1994a). However, it is now apparent that many different types of CWA proteins bind to the ECM and that some MSCRAMMs have additional functions other than promoting adhesion (Table 1). It was recently proposed that the term MSCRAMM be confined to proteins that have structural and functional similarities and a common mechanism of ligand binding performed by two adjacent IgG-like folded domains (Foster et al. 2014). The archetypal MSCRAMMs clumping factor A ClfA from *S. aureus* and SdrG from *Staphylococcus epidermidis* have N-terminal A regions composed of three separately folded subdomains N1, N2 and N3 with N2 and N3 comprising the adjacent IgG-like folds (Fig. 2).

3.1.2 Ligand Binding: Dock, Lock, and Latch

Proteins in the MSCRAMM family bind to their ligands by the dock, lock, and latch mechanism (Table 1; Fig. 3) (Ponnuraj et al. 2003; Foster et al. 2014). The N2 and N3 subdomains are the minimum required for ligand binding. N2 and N3 are folded separately and comprise 2 β-sheets that are arranged in a variation of the IgG fold (Deivanayagam et al. 2002). Located between N2 and N3 is a hydrophobic trench that accommodates unfolded peptide ligands, in the case of ClfA, FnBPA, and FnBPB, the extreme C-terminus of the γ-chain of fibrinogen (Fg) (Wann et al. 2000; Deivanayagam et al. 2002). ClfB binds the α-chain of Fg, cytokeratin 10, and loricrin (Ganesh et al. 2011; Mulcahy et al. 2012). Once the ligand is in place, a conformational change occurs in a short peptide attached to the C-terminus of subdomain N3 resulting in it folding over the bound peptide to lock it in place and forming an additional β-strand in one of the β-sheets of N2 called the latch (Ponnuraj et al. 2003).

Linking the A region to the proline-rich wall-spanning domain is a linker region that likely acts as a flexible stalk to project the N-terminal ligand-binding domain from the cell surface. The linker region is composed of either repeats of the dipeptide

Table 1 Structural and functional properties of cell wall-anchored surface proteins

Protein family	Ligand and binding mechanism	Function	References
MSCRAMMs			
Clumping factor A (ClfA)	Fg γ chain C-terminus (DLL)	Adhesion to immobilized fibrinogen, immune evasion by binding soluble fibrinogen	Deivanayagam et al. (2002), Ganesh et al. (2008)
	Complement factor I	Immune evasion, degradation of C3b	Hair et al. (2008, 2010)
Clumping factor B (ClfB)	Fg α-chain repeat 5, keratin 10, and loricrin (DLL)	Adhesion to desquamated epithelial cells, nasal colonization	Ganesh et al. (2011), Mulcahy et al. (2012), Walsh et al. (2004, 2008, Xiang et al. (2012)
Serine–aspartate repeat protein C (SdrC)	β-neurexin (DLL)	Unknown	Barbu et al. (2010)
	Unknown	Adhesion to desquamated epithelial cells. Nasal colonization	Corrigan et al. (2009)
	SdrC	Homophilic SdrC–SdrC interaction. Biofilm formation	Barbu et al. (2014)
Serine–aspartate repeat protein D (SdrD)	Unknown	Adhesion to desquamated epithelial cells. Nasal colonization	Corrigan et al. (2009)
Serine–aspartate repeat protein E (SdrE)	Complement factor H	Immune evasion, degradation of C3b	Sharp et al. (2012)
Bone sialoprotein-binding protein (isoform of SdrE)	Fg α-chain (DLL)	Adhesion to immobilized fibrinogen	Vazquez et al. (2011)
Fibronectin-binding proteins A (FnBPA) and B (FnBPB). A domains	FnBPA and FnBPB A domains bind Fg γ-chain C-terminus and elastin (DLL)	Adhesion to ECM	Burke et al. (2011), Keane et al. (2007)
	FnBPB A domain also binds Fn but not by DLL		Burke et al. (2011)
	FnBPA	FnBPA–FnBPA A domain homophilic interaction. Biofilm formation	Geoghegan et al. (2013), Herman-Bausier et al. (2015)

(continued)

Table 1 (continued)

Protein family	Ligand and binding mechanism	Function	References
C-terminal repeats	Fn (FnBPA and C-terminal repeats, tandem β-zipper)	Adhesion to ECM, invasion of mammalian cells	Peacock et al. (1999), Sinha et al. (1999)
Collagen-binding protein (Cna)	Collagen triple helix (collagen hug)	Adhesion to collagen-rich tissue	Zong et al. (2005)
	Complement protein C1q	Prevent classical pathway of complement activation	Kang et al. (2013)
NEAT motif family			
Iron-regulated surface protein A (IsdA)	Haem, Fg, Fn, cytokeratin 10, loricrin (N-terminal NEAT motif region)	Haem uptake and iron acquisition Adhesion to desquamated epithelial cells	Grigg et al. (2010), Clarke et al. (2004, 2006, 2009)
		Resistance to lactoferrin	Clarke and Foster (2008)
	Unknown ligand(s). C-terminal domain NEAT motif region	Resistance to bactericidal lipids and antimicrobial peptides Survival in neutrophils	Clarke et al. (2007)
IsdB	Haemoglobin, haem (N-terminal NEAT motif region)	Haem uptake and iron acquisition	Pilpa et al. (2009), Grigg et al. (2010)
	β3 integrins (NEAT motif regions)	Invasion of non-phagocytic cells	Zapotoczna et al. (2013)
IsdH	Haem, haemoglobin (N-terminal and/or C-terminal NEAT motif region)	Haem uptake and iron acquisition	Grigg et al. (2010)
	Unknown ligand(s). N-terminal NEAT motif region	Accelerated degradation of C3b	Visai et al. (2009)
Three-helical bundle			
Protein A Spa	Three-helical bundle domain		
	IgG Fc	Inhibits opsonophagocytosis	Graille et al. (2000)
	IgM Fab VH3 subclass	B cell superantigen	Silverman and Goodyear (2006)
	TNFR-1	Inflammation	Gomez et al. (2006)

(continued)

Table 1 (continued)

Protein family	Ligand and binding mechanism	Function	References
	von Willebrand factor	Endovascular infection. Endocarditis	O'Seaghdha et al. (2006)
	Region X Unknown ligand	Inflammation	Martin et al. (2009)
G5-E Domain Family			
S. aureus surface protein G (SasG) and plasmin-sensitive surface protein (Pls, SasG homolog in MRSA)	No known ligand	A domain. Adhesion to desquamated epithelial cells	Roche et al. (2003), Corrigan et al. (2007)
		G5-E repeats' homophilic interaction. Biofilm formation	Geoghegan et al. (2010), Gruszka et al. (2012)
Legume lectin-like Cadherin-like			
Serine-rich repeat protein (SraP) N-terminal legume lectin domain	N-acetyl neuraminic acid (Neu5AC) Salivary agglutinin gp340	Adhesion to and invasion of mammalian cells	Kukita et al. (2013), Yang et al. (2014)
SraP cadherin-like domain (CHDL)	SraP CHDL	Homophilic CHDL–CHDL interaction. Biofilm formation Projection of L-lectin domain	Yang et al. (2014)
5′-nucleotidase			
Adenosine synthase A (AdsA)	Converts ATP to adenosine	Survival in neutrophils—inhibit oxidative burst	Thammavongsa et al. (2009)
	2′-deoxyadenosine 3′ monophosphate. Degradation product of DNA converted to 2′-deoxyadenosine	Macrophage and monocyte apoptosis	Thammavongsa et al. (2013)
S. aureus surface protein X (SasX)	Ligands unknown	Biofilm, cell aggregation, squamous cell adhesion, neutrophil evasion	Li et al. (2012)
SasC N-terminal FIVAR-containing domain	Unknown	Promotes primary attachment and accumulation phases of biofilm formation	Schroeder et al. (2009)

(continued)

Table 1 (continued)

Protein family	Ligand and binding mechanism	Function	References
SasF	Unknown	Implicated in resistance to bactericidal effects of long-chain unsaturated fatty acids	Kenny et al. (2009)
SasB, SasD, SasF, SasJ, SasK, SasL		Putative LPXTG proteins identified from genome sequences. No known structure or function	

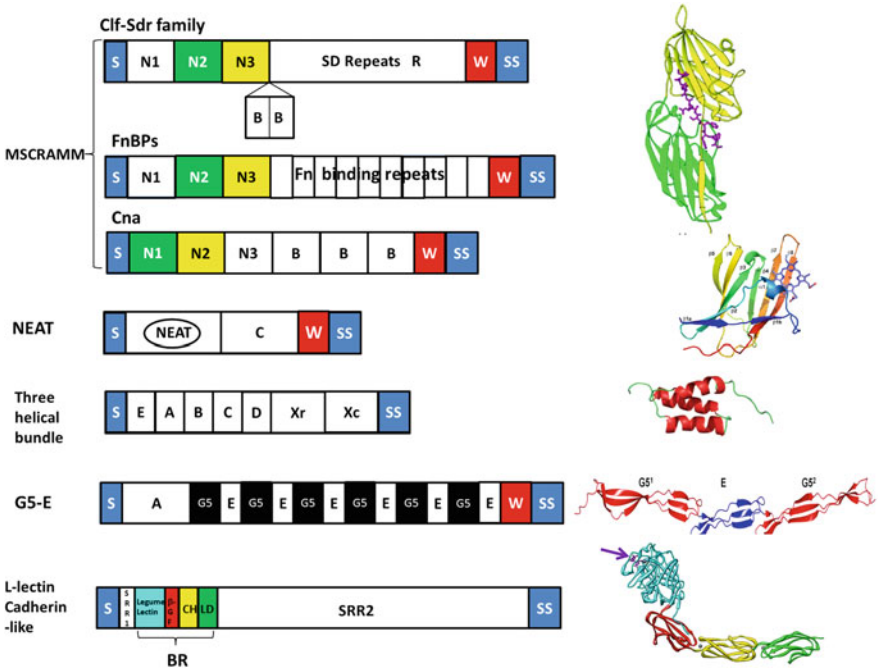
DLL, dock, lock, and latch; *ECM*, extracellular matrix; *NEAT*, near-iron transporter; *Pls*, plasmin sensitive; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *C1q, C3a*, complement components; *FIVAR*, found in various architectures; *Fg*, fibrinogen; *Fn* fibronectin

Ser-Asp (SD repeats) or a tandem array of 10 or 11 repeated fibronectin-binding domains (in fibronectin-binding proteins (FnBPs)). Serine residues in the SD domain of MSCRAMMs are glycosylated (see below).

The function of the N1 subdomain is unclear. Recently, a short stretch of residues close to the boundary between subdomains N1 and N2 was shown to be required for ClfA and FnBPA to be secreted efficiently across the membrane and displayed on the cell surface (McCormack et al. 2014). Interestingly, these residues were not required for display of a mutant form of ClfA lacking the SD repeat region or mutants of FnBPA lacking the intrinsically disordered Fn-binding repeat region leading to speculation that their role is to aid transport of long unfolded repetitive regions. As yet no function has been identified for the remainder of N1. The SdrC, SdrD, and SdrE proteins have two or more repeated domains of 110–113 residues (BSDR) located between the A region and the SD region (Fig. 2) (Josefsson et al. 1998a). The B_{SDR} repeats are folded separately and form rigid rodlike structures that depend on $Ca2^{+}$ for their structural integrity and have no known ligand-binding activity (Josefsson et al. 1998b). However, it has been suggested that the B1 domain of SdrD makes contact with the adjacent N3 subdomain of region A resulting in the ligand-binding groove between N2 and N3 opening further than seen in the structure of the A domain alone, possibly influencing binding activity (Wang et al. 2013).

3.1.3 Ligand Binding: The Collagen Hug

The collagen-binding protein Cna is also an MSCRAMM, but it differs from the Clf-Sdr-FnBP family in the organization of the ligand-binding A domain (Fig. 2). This comprises three separately folded subdomains with N1 and N2 rather than N2 and N3 providing ligand-binding activity (Zong et al. 2005). The A region is linked to the wall-spanning region by variable numbers of B_{CNA} repeated domains that



differ in sequence from B_{SDR} (Deivanayagam et al. 2000). Another difference is the lack of a flexible stalk.

Collagens are composed of a triple-helical array of three separate polypeptide chains. To accommodate this thick structure, the Cna protein N1 and N2 subdomains are separated by an extended linker region. The collagen is grabbed and hugged by the N1N2 subdomains and is stabilized by β-strand complementation between the linker that separates N2 and N3 and a β-sheet in N1 (Fig. 3) (Zong et al. 2005).

3.1.4 Post-Translational Modification

Proteases remove subdomain N1 from MSCRAMMs located on the bacterial cell surface. This can be mediated by a staphylococcal protease that cleaves close to the boundary between subdomains N1 and N2 of ClfA and ClfB (aureolysin) or, in the case of FnBPA, by the host protease thrombin. Following removal of N1, ClfB can no longer bind to Fg (McAleese et al. 2001). It is possible that removal of subdomain N1 reduces the capacity of *S. aureus* to adhere to Fg, loricrin, and cytokeratin 10 in vivo since these ligands share a common binding site in ClfB. The biological significance of the removal of subdomain N1 from ClfA and FnBPA is unclear since it does not reduce the ability of FnBPA to promote biofilm

◀ **Fig. 2** Cell wall-anchored surface proteins classified based on structural motifs. The primary translation products of all cell wall-anchored (*CWA*) proteins contain a signal sequence (*S*) at the amino terminus and a wall-spanning region (*W*) and sorting signal (*SS*) at the carboxyl terminus. The CWA proteins that are depicted are those for which structural analysis has facilitated classification into five distinct groups. (**A**) Microbial surface component recognizing adhesive matrix molecules (*MSCRAMMs*). The clumping factor (*Clf*)–serine–aspartate repeat (*Sdr*) group comprises proteins that are closely related to ClfA. ClfA and ClfB have a similar domain organization, whereas SdrC, SdrD, and SdrE contain additional B_{SDR} repeats that are located between the A domain and the serine–aspartate SD repeat R region. The N-terminal A region contains three separately folded domains, called N1, N2, and N3. Structurally, N2 and N3 form IgG-like folds that bind ligands by the 'dock, lock, and latch' mechanism. Fibronectin-binding protein A (FnBPA) and FnBPB have A domains that are structurally and functionally similar to the A domain of the Clf–Sdr group. Located in place of the serine–aspartate repeat region are tandemly repeated fibronectin-binding domains (11 in FnBPA, 10 in FnBPB). The A region of the collagen adhesin (*Cna*) protein is organized differently to other MSCRAMMs, with N1 and N2 comprising IgG-like folds that bind to ligands using the 'collagen hug' mechanism. The A region is linked to the wall-spanning and anchorage domains by variable numbers of BCNA repeats. The ribbon diagram shows ClfA N2N3 in complex with the C-terminal γ-chain peptide of fibrinogen. (**B**) Near-iron transporter (*NEAT*) motif protein family. The iron-regulated surface determinant (*Isd*) proteins have one (for *IsdA*), two (for *IsdB*), or three (for *IsdH*) NEAT motifs that bind to haemoglobin or haem. The figure depicts IsdA, which has a C-terminal hydrophilic stretch that reduces cell surface hydrophobicity and contributes to resistance to bactericidal lipids and antimicrobial peptides. The ribbon diagram shows a complex between a NEAT domain and haem. (**C**) Three-helical bundle motif protein A. The five N-terminal tandemly linked triple-helical bundle domains (known as *EABCD*) that bind to IgG and other ligands are followed by the repeat-containing Xr region and the non-repetitive Xc region. (**D**) G5–E repeat family. The alternating repeats of the G5 and E domains of *S. aureus* surface protein G (*SasG*) from *S. aureus* [and the accumulation-associated protein (*Aap*) from *S. epidermidis*] link the N-terminal A region to the wall-spanning and anchorage domains. If the A domain is removed, the G5–E region can promote cell aggregation. **(E)** Legume lectin, cadherin-like domain protein. The BR region of the serine-rich adhesin of platelet (*SraP*) protein is flanked by serine-rich repeat domains. The BR region comprises three distinct structural domains: the legume lectin-like, the β-grasp fold (β-GF), and the cadherin-like (*CHLD*) domains. The arrow pointing to the ribbon diagram indicates a sugar molecule bound to the L-lectin moiety

accumulation or of ClfA and FnBPA to mediate adherence to Fg (Geoghegan et al. 2013; McCormack et al. 2014). Under certain conditions, FnBPs are degraded by the *S. aureus* protease V8 (McGavin et al. 1997), reducing the ability of the bacteria to adhere to fibronectin.

Glycosyltransferases expressed by *S. aureus* (SdgA and SdgB) decorate the serine–aspartate repeat regions of Clf-Sdr family proteins with N-acetylglucosamine. This modification protects the SD repeat region from degradation by the human neutrophil serine protease cathepsin G (Hazenbos et al. 2013). Thus, the glycosylation of SD repeats is crucial for maintaining functional MSCRAMMs on the surface of *S. aureus* in vivo.

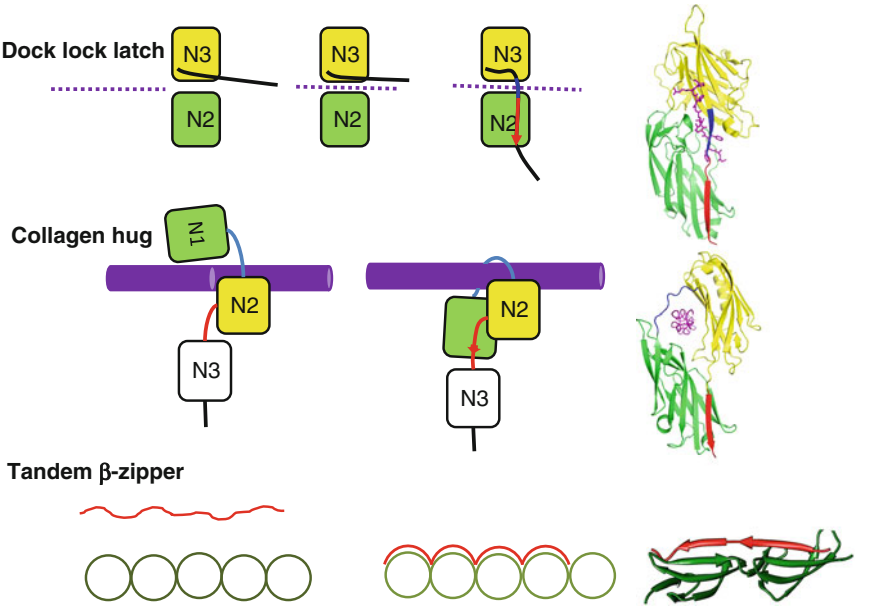


Fig. 3 Ligand-binding mechanisms of surface protein adhesins. The molecular mechanisms by which MSCRAMMs bind their ligands by the 'dock, lock, and latch' and 'collagen hug' mechanisms are shown, as well as the tandem β-zipper mechanism for fibronectin-binding proteins. A schematic diagram of each is shown alongside a *ribbon* diagram of the bacterial protein in complex with its ligand. The red strands with *arrowheads* indicate new β-strands that are formed when the complex is stabilized. In MSCRAMMs, the latching peptide, an unstructured extension of the N3 subdomain (*yellow*), binds to a β-strand in N2 subdomain (*green*). The *purple dashed* line represents the peptide ligand. In the collagen hug, an extended loop (*blue*) between N1 (*green*) and N2 (*yellow*) grabs the collagen triple helix (purple rod) and the linker between N2 and N3 (*red*) stabilizes the complex by forming an additional β-strand in N1. The fibronectin-binding repeats occur as an unstructured linker between the A region and the wall-spanning region. Each repeat can complex with the type I modules at the N-terminus of fibronectin (*green*) by forming an additional short β-strand (*red*, on *top*). The tandem array of β-strands is called β-zippering

3.2 G5-E Repeat Domains

The *S. aureus* surface protein SasG is very similar in structure and organization to the accumulation-associated protein Aap of *S. epidermidis* (Gruszka et al. 2012; Conrady et al. 2013). Both proteins promote cell aggregation and biofilm formation. The SasG protein has multiple alternating repeats of G5-E domains that are identical in sequence. They each form similar flat single-layered β-sheets lacking a compact hydrophobic core (Fig. 2). The alternation of G5 and E regions is a device that prevents misfolding that would otherwise occur in a tandem array of identical or very similar sequences. The unique structural organization of the G5-E repeats provides mechanical strength allowing the protein to maintain both its length and stability. This is due to the E domains forming stable interfaces (clamps) that couple

non-adjacent G5 domains by forming H bonds between β-strands in flanking G5 domains (Gruszka et al. 2015).

The G5-E domains become exposed by proteolytic removal of N-terminal A domain (Aap) (Rohde et al. 2005) or by limited cleavage with G5-E domain (SasG) (Geoghegan et al. 2010). This allows a specific homophilic interaction to occur between proteins located on adjacent cells, which promotes cell aggregation and biofilm accumulation.

3.3 Three-Helical Bundles

The N-terminal region of protein A comprises a tandem array of five separately folded three-helical bundles, each of which can bind to several different ligands (Fig. 2; Table 1). The A1 domain of von Willebrand factor, tumour necrosis factor receptor 1, and the Fc region of IgG each bind to the interface between helices 1 and 2, while the Fab region of IgM binds to the interface of helices 2 and 3. The three-helical bundles of Spa can exist in different conformations (Deis et al. 2015). This conformational plasticity facilitates binding to structurally different partners. Upon binding to the Fc region of IgG, there is considerable loss of conformational heterogeneity combined with structural rearrangements at the interface.

Protein A is the only CWA protein with three-helical bundles. However, the secreted immune evasion proteins SCIN and Efb (Lambris et al. 2008) and the *S. aureus* binder of Ig protein Sbi (Burman et al. 2008) contain domains that fold into three-helical bundles.

3.4 The NEAT Motif Family

Near-iron transporter (NEAT) domain proteins capture haem from haemoglobin and help bacteria to survive in the host where iron is restricted. Haem is transported via several CWA proteins called iron-regulated surface (Isd) proteins to a membrane transporter and then to the cytoplasm where haemoxygenases release free iron (Hammer and Skaar 2011; Cassat and Skaar 2012). The defining characteristic of Isd CWA proteins is the presence of one or more NEAT motifs, which bind either haemoglobin or haem (Fig. 2). The structures of NEAT domains have been solved and the molecular mechanisms of ligand binding defined (Grigg et al. 2010). Isd proteins have functions other than capturing and transporting haem. IsdA promotes adhesion to squamous epithelial cells and promotes nasal colonization (Clarke et al. 2006). It also confers resistance to bactericidal lipids, but this property is conferred by the C-terminal region, not the domain containing the NEAT motif (Clarke et al. 2007). IsdB binds to β-3-containing integrins and promotes platelet activation and invasion of mammalian cells (Miajlovic et al. 2010; Zapotoczna et al. 2013). IsdH

helps bacteria avoid phagocytosis by promoting degradation of complement opsonin C3b (Visai et al. 2009).

3.5 The Legume Lectin Domain

The serine-rich adhesin of platelet SraP is a member of a family of glycoproteins found in Gram-positive cocci (Lizcano et al. 2012). The *sraP* structural gene is part of a locus comprising a conserved array that includes genes encoding glycosyltransferases (GtfA and GtfB) and accessory secretion factors (SecY2 and SecA2). A short serine-rich repeat region (SSR1) occurs at the N-terminus of the mature protein followed by the ligand-binding BR domain (Fig. 2). Structural analysis of BR revealed four separately folded domains that form a rigid bent rod (Yang et al. 2014). Two cadherin-like domains and a β-grasp fold domain project the legume lectin domain. This region has structural similarity to legume lectins and binds Neu5Ac-containing glycoproteins located on the surface of mammalian cells. It also binds to salivary glycoprotein gp340 which is rich in a 5NeuAc-containing trisaccharide (Kukita et al. 2013). SraP promotes bacterial adhesion to and invasion of mammalian cells. This was observed in an experimental set-up where there was little or no fibronectin and with a strain that expressed low levels of FnBPs (see below), thus suppressing FnBP-mediated uptake.

3.6 Fibronectin Binding by Tandem β-Zipper

S. aureus has the ability to promote adherence to and invasion of mammalian cells that are not normally phagocytic (e.g. epithelial cells, endothelial cells, keratinocytes). This provides an intracellular niche where bacteria can avoid host defences and antibiotics. Fibronectin-binding proteins (FnBPs) provide a potent mechanism for cell invasion (Dziewanowska et al. 1999; Peacock et al. 1999; Sinha et al. 1999).

The fibronectin (Fn)-binding region is a long intrinsically disordered segment located between the A domain and the wall-spanning region (Fig. 2). It comprises up to 11 Fn-binding domains which differ considerably in sequence and in Fn-binding affinity (Schwarz-Linek et al. 2003, 2004). FnBPs bind to the N-terminal type 1 domains of Fn, each of which is composed of a small β-sandwich comprising two β-sheets that have 2 and 3 β-strands, respectively. High-affinity ligand-binding domains within Fn-binding repeats each bind β-strands in Fn type I modules forming sequential short β-strand complementation—the tandem β-zipper (Fig. 3). Thus, the intrinsically disordered Fn-binding sequences adopt a secondary structure determined by their binding to the Fn type I modules.

Module 13 in the centre of the elongated multidomain Fn molecule contains arginine–glycine–aspartate (RGD) which is recognized by the common and abundant integrin α5 β 1. Thus, Fn acts as a bridge between the FnBPs on the bacterial cell and integrins on the surface of host cells. The integrins cluster together which triggers phosphorylation of their cytoplasmic domain and in turn stimulates a signalling cascade resulting in cytoskeletal rearrangements and endocytosis (Schwarz-Linek et al. 2004, 2006).

The invasion promoted by FnBPs is regarded as the dominant and most efficient uptake mechanism for many well-studied strains. However, *S. aureus* can invade mammalian cells by other routes. These might serve as auxiliary or backup mechanisms. As mentioned above, the SraP legume lectin domain binds to glycoproteins on the surface of mammalian cells (Yang et al. 2014). Also, the iron-regulated surface determinant protein IsdB can promote invasion by direct interaction with β3-containing integrins (Zapotoczna et al. 2013).

3.7 Nucleotidase Motif

Bioinformatic analysis identified a nucleotidase motif in a previously uncharacterized CWA protein called SasH. This domain was shown to be enzymatically active and importantly contributed significantly to the ability of *S. aureus* to evade immune responses in the infected host (Thammavongsa et al. 2009, 2011). Neutrophils provide the first line of defence against infection. *S. aureus* expresses multiple factors that interfere with phagocytosis. However, once engulfed, the ability of SasH (now called adenosine synthase (AdsA)) to convert intracellular ATP to the potent immunoregulatory molecule adenosine inhibited the oxidative burst and promoted survival within the neutrophil.

Once infection becomes established, neutrophils contribute to defences by releasing DNA (neutrophil extracellular traps (NETs)) which can entrap bacteria and facilitate killing by entwined granule contents. *S. aureus* elaborates an extracellular DNase which degrades NET DNA producing phosphomononucleotides including 2′ deoxyadenosine 3′ monophosphate (Berends et al. 2010; Thammavongsa et al. 2013). This is converted by AdsA to 2′ deoxyadenosine which triggers caspase 3-promoted apoptosis in macrophages and monocytes (Thammavongsa et al. 2013). Thus, AdsA inhibits immune cells as they attempt to contain bacteria in a developing abscess by excluding macrophages and promoting persistent infection.

4 CWA Proteins as Colonization and Virulence Factors

4.1 Approaches to Elucidating the Contribution of CWA Proteins to the Virulence of S. aureus

A logical strategy to demonstrate that a CWA protein is a virulence factor is to isolate a mutant defective in the factor and to compare its performance to the wild-type strain in an animal infection model. However, functional redundancy sometimes makes it difficult to show conclusively that a mutant lacking a single factor has reduced virulence. For example, *S. aureus* can express several CWA proteins that bind Fg, and most strains elaborate two fibronectin-binding proteins. An alternative approach is to express the CWA protein in a surrogate host such as *Lactococcus lactis* (O'Brien et al. 2002; Que et al. 2005; Corrigan et al. 2009; Mulcahy et al. 2012) or *Staphylococcus carnosus* (Sinha et al. 2000). Adding to the problem in demonstrating a role in virulence has been the difficulty in isolating mutations in clinically relevant strains, so until recently, studies were confined to well-established laboratory strains such as Newman and derivatives of NCTC8325. It is now possible to circumvent restriction barriers that prevent transfer of plasmid DNA from *Escherichia coli* cloning hosts to *S. aureus*. Now, genetic manipulation of diverse clinical isolates is feasible (Monk and Foster 2012; Monk et al. 2012, 2015). Another important consideration is the difference in animal species compared to humans. Thus, the results of experimental infection studies must be interpreted with caution if the surface protein in question has a different affinity for the ligand from the animal host compared to the human version. For example, IsdB does not bind mouse haemoglobin, but a transgenic mouse expressing the human version of the protein can be used instead (Pishchany et al. 2010).

An overview of studies demonstrating that bacterial mutants defective in CWA proteins have reduced virulence in different infection (and colonization) models is shown in Table 2. A systematic analysis of individual surface proteins was conducted with mutants of the same strain (Newman) in a mouse model of bacteraemia, dissemination, and abscess formation (Cheng et al. 2009). Mice were injected intravenously with bacteria and the bacterial load in the kidneys, and the number of abscesses was measured after 5 days. This model measures the ability of bacteria to survive in the bloodstream as well as the ability of organisms to invade tissue and establish an abscess. This study showed that several mutants had a statistically significant >1 log reduction in the viable count in the kidneys indicating the importance of CWA proteins. It was limited by the fact that the strain used does not express Cna and lacks the ability to display FnBPs on its surface due to nonsense mutations at the 5′ end of the *fnbA* and *fnbB* genes, which prevents anchorage of the proteins to the cell wall (Grundmeier et al. 2004). The collagen-binding protein has been shown to promote virulence in models where adhesion to collagen-rich tissue is implicated (ocular keratitis and septic arthritis). In the latter model, the strength of adhesion of the recombinant protein to immobilized collagen in vitro is correlated with the severity of the disease (Xu et al. 2004).

Table 2 Cell wall-anchored proteins as colonization and virulence factors

Role in colonization or infection	CWA protein	Mechanism	References
Nasal or skin colonization	ClfB	Adhesion to loricrin on squames	Mulcahy et al. (2012)
	IsdA	Adhesion to squames	Clarke et al. (2006)
	SasX	Adhesion to squames	Li et al. (2012)
Endocarditis	ClfA	Adhesion to thrombus	Moreillon et al. (1995)
	FnBPA	Adhesion to thrombus. Invasion of adjacent endothelium	Que et al. (2005)
	ClfB	Adhesion to thrombus	Entenza et al. (2000)
	SraP	Adhesion to platelets. Colonization of thrombus	Siboo et al. (2005)
Mastitis	FnBP	Invasion of epithelial cells in mammary gland	Brouillette et al. (2003)
Pneumonia	Protein A	Enhanced inflammation of lung epithelium	Gomez et al. (2004)
Foreign body infection	FnBP	MRSA biofilm promoted Adhesion to intra-aortic patch	Arrecubieta et al. (2006), Vergara-Irigaray et al. (2009)
Ocular keratitis	Cna	Enhanced colonization and infection	Rhem et al. (2000)
Septic death Survival in blood	ClfA	Reduced opsonophagocytosis	Cheng et al. (2009), Josefsson et al. (2001)
	Spa		Palmqvist et al. (2002), Patel et al. (1987)
	IsdH		Visai et al. (2009)
	AdsA		Thammavongsa et al. (2009)
	SasX		Li et al. (2012)
Kidney abscess	AdsA, ClfA, ClfB, IsdA, IsdB, IsdC, SdrD, SrdE	Increased survival in bloodstream prior to kidney infection	Thammavongsa et al. (2009), Cheng et al. (2009)
Septic arthritis	ClfA, Spa	Enhanced survival in bloodstream prior to invasion of joint	Josefsson et al. (2001), Palmqvist et al. (2002)
	Cna	Enhanced survival in bloodstream. Adhesion to cartilage within joint	Patti et al. (1994b)

(continued)

Table 2 (continued)

Role in colonization or infection	CWA protein	Mechanism	References
Joint infection	Fg-bindingMSCRAMMS	Biofilm formation in synovial fluid	Dastgheyb et al. (2015)
Subcutaneous abscess	Spa	Abscess development, bacterial load	Patel et al. (1987)
	FnBPs SasF		Kwiecinski et al. (2014)
	ClfA		Kenny et al. (2009)

Studies of *S. aureus* strains that colonized cardiac devices in human patients compared to those isolated from cases of uncomplicated bacteraemia identified polymorphisms in FnBPA that increased the affinity of the protein for Fn in vitro (Lower et al. 2011). This implied that selection occurred in vivo for strains that had a high affinity for a ligand that coated the devices in the patient.

Rather than isolate null mutants that lack the ability to express a surface protein and based on the knowledge of the mechanism of ligand binding gained from in vitro studies with purified protein, it is sometimes possible to study bacteria expressing the protein on its cell surface with amino acid substitutions or a small deletion that cause loss of function. Mutants expressing binding-defective mutants of ClfA (Josefsson et al. 2008) and Cna (Xu et al. 2004) were shown to lack virulence in a model of septic arthritis, and a mutant expressing a defective AdsA survived less well than the wild type in bloodstream infection (Thammavongsa et al. 2009). An advantage of this approach is that possible pleiotropic effects of the missing surface protein on the elaboration of other CWA proteins are avoided.

Infections studied with a surrogate host such as *L. lactis* expressing one or two CWA proteins can be used to study aspects of the infection process. The ability of *L. lactis* to colonize a sterile thrombus on damaged heart valves in a rat model of endocarditis was dependent on bacteria first being able to adhere to fibrinogen/fibrin mediated either by ClfA or by the A domain of FnBPA (Que et al. 2005). Subsequent proliferation and expansion of the infected tissue required cells to be able to invade the surrounding endothelium promoted by the Fn-binding repeat region of FnBPA.

4.2 CWA Proteins Promote Colonization of the Host

ClfB and IsdA promote nasal colonization in rodents (Clarke et al. 2006; Schaffer et al. 2006; Mulcahy et al. 2012) and, in the case of ClfB, also in humans (Wertheim et al. 2008). ClfB binds to the C-terminal domain of cytokeratin 10, which is a major component of the interior of squamous cells and is exposed at the cell surface (Walsh et al. 2004). ClfB recognizes a region of cytokeratin 10 that

contains several omega (Ω) loops. These motifs are also present in loricrin, which is the dominant component of the cornified protein envelope of squames. Moreover, *S. aureus* has a decreased ability to colonize the nasal cavity of loricrin knockout mice, which shows that, in mice, loricrin is an important ligand for ClfB (Mulcahy et al. 2012).

IsdA also promotes the adhesion of *S. aureus* to squames. Colonization of the nasal cavity of cotton rats is reduced for mutants lacking IsdA and after immunization with recombinant IsdA (Clarke et al. 2006). SasX promotes colonization of the nares of mice and immunization with recombinant SasX or passive transfer of specific antibodies against SasX reduces nasal colonization (Liu et al. 2015). SdrC, SdrD, and SasG have also been reported to promote adhesion to squames in vitro (Corrigan et al. 2007, 2009), but the ligands that are involved are not known, and the ability of the proteins to promote colonization in vivo has not been tested.

4.3 CWA Protein Interactions with Fibrinogen/Fibrin

The association of *S. aureus* with soluble and immobilized Fg and fibrin is primarily mediated by the Fg-binding MSCRAMMs. ClfA, FnBPA, and FnBPB bind to the extreme C-terminus of the γ-chain of Fg. Fg-deficient mice are less susceptible to *S. aureus* infection, and mice producing a mutant form of Fg lacking the ClfA/FnBP binding motif are more resistant to challenge than wild-type mice, indicating that the interaction between *S. aureus* and Fg is crucial for the establishment of infection (Flick et al. 2013). *S. aureus* cells agglutinate in the presence of plasma through MSCRAMM-mediated adherence to Fg/fibrin so that large clumps of bacteria are formed (McAdow et al. 2011; Walker et al. 2013). The process of agglutination promotes the in vivo formation of thromboembolic lesions in heart tissue in mice. The recruitment of Fg/fibrin to the bacterial surface is an important immune evasion strategy since it shields opsonins from recognition by host phagocyte receptors (Higgins et al. 2006; Ko et al. 2011). The bifunctional secreted protein Efb binds to complement that has been deposited on the cell surface and cloaks the cell surface with a capsule of fibrinogen which protects the cells from neutrophils (Ko et al. 2013). Staphylococcal coagulases (Coa and vWBP) activate prothrombin to cleave Fg and promote the formation of fibrin cables in the bloodstream, thus allowing ClfA-mediated association of *S. aureus* with fibrin networks (McAdow et al. 2011). We postulate that Fg-binding CWA proteins contribute to this phenomenon. Also, Fg-binding MSCRAMMs also promote bacterial aggregation with fibrin in synovial fluid during joint infection leading to the formation of biofilm-like clumps of bacteria with increased resistance to antibiotics (Dastgheyb et al. 2015).

5 Discussion and Future Prospects

Cell wall-anchored surface proteins are of crucial importance in the interaction of *S. aureus* with its host both in the commensal state and during infection. In this review, we have categorized CWA proteins according to their structural motifs and their known functions.

In fact, the functions of several CWA proteins have yet to be discovered. Furthermore, the best characterized CWA proteins often have multiple functions, and new ligands will undoubtedly be found. Advances in procedures that facilitate detection of binding partners such as phage display and two-hybrid analysis as well as more sensitive detection systems for pull-down experiments will make this possible. For example, like many invasive bacteria [e.g. group A streptococci (Walker et al. 2014)], *S. aureus* has the ability to capture and activate plasminogen (Molkanen et al. 2002), but there is surprisingly little known about the surface proteins involved. It is worth speculating that one or more CWA proteins are involved in addition to Sbi (Koch et al. 2012), MntC (Salazar et al. 2014), and the moonlighting cytoplasmic enzyme enolase (Molkanen et al. 2002; Antikainen et al. 2007).

It is perhaps surprising that so little is known about the mechanistic basis of how CWA proteins help the bacterium avoid innate immune responses, in particular how they control complement activation. This strategy is commonly employed by invasive pathogens, most notably meningococci and streptococci (Lambris et al. 2008). Detailed analysis of the interaction of CWA proteins with complement components and host regulators is needed.

As *S. aureus* continues to evolve to colonize its hosts more efficiently and to avoid immune responses more effectively, it is likely that new CWA proteins will be acquired by horizontal gene transfer. The genes of several CWA proteins are encoded by mobile genetic elements (Malachowa and DeLeo 2010). The plasmin-sensitive surface protein Pls is encoded within a staphylococcal cassette chromosome responsible for resistance to methicillin (SCCmec type I) (Werbick et al. 2007), the recently discovered SasX protein is encoded by a lysogenic bacteriophage (Li et al. 2012), and the biofilm-associated protein Bap is encoded within the pathogenicity island SaPIbov2 that occurs exclusively in bovine strains (Novick et al. 2010).

Soluble forms of CWA proteins might have important biological functions. A substantial fraction of protein A is present in the supernatant of growing cultures as a result of autolytic activity and inefficient sorting, and this has been shown to help bacteria survive killing in whole human blood (O'Halloran et al. 2015). It is possible that released forms of other intact CWA proteins or proteolytically released fragments are biologically active analogously to the SpeB-released M protein fragments of group A streptococci (Walker et al. 2014).

In conclusion, cell wall-anchored surface proteins carry out a diverse array of functions and are essential for colonization of and survival in the host. Structural analysis has provided a framework for the classification of this important group of bacterial proteins.

References

Antikainen J, Kuparinen V, Lahteenmaki K, Korhonen TK (2007) Enolases from Gram-positive bacterial pathogens and commensal lactobacilli share functional similarity in virulence-associated traits. FEMS Immunol Med Microbiol 51:526–534

Arrecubieta C et al (2006) The role of Staphylococcus aureus adhesins in the pathogenesis of ventricular assist device-related infections. J Infect Dis 193:1109–1119

Barbu EM et al (2010) beta-Neurexin is a ligand for the Staphylococcus aureus MSCRAMM SdrC. PLoS Pathog 6:e1000726

Barbu EM, Mackenzie C, Foster TJ, Hook M (2014) SdrC induces staphylococcal biofilm formation through a homophilic interaction. Mol Microbiol 94:172–185

Becker S, Frankel MB, Schneewind O, Missiakas D (2014) Release of protein A from the cell wall of Staphylococcus aureus. Proc Natl Acad Sci U S A 111:1574–1579

Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Kockritz-Blickwede M (2010) Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. J Innate Immun 2:576–586

Brouillette E, Grondin G, Shkreta L, Lacasse P, Talbot BG (2003) In vivo and in vitro demonstration that Staphylococcus aureus is an intracellular pathogen in the presence or absence of fibronectin-binding proteins. Microb Pathog 35:159–168

Burke FM, Di Poto A, Speziale P, Foster TJ (2011) The A domain of fibronectin-binding protein B of Staphylococcus aureus contains a novel fibronectin binding site. FEBS J 278:2359–2371

Burman JD et al (2008) Interaction of human complement with Sbi, a staphylococcal immunoglobulin-binding protein: indications of a novel mechanism of complement evasion by Staphylococcus aureus. J Biol Chem 283:17579–17593

Cassat JE, Skaar EP (2012) Metal ion acquisition in Staphylococcus aureus: overcoming nutritional immunity. Semin Immunopathol 34:215–235

Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM (2009) Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues. FASEB J 23:3393–3404

Clarke SR et al (2006) Identification of in vivo-expressed antigens of Staphylococcus aureus and their use in vaccinations for protection against nasal carriage. J Infect Dis 193:1098–1108

Clarke SR et al (2007) The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin. Cell Host Microbe 1:199–212

Clarke SR, Andre G, Walsh EJ, Dufrene YF, Foster TJ, Foster SJ (2009) Iron-regulated surface determinant protein A mediates adhesion of Staphylococcus aureus to human corneocyte envelope proteins. Infect Immun 77:2408–2416

Clarke SR, Foster SJ (2008) IsdA protects Staphylococcus aureus against the bactericidal protease activity of apolactoferrin. Infect Immun 76:1518–1526

Clarke SR, Wiltshire MD, Foster SJ (2004) IsdA of Staphylococcus aureus is a broad spectrum, iron-regulated adhesin. Mol Microbiol 51:1509–1519

Conrady DG, Wilson JJ, Herr AB (2013) Structural basis for Zn2+-dependent intercellular adhesion in staphylococcal biofilms. Proc Natl Acad Sci U S A 110:E202–E211

Corrigan RM, Miajlovic H, Foster TJ (2009) Surface proteins that promote adherence of Staphylococcus aureus to human desquamated nasal epithelial cells. BMC Microbiol 9:22

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

Cregg KM, Wilding I, Black MT (1996) Molecular cloning and expression of the spsB gene encoding an essential type I signal peptidase from Staphylococcus aureus. J Bacteriol 178:5712–5718

Dastgheyb S, Parvizi J, Shapiro IM, Hickok NJ, Otto M (2015) Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment. J Infect Dis 211:641–650

DeDent A, Bae T, Missiakas DM, Schneewind O (2008) Signal peptides direct surface proteins to two distinct envelope locations of Staphylococcus aureus. EMBO J 27:2656–2668

Deis LN et al (2015) Suppression of conformational heterogeneity at a protein-protein interface. Proc Natl Acad Sci U S A 112:9028–9033

Deivanayagam CC et al (2000) Novel fold and assembly of the repetitive B region of the Staphylococcus aureus collagen-binding surface protein. Structure 8:67–78

Deivanayagam CC et al (2002) A novel variant of the immunoglobulin fold in surface adhesins of Staphylococcus aureus: crystal structure of the fibrinogen-binding MSCRAMM, clumping factor A. EMBO J 21:6660–6672

Dziewanowska K, Patti JM, Deobald CF, Bayles KW, Trumble WR, Bohach GA (1999) Fibronectin binding protein and host cell tyrosine kinase are required for internalization of Staphylococcus aureus by epithelial cells. Infect Immun 67:4673–4678

Entenza JM, Foster TJ, Ni Eidhin D, Vaudaux P, Francioli P, Moreillon P (2000) Contribution of clumping factor B to pathogenesis of experimental endocarditis due to Staphylococcus aureus. Infect Immun 68:5443–5446

Flick MJ et al (2013) Genetic elimination of the binding motif on fibrinogen for the S. aureus virulence factor ClfA improves host survival in septicemia. Blood 121:1783–1794

Foster TJ, Geoghegan JA, Ganesh VK, Hook M (2014) Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12:49–62

Ganesh VK et al (2008) A structural model of the Staphylococcus aureus ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. PLoS Pathog 4:e1000226

Ganesh VK et al (2011) Structural and biochemical characterization of Staphylococcus aureus clumping factor B/ligand interactions. J Biol Chem 286:25963–25972

Geoghegan JA et al (2010) Role of surface protein SasG in biofilm formation by Staphylococcus aureus. J Bacteriol 192:5663–5673

Geoghegan JA, Monk IR, O'Gara JP, Foster TJ (2013) Subdomains N2N3 of fibronectin binding protein A mediate Staphylococcus aureus biofilm formation and adherence to fibrinogen using distinct mechanisms. J Bacteriol 195:2675–2683

Gomez MI et al (2004) Staphylococcus aureus protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat Med 10:842–848

Gomez MI, O'Seaghdha M, Magargee M, Foster TJ, Prince AS (2006) Staphylococcus aureus protein A activates TNFR1 signaling through conserved IgG binding domains. J Biol Chem 281:20190–20196

Graille M et al (2000) Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci U S A 97:5399–5404

Grigg JC, Ukpabi G, Gaudin CF, Murphy ME (2010) Structural biology of heme binding in the Staphylococcus aureus Isd system. J Inorg Biochem 104:341–348

Grundmeier M, Hussain M, Becker P, Heilmann C, Peters G, Sinha B (2004) Truncation of fibronectin-binding proteins in Staphylococcus aureus strain Newman leads to deficient adherence and host cell invasion due to loss of the cell wall anchor function. Infect Immun 72:7155–7163

Gruszka DT et al (2012) Staphylococcal biofilm-forming protein has a contiguous rod-like structure. Proc Natl Acad Sci U S A 109:E1011–E1018

Gruszka DT et al (2015) Cooperative folding of intrinsically disordered domains drives assembly of a strong elongated protein. Nat Commun 6:7271

Hair PS et al (2010) Clumping factor A interaction with complement factor I increases C3b cleavage on the bacterial surface of Staphylococcus aureus and decreases complement-mediated phagocytosis. Infect Immun 78:1717–1727

Hair PS, Ward MD, Semmes OJ, Foster TJ, Cunnion KM (2008) Staphylococcus aureus clumping factor A binds to complement regulator factor I and increases factor I cleavage of C3b. J Infect Dis 198:125–133

Hammer ND, Skaar EP (2011) Molecular mechanisms of Staphylococcus aureus iron acquisition. Annu Rev Microbiol 65:129–147

Hazenbos WL et al (2013) Novel staphylococcal glycosyltransferases SdgA and SdgB mediate immunogenicity and protection of virulence-associated cell wall proteins. PLoS Pathog 9: e1003653

Herman-Bausier P, El-Kirat-Chatel S, Foster TJ, Geoghegan JA, Dufrene YF (2015) Staphylococcus aureus Fibronectin-Binding Protein A Mediates Cell-Cell Adhesion through Low-Affinity Homophilic Bonds. MBio 6:e00413–e00415

Higgins J, Loughman A, van Kessel KP, van Strijp JA, Foster TJ (2006) Clumping factor A of Staphylococcus aureus inhibits phagocytosis by human polymorphonuclear leucocytes. FEMS Microbiol Lett 258:290–296

Josefsson E et al (1998a) Three new members of the serine-aspartate repeat protein multigene family of Staphylococcus aureus. Microbiology 144:3387–3395

Josefsson E, O'Connell D, Foster TJ, Durussel I, Cox JA (1998b) The binding of calcium to the B-repeat segment of SdrD, a cell surface protein of Staphylococcus aureus. J Biol Chem 273:31145–31152

Josefsson E, Hartford O, O'Brien L, Patti JM, Foster T (2001) Protection against experimental Staphylococcus aureus arthritis by vaccination with clumping factor A, a novel virulence determinant. J Infect Dis 184:1572–1580

Josefsson E, Higgins J, Foster TJ, Tarkowski A (2008) Fibrinogen binding sites P336 and Y338 of clumping factor A are crucial for Staphylococcus aureus virulence. PLoS ONE 3:e2206

Kang M et al (2013) Collagen-binding Microbial Surface Components Recognizing Adhesive Matrix Molecule (MSCRAMM) of Gram-positive Bacteria Inhibit Complement Activation via the Classical Pathway. J Biol Chem 288:20520–20531

Keane FM, Loughman A, Valtulina V, Brennan M, Speziale P, Foster TJ (2007) Fibrinogen and elastin bind to the same region within the A domain of fibronectin binding protein A, an MSCRAMM of Staphylococcus aureus. Mol Microbiol 63:711–723

Kenny JG et al (2009) The Staphylococcus aureus response to unsaturated long chain free fatty acids: survival mechanisms and virulence implications. PLoS ONE 4:e4344

Ko YP et al (2013) Phagocytosis escape by a Staphylococcus aureus protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog 9:e1003816

Ko YP, Liang X, Smith CW, Degen JL, Hook M (2011) Binding of Efb from Staphylococcus aureus to fibrinogen blocks neutrophil adherence. J Biol Chem 286:9865–9874

Koch TK et al (2012) Staphylococcus aureus proteins Sbi and Efb recruit human plasmin to degrade complement C3 and C3b. PLoS ONE 7:e47638

Kukita K et al (2013) Staphylococcus aureus SasA is responsible for binding to the salivary agglutinin gp340, derived from human saliva. Infect Immun 81:1870–1879

Kwiecinski J, Jin T, Josefsson E (2014) Surface proteins of Staphylococcus aureus play an important role in experimental skin infection. APMIS 122:1240–1250

Lambris JD, Ricklin D, Geisbrecht BV (2008) Complement evasion by human pathogens. Nat Rev Microbiol 6:132–142

Li M et al (2012) MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med 18:816–819

Liu Q et al (2015) Targeting surface protein SasX by active and passive vaccination to reduce Staphylococcus aureus colonization and infection. Infect Immun 83:2168–2174

Lizcano A, Sanchez CJ, Orihuela CJ (2012) A role for glycosylated serine-rich repeat proteins in gram-positive bacterial pathogenesis. Mol Oral Microbiol 27:257–269

Lower SK et al (2011) Polymorphisms in fibronectin binding protein A of Staphylococcus aureus are associated with infection of cardiovascular devices. Proc Natl Acad Sci U S A 108:18372–18377

Malachowa N, DeLeo FR (2010) Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci 67:3057–3071

Marraffini LA, Schneewind O (2005) Anchor structure of staphylococcal surface proteins. V. Anchor structure of the sortase B substrate IsdC. J Biol Chem 280:16263–16271

Martin FJ et al (2009) Staphylococcus aureus activates type I IFN signaling in mice and humans through the Xr repeated sequences of protein A. J Clin Invest 119:1931–1939

Mazmanian SK, Liu G, Ton-That H, Schneewind O (1999) Staphylococcus aureus sortase, an enzyme that anchors surface proteins to the cell wall. Science 285:760–763
Mazmanian SK, Ton-That H, Schneewind O (2001) Sortase-catalysed anchoring of surface proteins to the cell wall of Staphylococcus aureus. Mol Microbiol 40:1049–1057
McAdow M, Kim HK, Dedent AC, Hendrickx AP, Schneewind O, Missiakas DM (2011) Preventing Staphylococcus aureus sepsis through the inhibition of its agglutination in blood. PLoS Pathog 7:e1002307
McAleese FM, Walsh EJ, Sieprawska M, Potempa J, Foster TJ (2001) Loss of clumping factor B fibrinogen binding activity by Staphylococcus aureus involves cessation of transcription, shedding and cleavage by metalloprotease. J Biol Chem 276:29969–29978
McCarthy AJ, Lindsay JA (2010) Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173
McCormack N, Foster TJ, Geoghegan JA (2014) A short sequence within subdomain N1 of region A of the Staphylococcus aureus MSCRAMM clumping factor A is required for export and surface display. Microbiology 160:659–670
McGavin MJ, Zahradka C, Rice K, Scott JE (1997) Modification of the Staphylococcus aureus fibronectin binding phenotype by V8 protease. Infect Immun 65:2621–2628
Miajlovic H, Zapotoczna M, Geoghegan JA, Kerrigan SW, Speziale P, Foster TJ (2010) Direct interaction of iron-regulated surface determinant IsdB of Staphylococcus aureus with the GPIIb/IIIa receptor on platelets. Microbiology 156:920–928
Molkanen T, Tyynela J, Helin J, Kalkkinen N, Kuusela P (2002) Enhanced activation of bound plasminogen on Staphylococcus aureus by staphylokinase. FEBS Lett 517:72–78
Monk IR, Foster TJ (2012) Genetic manipulation of Staphylococci-breaking through the barrier. Front Cell Infect Microbiol 2:49
Monk IR, Shah IM, Xu M, Tan MW, Foster TJ (2012) Transforming the untransformable: application of direct transformation to manipulate genetically Staphylococcus aureus and Staphylococcus epidermidis. MBio 3:e00277-11
Monk IR, Tree JJ, Howden BP, Stinear TP, Foster TJ (2015) Complete Bypass of Restriction Systems for Major Staphylococcus aureus Lineages. MBio 6:e00308–e00315
Moreillon P et al (1995) Role of Staphylococcus aureus coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect Immun 63:4738–4743
Mulcahy ME et al (2012) Nasal colonisation by Staphylococcus aureus depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathog 8: e1003092
Novick RP, Christie GE, Penades JR (2010) The phage-related chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol 8:541–551
O'Brien LM, Walsh EJ, Massey RC, Peacock SJ, Foster TJ (2002) Staphylococcus aureus clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell Microbiol 4:759–770
O'Halloran DP, Wynne K, Geoghegan JA (2015) Protein A is released into the Staphylococcus aureus culture supernatant with an unprocessed sorting signal. Infect Immun 83:1598–1609
O'Seaghdha M et al (2006) Staphylococcus aureus protein A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. FEBS J 273:4831–4841
Palmqvist N, Foster T, Tarkowski A, Josefsson E (2002) Protein A is a virulence factor in Staphylococcus aureus arthritis and septic death. Microb Pathog 33:239–249
Patel AH, Nowlan P, Weavers ED, Foster T (1987) Virulence of protein A-deficient and alpha-toxin-deficient mutants of Staphylococcus aureus isolated by allele replacement. Infect Immun 55:3103–3110
Patti JM, Allen BL, McGavin MJ, Hook M (1994a) MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585–617
Patti JM et al (1994b) The Staphylococcus aureus collagen adhesin is a virulence determinant in experimental septic arthritis. Infect Immun 62:152–161

Peacock SJ, Foster TJ, Cameron BJ, Berendt AR (1999) Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of Staphylococcus aureus to resting human endothelial cells. Microbiology 145:3477–3486

Perry AM, Ton-That H, Mazmanian SK, Schneewind O (2002) Anchoring of surface proteins to the cell wall of Staphylococcus aureus. III. Lipid II is an in vivo peptidoglycan substrate for sortase-catalyzed surface protein anchoring. J Biol Chem 277:16241–16248

Pilpa RM, Robson SA, Villareal VA, Wong ML, Phillips M, Clubb RT (2009) Functionally distinct NEAT (NEAr Transporter) domains within the Staphylococcus aureus IsdH/HarA protein extract heme from methemoglobin. J Biol Chem 284:1166–1176

Pishchany G et al (2010) Specificity for human hemoglobin enhances Staphylococcus aureus infection. Cell Host Microbe 8:544–550

Ponnuraj K et al (2003) A "dock, lock, and latch" structural model for a staphylococcal adhesin binding to fibrinogen. Cell 115:217–228

Que YA et al (2005) Fibrinogen and fibronectin binding cooperate for valve infection and invasion in Staphylococcus aureus experimental endocarditis. J Exp Med 201:1627–1635

Rhem MN et al (2000) The collagen-binding adhesin is a virulence factor in Staphylococcus aureus keratitis. Infect Immun 68:3776–3779

Roche FM, Meehan M, Foster TJ (2003) The Staphylococcus aureus surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. Microbiology 149:2759–2767

Rohde H et al (2005) Induction of Staphylococcus epidermidis biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. Mol Microbiol 55:1883–1895

Salazar N et al (2014) Staphylococcus aureus manganese transport protein C (MntC) is an extracellular matrix- and plasminogen-binding protein. PLoS ONE 9:e112730

Schaffer AC et al (2006) Immunization with Staphylococcus aureus clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect Immun 74:2145–2153

Schneewind O, Mihaylova-Petkov D, Model P (1993) Cell wall sorting signals in surface proteins of gram-positive bacteria. EMBO J 12:4803–4811

Schneewind O, Model P, Fischetti VA (1992) Sorting of protein A to the staphylococcal cell wall. Cell 70:267–281

Schroeder K et al (2009) Molecular characterization of a novel Staphylococcus aureus surface protein (SasC) involved in cell aggregation and biofilm accumulation. PLoS ONE 4:e7567

Schwarz-Linek U et al (2003) Pathogenic bacteria attach to human fibronectin through a tandem beta-zipper. Nature 423:177–181

Schwarz-Linek U, Hook M, Potts JR (2004) The molecular basis of fibronectin-mediated bacterial adherence to host cells. Mol Microbiol 52:631–641

Schwarz-Linek U, Hook M, Potts JR (2006) Fibronectin-binding proteins of gram-positive cocci. Microbes Infect 8:2291–2298

Sharp JA et al (2012) Staphylococcus aureus surface protein SdrE binds complement regulator factor H as an immune evasion tactic. PLoS ONE 7:e38407

Siboo IR, Chaffin DO, Rubens CE, Sullam PM (2008) Characterization of the accessory Sec system of Staphylococcus aureus. J Bacteriol 190:6188–6196

Siboo IR, Chambers HF, Sullam PM (2005) Role of SraP, a Serine-Rich Surface Protein of Staphylococcus aureus, in binding to human platelets. Infect Immun 73:2273–2280

Silverman GJ, Goodyear CS (2006) Confounding B-cell defences: lessons from a staphylococcal superantigen. Nat Rev Immunol 6:465–475

Sinha B et al (1999) Fibronectin-binding protein acts as Staphylococcus aureus invasin via fibronectin bridging to integrin alpha5beta1. Cell Microbiol 1:101–117

Sinha B et al (2000) Heterologously expressed Staphylococcus aureus fibronectin-binding proteins are sufficient for invasion of host cells. Infect Immun 68:6871–6878

Thammavongsa V, Kern JW, Missiakas DM, Schneewind O (2009) Staphylococcus aureus synthesizes adenosine to escape host immune responses. J Exp Med 206:2417–2427

Thammavongsa V, Missiakas DM, Schneewind O (2013) Staphylococcus aureus degrades neutrophil extracellular traps to promote immune cell death. Science 342:863–866

Thammavongsa V, Schneewind O, Missiakas DM (2011) Enzymatic properties of Staphylococcus aureus adenosine synthase (AdsA). BMC Biochem 12:56

Vazquez V et al (2011) Fibrinogen is a ligand for the Staphylococcus aureus microbial surface components recognizing adhesive matrix molecules (MSCRAMM) bone sialoprotein-binding protein (Bbp). J Biol Chem 286:29797–29805

Vergara-Irigaray M et al (2009) Relevant role of fibronectin-binding proteins in Staphylococcus aureus biofilm-associated foreign-body infections. Infect Immun 77:3978–3991

Visai L et al (2009) Immune evasion by Staphylococcus aureus conferred by iron-regulated surface determinant protein IsdH. Microbiology 155:667–679

Walker JN et al (2013) The Staphylococcus aureus ArlRS two-component system is a novel regulator of agglutination and pathogenesis. PLoS Pathog 9:e1003819

Walker MJ et al (2014) Disease manifestations and pathogenic mechanisms of group a Streptococcus. Clin Microbiol Rev 27:264–301

Walsh EJ, Miajlovic H, Gorkun OV, Foster TJ (2008) Identification of the Staphylococcus aureus MSCRAMM clumping factor B (ClfB) binding site in the alphaC-domain of human fibrinogen. Microbiology 154:550–558

Walsh EJ, O'Brien LM, Liang X, Hook M, Foster TJ (2004) Clumping factor B, a fibrinogen-binding MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesin of Staphylococcus aureus, also binds to the tail region of type I cytokeratin 10. J Biol Chem 279:50691–50699

Wang X, Ge J, Liu B, Hu Y, Yang M (2013) Structures of SdrD from Staphylococcus aureus reveal the molecular mechanism of how the cell surface receptors recognize their ligands. Protein Cell 4:277–285

Wann ER, Gurusiddappa S, Hook M (2000) The fibronectin-binding MSCRAMM FnbpA of Staphylococcus aureus is a bifunctional protein that also binds to fibrinogen. J Biol Chem 275:13863–13871

Werbick C et al (2007) Staphylococcal chromosomal cassette mec type I, spa type, and expression of Pls are determinants of reduced cellular invasiveness of methicillin-resistant Staphylococcus aureus isolates. J Infect Dis 195:1678–1685

Wertheim HF et al (2008) Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med 5:e17

Xiang H et al (2012) Crystal structures reveal the multi-ligand binding mechanism of Staphylococcus aureus ClfB. PLoS Pathog 8:e1002751

Xu Y, Rivas JM, Brown EL, Liang X, Hook M (2004) Virulence potential of the staphylococcal adhesin CNA in experimental arthritis is determined by its affinity for collagen. J Infect Dis 189:2323–2333

Yang YH et al (2014) Structural insights into SraP-mediated Staphylococcus aureus adhesion to host cells. PLoS Pathog 10:e1004169

Zapotoczna M, Jevnikar Z, Miajlovic H, Kos J, Foster TJ (2013) Iron-regulated surface determinant B (IsdB) promotes Staphylococcus aureus adherence to and internalization by non-phagocytic human cells. Cell Microbiol 15:1026–1041

Zong Y et al (2005) A 'Collagen Hug' model for Staphylococcus aureus CNA binding to collagen. EMBO J 24:4224–4236

Staphylococcus aureus Pore-Forming Toxins

Tamara Reyes-Robles and Victor J. Torres

Abstract *Staphylococcus aureus* (*S. aureus*) is a formidable foe equipped with an armamentarium of virulence factors to thwart host defenses and establish a successful infection. Among these virulence factors, *S. aureus* produces several potent secreted proteins that act as cytotoxins, predominant among them the beta-barrel pore-forming toxins. These toxins play several roles in pathogenesis, including disruption of cellular adherens junctions at epithelial barriers, alteration of intracellular signaling events, modulation of host immune responses, and killing of eukaryotic immune and non-immune cells. This chapter provides an updated overview on the *S. aureus* beta-barrel pore-forming cytotoxins, the identification of toxin receptors on host cells, and their roles in pathogenesis.

Abbreviations

ADAM10	A disintegrin and metalloprotease 10
CA-MRSA	Community-acquired methicillin-resistant *Staphylococcus aureus*
CCR2	C-C chemokine receptor type 2
CCR5	C-C chemokine receptor type 5
CD11b	Cluster of differentiation molecule 11b
cDNA	Complementary deoxyribonucleic acid
CXCR1	C-X-C chemokine receptor type 1
CXCR2	C-X-C chemokine receptor type 2
DARC	Duffy antigen receptor for chemokines
HEK	Human embryonic kidney cells
Hla	Alpha-toxin (also known as alpha-hemolysin)
HlgAB	Gamma-hemolysin AB
HlgACB	Gamma-hemolysins

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HlgCB	Gamma-hemolysin CB
LukAB/HG	Leukocidin AB/HG
LukED	Leukocidin ED
LukSF-PV/PVL	Panton–Valentine Leukocidin
MAC-1	Macrophage-1 integrin
PLEKHA7	Pleckstrin homology domain-containing family A member 7
PMNs	Polymorphonuclear neutrophils
S. aureus	*Staphylococcus aureus*
SSTI	Skin and soft tissue infection
WT	Wild type

Contents

1 Identification of *S. aureus* Toxins: An Overview

The host cell membrane is a main target of virulence factors produced by pathogens (DuMont and Torres 2014). Within these factors, bacterial pore-forming toxins are amongst the most thoroughly investigated group of virulence factors due to their breadth of targets and effects on host cells (DuMont and Torres 2014). Unlike the majority of bacterial pathogens, which produce one or two pore-forming toxins at most, *S. aureus* can produce more than 5 beta-barrel pore-forming toxins with distinct and sometimes redundant roles (Alonzo and Torres 2014; DuMont and Torres 2014).

The identification of *S. aureus* cytolytic factors began in the 1800s (Alonzo and Torres 2014). A pus-causing factor within culture supernatants caused immune cell (leukocyte) killing, erythrocyte lysis, and consequent mortality in vivo (Denys and Van de Velde 1895; Van de Velde 1894). In 1894, Van de Velde described a *S. aureus* factor that killed rabbit leukocytes which was termed "leukocidin" (Alonzo and Torres 2014). The Van de Velde studies were followed up by several groups in the early 1900s, where attempts were made to separate the leukocidal activity from the hemolytic activity previously observed in culture supernatants (Alonzo and Torres 2014). Work by Neisser and Wechsberg demonstrated that leukocyte and erythrocyte killing were the result of two separate activities, as demonstrated by adsorption experiments of either hemolysin or leukocidin to their respective cell types (erythrocytes and leukocytes) (Neisser and Wechsberg 1901). After being commissioned to investigate lethality of children following administration of a diphteria toxin vaccine in the 1920s, Burnet discovered that culture supernatants obtained from contaminating *S. aureus* found in diphtheria toxin vaccines accidentally administered to children in Bundaberg, Australia, had hemolytic activity and were lethal when administered to rabbits (Berube and Bubeck Wardenburg 2013; Burnet 1929, 1930; Green 1928). In line with Neisser and Wechsberg's work, Julianelle found independent hemolytic and leukocidal activities across several *S. aureus* strains, some of which were unrelated to each other (Julianelle 1922; Alonzo and Torres 2014). After the Burnet studies, Glenny and Stevens described two secreted, immunologically divergent molecules, named alpha- and beta-toxin, that displayed hemolytic activity in a host species-specific manner. Of these molecules, the rabbit-tropic hemolytic factor is now known to be alpha-toxin (Glenny and Stevens 1935; Berube and Bubeck Wardenburg 2013).

Problems with reproducibility of experimental results by others questioned the hypothesis of the two different toxinogenic molecules by Neisser and Wechsberg (Burnet 1929; Weld and Gunther 1931; Wright 1936; Alonzo and Torres 2014). The controversy surrounding their existence was put to rest by work from Panton and Valentine in 1932 (Panton and Valentine 1932). The authors performed extensive studies using 22 *S. aureus* strains to evaluate erythrocyte hemolysis and skin lesions in rabbits, and human leukocyte killing, ultimately demonstrating that two distinct factors were responsible for the hemolytic and leukocidal activities (Alonzo and Torres 2014; Panton and Valentine 1932). Follow-up work confirmed their findings, resulting in the renaming of the molecule with leukocytolytic activity as Panton-Valentine Leukocidin, or PVL (Alonzo and Torres 2014). Future studies by Gladstone and van Heyningen confirmed the observations made by Van de Velde, where the leukocidal factor was indeed a two-component leukocidin (Gladstone and Van Heyningen 1957), potentially one of the five bicomponent leukocidins associated with human infections that are currently known (Alonzo and Torres 2014; DuMont and Torres 2014).

It is important to note that all these studies were performed before it was known that a single *S. aureus* strain can encode several cytotoxins (Alonzo and Torres 2014; Vandenesch et al. 2012) some of which have both redundant activities and target cell types (Spaan et al. 2015a).

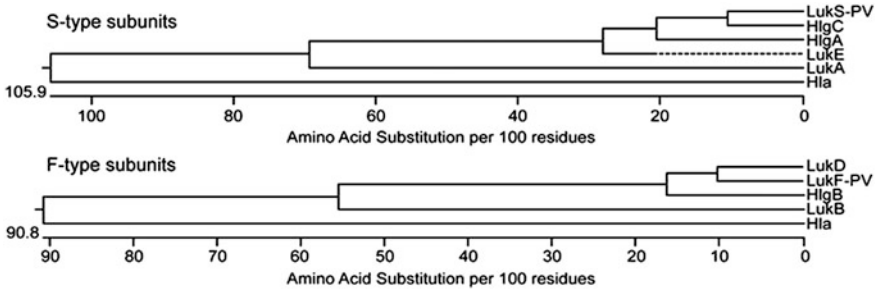

Fig. 1 Sequence identity between toxin subunits. Phylogenetic trees of the amino acid sequences of the mature S-type subunits and alpha-toxin (Hla) (*top*), and the F-type subunits and alpha-toxin (Hla) (*bottom*) constructed using DNAStar MegAlign ClustalW

Importantly, the discovery of the first leukocidin by Panton and Valentine paved the way for the uncovering of additional pore-forming toxins. From these, the beta-barrel pore-forming toxins constitute a large and clinically relevant group of cytolytic factors produced by *S. aureus* (Fig. 1). These are small (~32–40 kDa) secreted proteins that bind receptors on the host plasma membrane, leading to the stepwise assembly of a toxin oligomer, resulting in the formation of a functional pore of ~25 Å that pierces through the plasma membrane and kills eukaryotic cells by osmotic dysregulation mediated by flux of Ca^{2+}, K^{+}, ATP, and other small molecules through the pore (Alonzo and Torres 2014; DuMont and Torres 2014; Yoong and Torres 2013; Berube and Bubeck Wardenburg 2013). *S. aureus* strains associated with human disease can produce up to six of these pore-forming toxins, which are divided into the small beta-barrel homo-oligomeric pore-forming toxin (alpha-toxin), and a collection of bicomponent pore-forming toxins, of which their properties and activities on host cells are described below.

1.1 Alpha-Toxin: The Prototypical Beta-Barrel Pore-Forming Toxin

Alpha-toxin (also known as alpha-hemolysin or Hla) is a ~33-kDa toxin identified in the early 1900s, followed by the elucidation of its gene sequence in 1984 (Gray and Kehoe 1984). This toxin is a highly conserved (99 %), core genome-encoded cytotoxin that assembles into a homo-heptameric pore (Gouaux et al. 1994). Unlike other staphylococcal beta-barrel pore-forming proteins, human PMNs are largely refractory to alpha-toxin-mediated intoxication and this is due to low levels of its receptor (discussed in Sect. 4.1) (Berube and Bubeck Wardenburg 2013). Instead, alpha-toxin targets B and T lymphocytes, monocytes, macrophages, platelets, erythrocytes, and other non-myeloid cells such as epithelial and endothelial cells, with varying host-dependent susceptibilities (i.e., rabbit, mouse, and human)

(Bhakdi and Tranum-Jensen 1991; Bubeck Wardenburg et al. 2007b; Cassidy and Harshman 1976; Craven et al. 2009; Hildebrand et al. 1991; Nygaard et al. 2012; Powers et al. 2012, 2015, Wilke and Bubeck Wardenburg 2010). In addition, alpha-toxin has been shown to contribute to *S. aureus* pathogenesis using murine models of skin infection and pneumonia (Berube and Bubeck Wardenburg 2013), inhibit macrophage phagocytosis (Scherr et al. 2015), and promote death of these phagocytes in concert with secreted LukAB from *S. aureus* biofilms in a murine biofilm surgical implant model (Scherr et al. 2015). Alpha-toxin also upregulates host autophagy, allowing *S. aureus* to become tolerated by the host by downregulating expression of the toxin receptor, thus minimizing *S. aureus*-induced disease (Maurer et al. 2015). Of note, this toxin is notorious for its potent hemolytic activity toward rabbit and murine erythrocytes, thus its alternate name alpha-hemolysin. However, alpha-toxin is a poor hemolysin toward human erythrocytes due to low expression of the toxin receptor by these human cells (Berube and Bubeck Wardenburg 2013).

2 Beta-Barrel Bicomponent Pore-Forming Leukocidins

The bicomponent pore-forming leukocidins constitute a sizeable group within the beta-barrel pore-forming toxins (Alonzo and Torres 2014). These cytotoxins are secreted as two water-soluble protein monomers classified as S (for slow)- or F (for fast)-components based on their elution in liquid chromatography when initially identified (Woodin 1960). Unlike alpha-toxin, these assemble into hetero-octameric pores of alternating S- and F-components on the cellular surface of numerous immune and non-immune cell populations (Colin et al. 1994; Miles et al. 2002; Yamashita et al. 2011; Yokota and Kamio 2000). Highly virulent clinical strains that cause disease in humans can produce up to five of these bicomponent leukocidins: Panton–Valentine Leukocidin (LukSF-PV or PVL), gamma-hemolysin AB (HlgAB), gamma-hemolysin CB (HlgCB), Leukocidin AB (LukAB, also known as LukHG), and Leukocidin ED (LukED). The toxin S-subunits are LukS-PV, HlgA, HlgC, LukE, and LukA/H, while the F-subunits are LukF-PV, HlgB, LukD, and LukB/G (Yoong and Torres 2013). These bicomponent pore-forming toxins share high sequence homology among S- and F-subunits (60–80 %), with the exception of LukAB (30–40 %) (Fig. 1) and are discussed below.

2.1 Panton–Valentine Leukocidin (LukSF-PV/PVL)

PVL is a potent cytotoxin to human and rabbit PMNs first described in 1932 by Panton and Valentine (Panton and Valentine 1932). Although prophage-encoded and present in only 2–4 % of all contemporary *S. aureus* strains, the *pvl* sequence is conserved and present in the large majority of the SSTI CA-MRSA isolates in the

USA (Lina et al. 1999; Naimi et al. 2003; Vandenesch et al. 2003). However, the role of this toxin in the pathogenesis of CA-MRSA has been highly controversial.

Interestingly, this toxin is remarkably incompatible with murine cells (Bubeck Wardenburg et al. 2008; Loffler et al. 2010; Spaan et al. 2013), a phenotype explained by its receptor (discussed in Sect. 4.3) (Spaan et al. 2013). Confounding results using small animal models of infection are now known to be due to the host specificity of the toxin (Bubeck Wardenburg et al. 2007a; Labandeira-Rey et al. 2007; Vandenesch et al. 2010; Zivkovic et al. 2011). Notably among these, the pathology of necrotizing pneumonia observed in murine models has been correlated with a robust proinflammatory response (Labandeira-Rey et al. 2007; Vandenesch et al. 2010; Yoong and Pier 2012). Taken together, the data reported to date regarding the PVL proinflammatory activity in vivo using murine models of infection must be cautiously considered given the discrepancies regarding in vivo targeting of murine cells (Bubeck Wardenburg et al. 2007a; Cremieux et al. 2009; Diep et al. 2010; Graves et al. 2012; Loffler et al. 2010, 2013; Yoong and Pier 2012).

2.2 Gamma-Hemolysin HlgACB

The tripartite gamma-hemolysin HlgACB was identified in 1938 and can form two functional toxins sharing the same F-subunit, HlgB: HlgAB and HlgCB. The three open reading frames encoding the gamma-hemolysins HlgAB and HlgCB are transcribed from two different promoters in the core genome, with the *hlgA* locus being transcribed from its own promoter upstream of the *hlgCB* locus (Cooney et al. 1993). The genes encoding these toxins show almost full conservation among all sequenced *S. aureus* strains (Alonzo and Torres 2014). The gamma-hemolysins are two of three hemolytic leukocidins secreted by *S. aureus* (Fackrell and Wiseman 1976), the third one being LukED (Gravet et al. 1998; Morinaga et al. 2003). Studies using isogenic strains in several infection models have suggested roles for the gamma-hemolysins in murine systemic infection (Malachowa et al. 2011), septic arthritis (Nilsson et al. 1999), and ocular models of infection in rabbits (Dajcs et al. 2002a, b; Siqueira et al. 1997; Supersac et al. 1998). Importantly, and despite their proximity on the genome, HlgAB and HlgCB display distinct receptor and species specificity (discussed in Sects. 4.4 and 4.5) (Spaan et al. 2014).

2.3 Leukocidin ED (LukED)

Leukocidin ED (LukED) was identified in 1998 in a study searching for molecules immunologically similar to PVL and gamma-hemolysin components in culture supernatants from *S. aureus* strain Newman (Gravet et al. 1998). Subsequent work from Morinaga et al. in 2003 showed that the LukED sequence described by Gravet

et al. was different from the LukED variants (LukEv and LukDv) identified from their study (Morinaga et al. 2003). Unlike the majority of the leukocidins, this toxin is encoded on the stable *S. aureus* pathogenicity island (SaPI) vSaβ (Novick and Subedi 2007), is lytic toward human and rabbit erythrocytes, and can induce dermonecrosis when administered into rabbit skin (Morinaga et al. 2003). In 2012, Alonzo et al. demonstrated that LukED potently targets murine cells (Alonzo et al. 2012). Importantly, experiments with *lukED*-isogenic mutant strains demonstrated the requirement of this toxin for the lethality observed in a murine model of bacteremia and demonstrated that toxin-mediated depletion of phagocytes contributes to *S. aureus*-mediated sepsis (Alonzo et al. 2012). Further sequence analyses by Alonzo et al. and McCarthy and Lindsay (McCarthy and Lindsay 2013) confirmed that the Morinaga et al. *lukED* sequence is indeed the correct sequence (Alonzo et al. 2012). Moreover, it was later shown that most of the sequenced strains contain the *lukED* locus with the exception of the clonal complex 30 and that the *lukED* sequences are highly conserved among strains (Alonzo and Torres 2014; McCarthy and Lindsay 2013).

2.4 Leukocidin AB (LukAB)

Leukocidin AB (LukAB, also known as LukHG) is the newest member of the bicomponent leukocidins, which was described by two groups in late 2010 and early 2011 (DuMont et al. 2011; Ventura et al. 2010). LukAB specifically targets human phagocytic cells (DuMont et al. 2011, 2013a; Malachowa et al. 2012). This core genome-encoded toxin is found in all sequenced *S. aureus* strains (Alonzo and Torres 2014), but unlike most of the leukocidins, several allelic variants of *lukAB* exist (DuMont et al. 2014). Remarkably, LukAB is the only toxin that can be found as a dimer in solution (DuMont et al. 2014). The LukAB pre-dimerization state challenges the established paradigm of membrane assembly for the leukocidins, where toxin subunits assemble in a stepwise fashion (Yamashita et al. 2011). Like PVL, however, this toxin is highly specific toward human leukocytes (DuMont et al. 2011, 2013a; Malachowa et al. 2012). As with the other leukocidins, the cellular and species specificity of LukAB is dictated by receptor targeting (discussed in Sect. 4.6). Additionally, a study evaluating antibody production against *S. aureus*-secreted factors in children with invasive infection demonstrated high anti-LukAB neutralizing antibody titers during acute infection, indicating that LukAB is produced in vivo (Thomsen et al. 2014). To date, LukAB is the only bicomponent leukocidin known to lyse PMNs from within (DuMont et al. 2013b), can elicit the formation of neutrophil extracellular traps (Malachowa et al. 2013) and, in concert with Hla, can protect *S. aureus* biofilms by inhibiting macrophage phagocytosis and promoting their death (Scherr et al. 2015).

Importantly, LukAB shares only 30 % sequence homology when compared to the S- or F-subunits of the other leukocidins (DuMont et al. 2014; Yoong and Torres 2013) and contains unique N- and C-terminal extensions that are absent from

all other staphylococcal leukocidins (DuMont et al. 2014; Yoong and Torres 2013). Taken together, these peculiarities also help explain the unique activities of LukAB.

In summary, numerous cytolytic factors are produced by *S. aureus* to target and kill host immune cells. Of these, the beta-barrel, pore-forming leukocidins are a substantial group both in number and cellular targets, which are employed by *S. aureus* to promote its survival and replication within the host.

3 Mode of Action of *S. aureus* Beta-Barrel Pore-Forming Leukocidins

The mechanism of action of the *S. aureus* beta-barrel pore-forming leukocidins has been investigated using biophysical and biochemical methods. These studies have been greatly aided by the X-ray crystallization of alpha-toxin (Song et al. 1996), LukS-PV (Guillet et al. 2004a; b), LukF-PV (Olson et al. 1999; Pedelacq et al. 1999), HlgB (Olson et al. 1999), HlgC (Yamashita et al. 2011), LukE and LukD (Galy et al. 2012), and the functional octameric pore formed by the gamma-hemolysin HlgAB (Yamashita et al. 2014, 2011) and LukAB (Badarau et al. 2015). These studies revealed several key structural features found in the toxin monomers (Fig. 2). Among those are the cap, which contains the amino latch, β-sandwich, and folded pre-stem domains. Another domain is composed by the amino latch, which is hypothesized to play a role in the anchoring of stem domains during the transition of the alpha-toxin subunits from monomers to oligomers, but its function in bicomponent leukocidin pore assembly remains poorly understood. In contrast, the β-sandwich harbors crucial residues for intersubunit contacts, while the rim domain contains mostly aromatic residues which have been proposed to be involved in binding to the host cell plasma membrane (Alonzo and Torres 2014; Joubert et al. 2007; Walker et al. 1992).

The proposed model regarding pore formation and assembly based on studies with gamma-hemolysin indicates that the S-component of the toxin binds to the host cell plasma membrane, causing the recruitment of the F-component, followed by the formation of toxin complexes, which subsequently oligomerize into non-lytic pre-pores comprised of an octamer of alternating S- and F-subunits that is stabilized by the interactions between the rim domains of the toxin monomers (Fig. 3) (Jayasinghe and Bayley 2005; Yamashita et al. 2011).

The final step is the formation of the membrane-perforating pore. For this, the pre-stem domains of each toxin subunit undergo a conformational change, through a mechanism still not fully understood, that extends and anchors the stem domains into the host cell plasma membrane, leading to the assembly of the beta-barrel structure (Fig. 3) (Yamashita et al. 2011, 2014).

Given the high sequence homology between the leukocidins, this model is regarded as the mechanism of pore formation for the other leukocidins (Yamashita et al. 2011; Yoong and Torres 2013). Membrane lipids, including

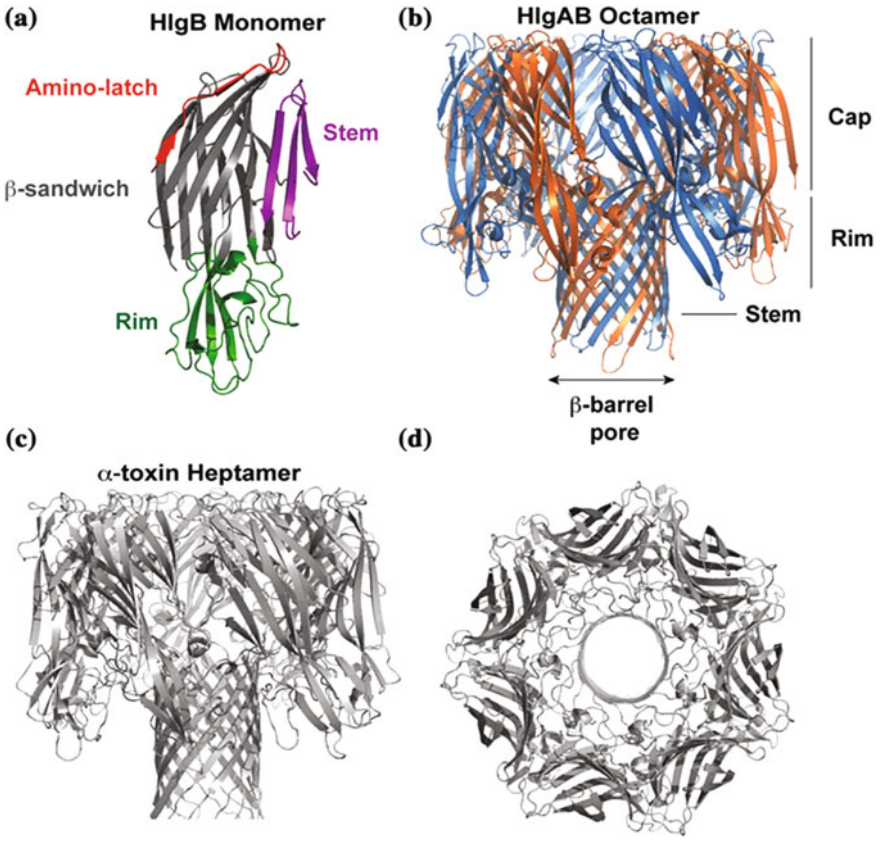

Fig. 2 Toxin structural features. **a** HlgB monomer. Structural information about the HlgB monomer was obtained from the Protein Data Bank (PDB), accession number 1LKF. **b** HlgAB octamer. Structural information about the HlgAB octamer was obtained from the PDB, accession number 3B07. A similar monomeric structure was described for alpha-toxin (Sugawara et al. 2015). **c** Alpha-toxin heptamer. **d** *Top view* of the alpha-toxin heptamer. Structural information about the alpha-toxin heptamer was obtained from the PDB, accession number 7AHL. Adapted from Alonzo and Torres (2014)

phosphatidylcholine, have also been suggested to play a role in mediating pore formation (Menestrina et al. 2003; Monma et al. 2004; Noda et al. 1980; Potrich et al. 2009). However, the ubiquitous presence of lipids does not explain the exquisite cellular tropism exhibited by these leukocidins. Importantly, the recent identification of cellular receptors of proteinaceous origin for all these pore-forming leukocidins refutes the hypothesis of membrane lipid components as the critical receptors (DuMont and Torres 2014). Nevertheless, it is conceivable that membrane lipids contribute to pore formation. Thus, the requirement of a choline-containing membrane lipid or lipid raft during one or more steps of receptor recognition, toxin assembly, and oligomerization on the cell surface is not completely ruled out.

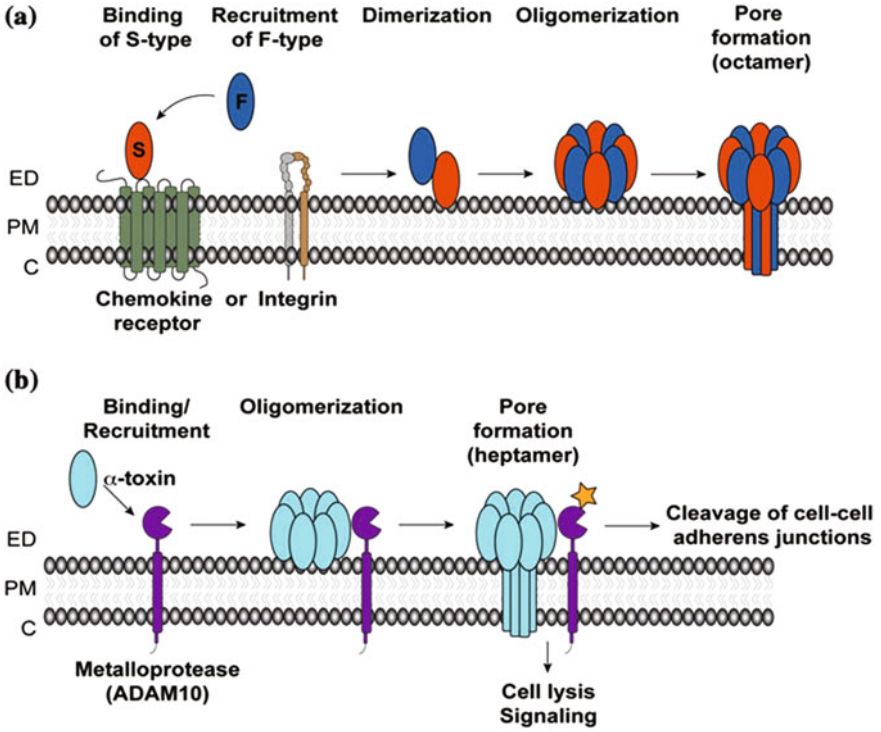

Fig. 3 Pore assembly on the host cell lipid bilayer. **a** Pore formation by a bicomponent toxin. The current model for pore formation proposes that the S-subunit binds to the host cell plasma membrane through a proteinaceous cellular receptor, either a chemokine receptor or an integrin (for LukAB). Recruitment of the F-subunit by the S-subunit is followed by dimerization and oligomerization of alternating S- and F-subunits, resulting in an octameric pore. A conformational change triggers the insertion of the toxin stem domains, forming the membrane-piercing beta-barrel structure that lyses host cells. **b** Pore formation by α-toxin. Recognition of ADAM10 by alpha-toxin leads to the formation of a homo-heptameric pore and the upregulation of the metalloprotease activity of ADAM10, resulting in cleavage of cell–cell junctions and intracellular signaling. Adapted from Alonzo and Torres (2014) and Berube and Bubeck Wardenburg (2013)

4 Identification of Proteinaceous Cellular Receptors for the Leukocidins

The early identification of cytolytic factors secreted by *S. aureus* was troublesome due to the confounding activities of some of these toxins. Ever since their discovery approximately 100 years ago, knowledge about *S. aureus* toxin receptors was lacking. Moreover, the species specificity and tropism toward immune cells exhibited by these toxins pointed toward the utilization of specific cellular receptors. For instance, all the bicomponent pore-forming toxins effectively target primary human PMNs, yet only a select few display tropism toward primary murine

PMNs. Interestingly, the same observation holds true for other immune cell types. Given the high sequence homology and shared cell-type targets, these toxins were believed to be redundant staphylococcal virulence factors. However, the last six years have welcomed a revival on the search for toxin receptors on host cells that has led to the discovery of proteinaceous cellular receptors for all the beta-barrel *S. aureus* pore-forming toxins.

4.1 Alpha-Toxin and ADAM10

Given the elusiveness of a proteinaceous receptor for alpha-toxin, previous reports attempted to elucidate the "membrane requirements" for the cytotoxic activity of alpha-toxin by focusing on various lipid components of the plasma membrane. Experiments employing biochemical and biophysical methods using multilamellar liposome membranes prepared from cholesterol and other phospholipids demonstrated that phosphocholine and sphingomyelin play a role on alpha-toxin oligomerization on lipid membranes and became the working model for toxin-engagement to cell membranes (Schwiering et al. 2013; Tomita et al. 1993; Valeva et al. 2006; Watanabe et al. 1987). Nevertheless, this hypothesis failed to explain the species specificity of alpha-toxin-induced hemolysis of erythrocytes. The hypothesis of a "lipid as a receptor" was put to rest in 2010 by seminal experiments exploiting the distinct susceptibilities of human and rabbit erythrocytes (Wilke and Bubeck Wardenburg 2010). In this study, it was demonstrated that a zinc-dependent metalloprotease known as a disintegrin and metalloprotease 10 (ADAM10) is the proteinaceous receptor for alpha-toxin (Wilke and Bubeck Wardenburg 2010). The susceptibility of different host cells to alpha-toxin was associated with ADAM10 surface levels. Pull-down experiments using the non-toxigenic form of alpha-toxin, Hla H35L, demonstrated that this toxin physically interacts with ADAM10 on the surface of rabbit, but not human erythrocytes, further confirming its species selectivity. Importantly, ADAM10 was found to be necessary and sufficient for alpha-toxin-induced cell death of nucleated cells (Wilke and Bubeck Wardenburg 2010). However, a direct interaction between alpha-toxin and purified ADAM10 has not been reported (Berube and Bubeck Wardenburg 2013; Wilke and Bubeck Wardenburg 2010).

Subsequent studies evaluating the role of ADAM10 in pathogenesis in vivo using conditional mice targeting the receptor in tissues that are particularly affected by alpha-toxin such as the lung and the skin revealed that alpha-toxin-mediated upregulation of the metalloprotease activity of ADAM10 results in disruption of E-cadherin, an adherens junction protein crucial to maintaining the integrity of the epidermis and the alveolar epithelium, leading to the severe dermonecrosis and lethal pneumonia caused by this toxin (Inoshima et al. 2011). Moreover, follow-up reports looking at additional roles of alpha-toxin in *S. aureus* pathogenesis demonstrated that this receptor plays predominant roles in neutrophil–platelet

aggregation in vivo, resulting in depletion of both platelets and cells of myeloid origin critical to control infection and restore epithelial integrity (Powers et al. 2015).

Recently, a high-throughput genetic screen using haploid cells identified the adherens junction protein known as the pleckstrin homology domain-containing protein 7 (PLEKHA7) as the second most enriched targeted gene upon alpha-toxin intoxication (ADAM10 being the primary target identified by the screen, validating previous reports) (Popov et al. 2015). Disruption of cell–cell contacts through PLEKHA7 was demonstrated to play a role downstream of alpha-toxin-mediated osmotic lysis. Cells lacking this junctional protein adeptly recovered from alpha-toxin-mediated cytotoxicity, while in vivo, PLEKHA7$^{-/-}$ mice subjected to either skin infection or lethal pneumonia showed improved recovery of skin lesions and increased survivability compared to WT mice, respectively. Taken together, this indicates the presence of additional, yet indirect targets of alpha-toxin that play roles beyond the pore formation process, which contribute to *S. aureus* virulence (Lubkin and Torres 2015).

4.2 LukED: CCR5, CXCR1, CXCR2, and DARC

The first receptor for a bicomponent leukocidin was described in 2012 (Alonzo et al. 2013). Alonzo et al. demonstrated that a T lymphocyte-like cell line stably transduced to overexpress the 7-transmembrane G-protein-coupled receptor (and HIV co-receptor) CCR5 was uniquely susceptible to LukED-mediated cytotoxicity and not to other leukocidins evaluated in this assay (LukAB and LukSF-PV/PVL). Importantly, LukED was not cytotoxic against other T lymphocyte cell lines, human, or murine primary cells lacking this receptor, indicating that the presence of CCR5 was required for the cytolytic activity of LukED.

Binding to CCR5 by LukE, the binding component of LukED was specific and inhibited by a CCR5 antagonist, maraviroc, highlighting the potential use of commercially available receptor antagonists to confer protection against this leukocidin. The in vitro and in vivo targeting and killing of CCR5-expressing adaptive immune cells, including memory T lymphocytes, macrophages, and dendritic cells, further supported these findings. Importantly, CCR5-targeting was demonstrated to play an important role in *S. aureus* pathogenesis, as infection of *Ccr5*$^{-/-}$ mice with WT *S. aureus* significantly protected mice, a phenotype recapitulated during infection of WT mice with a Δ*lukED* strain (Alonzo et al. 2012, 2013). Of note, these findings also illustrated that leukocidins have the potential to target and kill adaptive immune cells.

The identification of CCR5 as a LukED receptor did not explain the susceptibility of human and murine PMNs to this toxin, as these cells lack CCR5. A follow-up study screened a collection of chemokine receptors for their ability to render mammalian cells susceptible to LukED. These studies identified the

chemokine receptors CXCR1 and CXCR2, also known as the IL-8 receptors, as the LukED targets on PMNs and monocytes (Reyes-Robles et al. 2013). Binding to the receptors on primary human PMNs was also dependent on the binding subunit, LukE. Importantly, by exploiting the sequence homology between LukE and LukS-PV (the binding components of LukED and PVL, respectively), a LukE domain, described as Divergence Region 4 (DR4, residues 182–196), was found to be required for LukED targeting of both human and murine PMNs in vitro and in vivo in established murine infection models (Reyes-Robles et al. 2013). The discovery of CXCR1 and CXCR2 as LukED receptors complemented the findings of CCR5 by demonstrating targeting of both innate and adaptive immune cells by a single leukocidin, thus virtually disarming host immunity. As such, through the use of these receptors, LukED has the potential to eliminate cells necessary for bacterial clearance (Alonzo et al. 2013; Alonzo and Torres 2014; Reyes-Robles et al. 2013).

LukED also displays hemolytic activity on rabbit erythrocytes in vitro (Morinaga et al. 2003). The identification of proteinaceous cellular targets for LukED on immune cells did not provide an explanation for the hemolytic activity of LukED on erythrocytes. Erythrocyte lysis is critical for *S. aureus* survival in the host as this process releases iron sequestered within hemoglobin inside erythrocytes (Skaar and Schneewind 2004). Iron is a key cofactor in metabolic processes for pathogenic bacteria, including respiration, resistance to oxidative stress, and DNA synthesis (Andreini et al. 2008; Becker and Skaar 2014; Skaar and Raffatellu 2015). Interestingly, free iron within the host is very limited, a process considered as an innate immune response known as "nutritional immunity" (Andrews et al. 2003; Schaible and Kaufmann 2004; Weinberg 1975). Although iron metabolism has been significantly studied in *S. aureus*, the means by which iron is released from erythrocytes had remained elusive.

In 2015, the Duffy Antigen Receptor for Chemokines (DARC) was identified as a LukED receptor on human and murine erythrocytes (Spaan et al. 2015a). Variable susceptibility levels of human erythrocytes to LukED directly correlated with the presence of DARC on the surface of erythrocytes. As with CCR5, CXCR1 and CXCR2, DARC was found to be necessary and sufficient for rendering host cells susceptible to LukED. Importantly, in vitro hemolytic assays utilizing *S. aureus* WT, an isogenic strain lacking the genes encoding the surface receptors for the bacterium's iron uptake system (*isdBH*) and purified LukED demonstrated that LukED-mediated erythrocyte lysis promotes staphylococcal growth in a hemoglobin-dependent manner (Spaan et al. 2015a). These findings demonstrate the exploitation of a receptor in a non-immune cell by these pore-forming toxins is required for bacterial survival.

Taken together, these studies demonstrate that LukED is a unique example of a single virulence factor that effectively targets innate and adaptive immune cells, and non-immune cells critical for the bacterium survival and proliferation within the host.

4.3 LukSF-PV/PVL: C5aR and C5L2

In 2013, a study attempting to uncover the PVL receptors on leukocytes confirmed that LukS-PV bound to neutrophils and monocytes, but not lymphocytes (Gauduchon et al. 2001; Spaan et al. 2013). Importantly, an antibody screen comprised of anti-leukocyte surface receptors found that upon incubation in the presence of LukS-PV, antibodies targeting the complement receptor C5aR displayed decreased binding to these cells, and this interaction was also reduced upon incubation with C5a ligands, indicating that PVL engages C5aR on the surface of primary human PMNs. The complement receptors C5aR and C5L2 (also known as C5aR1 and C5aR2, respectively) were both found to render otherwise resistant cells sensitive to PVL (Spaan et al. 2013). Importantly, targeting C5aR/C5L2 determined PVL's species specificity, as human and rabbit PMNs, not murine or macaque, were susceptible to the toxin, in accordance with previous observations regarding full resistance of murine cells to PVL (Bubeck Wardenburg et al. 2008; Loffler et al. 2010). PVL targeting of C5aR and C5L2 explains the toxin's inability to target murine cells and thus the limitation of murine models to study this toxin. Currently, rabbits seem to be the only appropriate model to study the role of PVL in *S. aureus* pathogenesis (Diep et al. 2010), albeit this system is still suboptimal as rabbit PMNs are less sensitive compared to human PMNs (Loffler et al. 2010; Malachowa et al. 2012).

4.4 HlgAB: CXCR1, CXCR2, CCR2, and DARC

Despite being identified in the early twentieth century, the lytic activities of the gamma-hemolysin proved difficult to uncouple. In the 1970's, Fackrell and Wiseman (1976) described that this toxin not only has hemolytic, but leukocidal activity when applied to human lymphoblasts and leukocytes. Their studies were further confirmed shortly after by Szmigielski et al. (1976) using rabbit leukocytes. Nevertheless, no cellular receptor was ascribed to this toxin. A study in 2014 sought after the cellular targets of HlgAB (Spaan et al. 2014). First, targeting of human PMNs and monocytes was confirmed using purified recombinant HlgAB, while human lymphocytes were minimally susceptible to this toxin. To determine the cellular factors responsible for toxin-mediated death of these cell types, several immune cell types were subjected to purified recombinant HlgAB and their viability evaluated. Additionally, human embryonic kidney (HEK) cells were transfected with cDNAs encoding several chemokine receptors present on these susceptible cell types and treated with HlgAB and HlgCB. The results of these experiments indicated that, like LukED, the chemokine receptors CXCR1 and CXCR2 are also the receptors for HlgAB on primary human PMNs (Spaan et al. 2014). Moreover, the presence of these receptors in the susceptible immune cell types is directly correlated with their death. Interestingly, experiments employing primary murine leukocytes as

well as cells transfected with the murine version of the receptors indicated that primary murine PMNs are refractory to HlgAB-mediated intoxication despite CXCR2 being present on the surface of murine PMNs (murine PMNs lack detectable CXCR1), indicating that receptor targeting by HlgAB is species-selective (Spaan et al. 2014). Unlike LukED, murine monocytes and macrophages are susceptible to HlgAB through the chemokine receptor CCR2 (Spaan et al. 2014). HlgAB binds directly to and displays high affinity toward its receptors, as demonstrated by surface plasmon resonance (SPR) experiments. The relevance of receptor targeting in vitro was further confirmed ex vivo and in vivo, where CCR2 targeting by HlgAB was demonstrated to play a critical role during murine infection (Spaan et al. 2014).

HlgAB shares several commonalities with LukED, including immune cell receptor targeting (CXCR1 and CXCR2) and hemolytic activity toward human erythrocytes (Ozawa et al. 1995; Tomita et al. 2011). As with LukED, DARC was found to be the receptor for HlgAB on human and murine erythrocytes (Spaan et al. 2015a). Interestingly, infection of erythrocytes, either $DARC^+$ and $DARC^-$, with *S. aureus* WT and Δ*hlgA* isogenic strains demonstrated that *S. aureus*-mediated ex vivo hemolysis is HlgAB- and DARC-dependent (LukED activity was not evaluated due to negligible production of the toxin in vitro) (Spaan et al. 2015a).

4.5 HlgCB: C5aR and C5L2

It has been known for quite some time that HlgCB and PVL share similar targets and thus were hypothesized to share a common receptor (Gauduchon et al. 2001). In fact, studies evaluating binding of HlgC and LukS-PV (the binding subunits of HlgCB and LukSF-PV/PVL, respectively) demonstrated that these two toxins share the same binding site on primary human PMNs (Gauduchon et al. 2001; Prevost et al. 2001). In 2014, Spaan and colleagues demonstrated that despite their similarity, HlgAB and HlgCB targeted different receptors (Spaan et al. 2014). HlgAB targets the chemokine receptors CXCR1 and CXCR2 on human PMNs, while HlgCB targets the same receptors as PVL, the chemokine receptors C5aR and C5L2. Like PVL, HlgCB is also not compatible with murine C5aR. A follow-up study by the same group demonstrated that the tropism shown by HlgCB and PVL toward C5aR is dictated by specific receptor domains that differ between species (Spaan et al. 2015b).

4.6 LukAB: CD11b

The identification of the PVL receptors was followed up by the discovery of the LukAB receptor on phagocytes in 2013 (DuMont et al. 2013a). To identify host proteins bound by LukAB, a pull-down approach on primary human PMNs was performed, followed by mass spectrometry to identify the toxin-interacting partners. The most abundant host proteins identified by the pull-down were the α- and

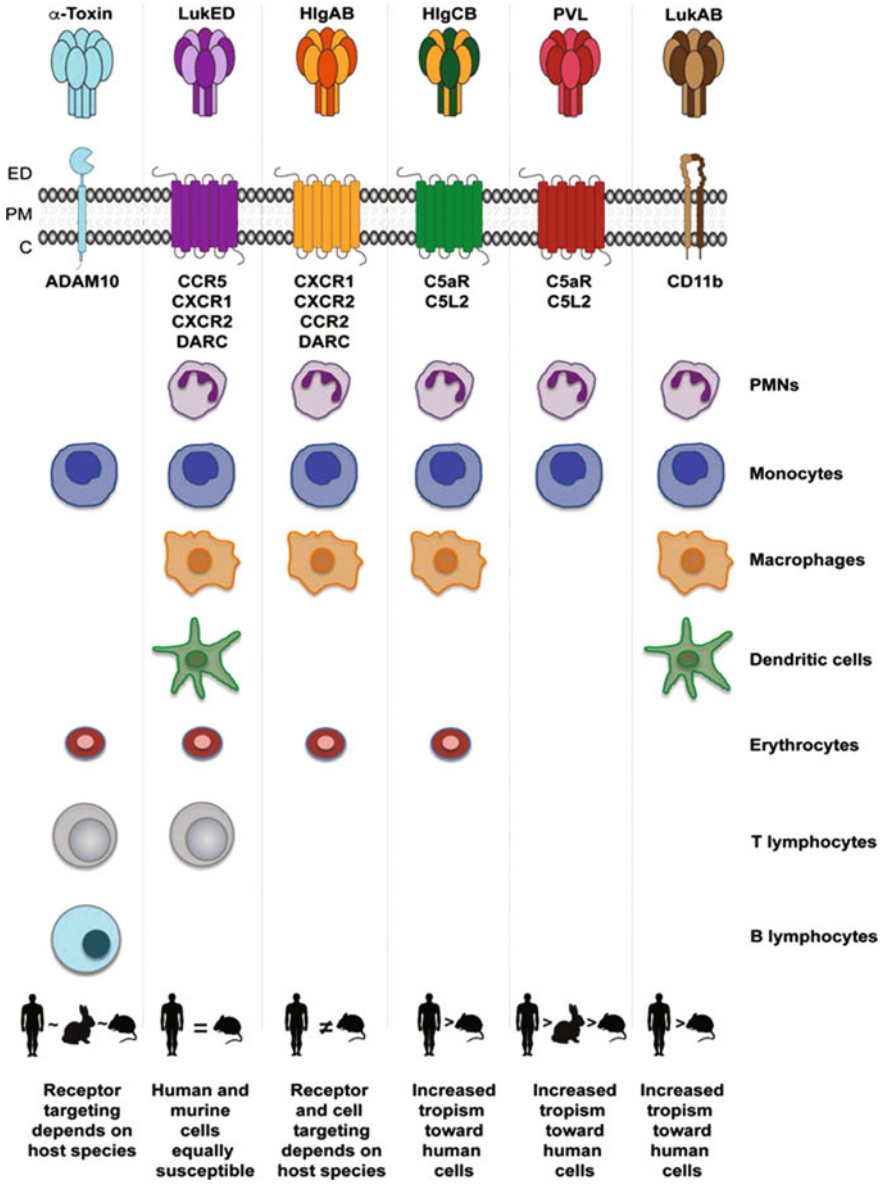

Fig. 4 Cellular receptors on hematopoietic cells targeted by *S. aureus* beta-barrel pore-forming toxins. The distinct bicomponent pore-forming cytotoxins are colored with two colors, while alpha-toxin is depicted in a single color. Receptors, cell targets, and host specificities are indicated under the corresponding toxin. Of note, while alpha-toxin does not exhibit species specificity on nucleated cells, lysis of erythrocytes is species-dependent. Adapted from DuMont and Torres (2014)

β-components of the macrophage-1 integrin (MAC-1) complex, also known as CD11b and CD18, respectively. Of the two components, only CD11b was determined to be necessary and sufficient for LukAB binding, and for rendering primary human PMNs and monocytes susceptible to the toxin. Contrary to the other leukocidins, which share high sequence homology (Fig. 1), LukAB is the only bicomponent leukocidin that does not employ a chemokine receptor to kill cells (Fig. 4). The divergent receptor targeting by LukAB seems to be dictated by the presence of unique C-terminal extensions absent in other leukocidins. Within the C-terminus, a single glutamic acid residue at position 323 was found to be required for toxin binding and subsequent cytotoxicity on CD11b-positive cells (DuMont et al. 2014). By screening antibodies against various domains of CD11b, DuMont and colleagues demonstrated that the I domain of CD11b is the interaction domain for LukAB. This interaction dictates LukAB's species specificity, as the toxin only recognizes human, but not murine, I domain (DuMont et al. 2013a).

5 Toxin Redundancy

All the bicomponent pore-forming toxins potently lyse primary human PMNs (Alonzo and Torres 2014; Vandenesch et al. 2012). In addition to PMNs, other cell-type targets are shared among some of these toxins (i.e., monocytes and macrophages are targeted by LukED, PVL, and HlgACB) (Fig. 4) (Alonzo et al. 2013; Melehani et al. 2015; Perret et al. 2012; Reyes-Robles et al. 2013; Spaan et al. 2014). Given the high sequence homology shared between S- and F-subunits (with the exception of LukAB) (Fig. 1), and the production of two or more toxins under any given conditions, these toxins have been considered to be redundant virulence factors produced by *S. aureus*. Remarkably, experiments using purified recombinant toxins demonstrated that in addition to assembling into functional "native" toxins, the bicomponent pore-forming toxin subunits are competent at oligomerizing into "non-native" pairs that still lyse primary human PMNs with various potencies, as well as other cell types (Dalla Serra et al. 2005; Gravet et al. 1998; Morinaga et al. 2003; Rouha et al. 2015; Yanai et al. 2014). Moreover, these mixed pairings have the potential of acting as inhibitory complexes regulating the potency of other toxins. For instance, LukED and PVL inhibit their in vitro and in vivo hemolytic and leukocidal activities, respectively, in a unique example of leukocidin antagonism (Yoong and Torres 2015). Thus, it is plausible that the large number of toxins produced by *S. aureus* expands their breadth of cellular targets, potentiates their activities, or can also be regulatory to reduce the damaging inflammatory effects of cell lysis and successfully maintain an infection without killing the host.

Altogether, the identification of proteinaceous receptors for each for the beta-barrel pore-forming toxins challenges the notion that these toxins are redundant by demonstrating that despite sharing similar host targets, their receptors dictate their cellular and species tropism.

6 Conclusions

S. aureus is an important human opportunistic pathogen causing significant morbidity and mortality worldwide in both hospital and community settings, for which treatment options are becoming limited due to rapid acquisition of antibiotic resistance. Contributing to *S. aureus* disease are toxins that target and kill host immune and non-immune cells. Despite their role in *S. aureus* pathogenesis, little was known about the molecular mechanisms that dictate their cellular targeting and cytotoxic activity. Here, we provided an updated view on *S. aureus* pore-forming toxins, their host membrane receptors, and how they positively influence staphylococcal pathogenesis and disease by injuring host cells. Additionally, the discovery of receptors laid long-standing toxin redundancy misconceptions to rest, provided further insights regarding toxin activity, their targets on host cells, and how this drives their cell and species tropism. Given the large repertoire of cytotoxins *S. aureus* produces compared to other bacterial pathogens relevant to human disease, renewed interest on these *S. aureus* toxins as it relates to their use in vaccine development has been gathered. Importantly, the uncovering of toxin receptors provides novel targeted avenues to explore in the development of anti-*S. aureus* therapeutics.

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References

Alonzo F III, Benson MA, Chen J, Novick RP, Shopsin B, Torres VJ (2012) *Staphylococcus aureus* leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. Mol Microbiol 83:423–435

Alonzo F III, Kozhaya L, Rawlings SA, Reyes-Robles T, DuMont AL, Myszka DG, Landau NR, Unutmaz D, Torres VJ (2013) CCR5 is a receptor for *Staphylococcus aureus* leukotoxin ED. Nature 493:51–55

Alonzo F III, Torres VJ (2014) The bicomponent pore-forming leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78:199–230

Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM (2008) Metal ions in biological catalysis: from enzyme databases to general principles. J Biol Inorg Chem 13:1205–1218

Andrews SC, Robinson AK, Rodriguez-Quinones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237

Badarau A, Rouha H, Malafa S, Logan DT, Hakansson M, Stulik L, Dolezilkova I, Teubenbacher A, Gross K, Maierhofer B et al (2015) Structure-function analysis of heterodimer formation, oligomerization, and receptor binding of the *Staphylococcus aureus* bi-component toxin LukGH. J Biol Chem 290:142–156

Becker KW, Skaar EP (2014) Metal limitation and toxicity at the interface between host and pathogen. FEMS Microbiol Rev 38:1235–1249

Berube BJ, Bubeck Wardenburg J (2013) *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166

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

Bubeck Wardenburg J, Bae T, Otto M, Deleo FR, Schneewind O (2007a) Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in *Staphylococcus aureus* pneumonia. Nat Med 13:1405–1406

Bubeck Wardenburg J, Patel RJ, Schneewind O (2007b) Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun 75:1040–1044

Bubeck Wardenburg J, Palazzolo-Ballance AM, Otto M, Schneewind O, DeLeo FR (2008) Panton-Valentine leukocidin is not a virulence determinant in murine models of community-associated methicillin-resistant *Staphylococcus aureus* disease. J Infect Dis 198:1166–1170

Burnet FM (1929) The exotoxins of *Staphylococcus pyogenes aureus*. J Pathol Bacteriol 32:717–734

Burnet FM (1930) The production of staphylococcal toxin. J Pathol Bacteriol 33:1–16

Cassidy P, Harshman S (1976) Studies on the binding of staphylococcal 125I-labeled alpha-toxin to rabbit erythrocytes. Biochemistry 15:2348–2355

Colin DA, Mazurier I, Sire S, Finck-Barbancon V (1994) Interaction of the two components of leukocidin from *Staphylococcus aureus* with human polymorphonuclear leukocyte membranes: sequential binding and subsequent activation. Infect Immun 62:3184–3188

Cooney J, Kienle Z, Foster TJ, O'Toole PW (1993) The gamma-hemolysin locus of *Staphylococcus aureus* comprises three linked genes, two of which are identical to the genes for the F and S components of leukocidin. Infect Immun 61:768–771

Craven R, Gao X, Allen I, Gris D, Bubeck Wardenburg J, McElvania-Tekippe E, Ting J, Duncan J (2009) *Staphylococcus aureus* alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. PloS One 4

Cremieux AC, Dumitrescu O, Lina G, Vallee C, Cote JF, Muffat-Joly M, Lilin T, Etienne J, Vandenesch F, Saleh-Mghir A (2009) Panton-valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. PLoS One 4:e7204

Dajcs JJ, Austin MS, Sloop GD, Moreau JM, Hume EB, Thompson HW, McAleese FM, Foster TJ, O'Callaghan RJ (2002a) Corneal pathogenesis of *Staphylococcus aureus* strain Newman. Invest Ophthalmol Vis Sci 43:1109–1115

Dajcs JJ, Thibodeaux BA, Girgis DO, O'Callaghan RJ (2002b) Corneal virulence of *Staphylococcus aureus* in an experimental model of keratitis. DNA Cell Biol 21:375–382

Dalla Serra M, Coraiola M, Viero G, Comai M, Potrich C, Ferreras M, Baba-Moussa L, Colin DA, Menestrina G, Bhakdi S et al (2005) *Staphylococcus aureus* bicomponent gamma-hemolysins, HlgA, HlgB, and HlgC, can form mixed pores containing all components. J Chem Inf Model 45:1539–1545

Denys J, Van de Velde H (1895) Sur la production d'une antileucocidine chez les lapin vaccinés contre le staphylocoque pyogène. La Cellule, 359–372

Diep BA, Chan L, Tattevin P, Kajikawa O, Martin TR, Basuino L, Mai TT, Marbach H, Braughton KR, Whitney AR et al (2010) Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci USA 107:5587–5592

DuMont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, Shopsin B, Unutmaz D, Voyich JM, Torres VJ (2011) Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. Mol Microbiol 79:814–825

DuMont AL, Torres VJ (2014) Cell targeting by the *Staphylococcus aureus* pore-forming toxins: it's not just about lipids. Trends Microbiol 22:21–27

DuMont AL, Yoong P, Day CJ, Alonzo F III, McDonald WH, Jennings MP, Torres VJ (2013a) *Staphylococcus aureus* LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci USA 110:10794–10799

DuMont AL, Yoong P, Surewaard BG, Benson MA, Nijland R, van Strijp JA, Torres VJ (2013b) *Staphylococcus aureus* elaborates leukocidin AB to mediate escape from within human neutrophils. Infect Immun 81:1830–1841

DuMont AL, Yoong P, Liu X, Day CJ, Chumbler NM, James DB, Alonzo F III, Bode NJ, Lacy DB, Jennings MP et al (2014) Identification of a crucial residue required for *Staphylococcus aureus* LukAB cytotoxicity and receptor recognition. Infect Immun 82:1268–1276

Fackrell HB, Wiseman GM (1976) Properties of the gamma haemolysin of *Staphylococcus aureus* 'Smith 5R'. J Gen Microbiol 92:11–24

Galy R, Bergeret F, Keller D, Mourey L, Prévost G, Maveyraud L (2012) Crystallization and preliminary crystallographic studies of both components of the staphylococcal LukE-LukD leukotoxin. Acta Cryst 68:663–667

Gauduchon V, Werner S, Prevost G, Monteil H, Colin DA (2001) Flow cytometric determination of Panton-Valentine leucocidin S component binding. Infect Immun 69:2390–2395

Gladstone GP, Van Heyningen WE (1957) Staphylococcal leucocidins. Br J Exp Pathol 38: 123–137

Glenny AT, Stevens MF (1935) Staphylococcus toxins and antitoxins. J Pathol Bacteriol 40:201–210

Gouaux JE, Braha O, Hobaugh MR, Song L, Cheley S, Shustak C, Bayley H (1994) Subunit stoichiometry of staphylococcal alpha-hemolysin in crystals and on membranes: a heptameric transmembrane pore. Proc Natl Acad Sci USA 91:12828–12831

Graves SF, Kobayashi SD, Braughton KR, Whitney AR, Sturdevant DE, Rasmussen DL, Kirpotina LN, Quinn MT, DeLeo FR (2012) Sublytic concentrations of *Staphylococcus aureus* Panton-Valentine leukocidin alter human PMN gene expression and enhance bactericidal capacity. J Leukoc Biol 92:361–374

Gravet A, Colin DA, Keller D, Girardot R, Monteil H, Prevost G (1998) Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. FEBS Lett 436:202–208

Gray GS, Kehoe M (1984) Primary sequence of the alpha-toxin gene from *Staphylococcus aureus* wood 46. Infect Immun 46:615–618

Green HJ (Ed) (1928) Royal commission of inquiry into fatalities at Bundaberg. Report of the Royal Commission of Inquity into Fatalities at Bundaberg, Together with Appendices. Government Printer; Melbourne, Australia

Guillet V, Keller D, Prevost G, Mourey L (2004a) Crystallization and preliminary crystallographic data of a leucotoxin S component from *Staphylococcus aureus*. Acta Crystallogr D Biol Crystallogr 60:310–313

Guillet V, Roblin P, Werner S, Coraiola M, Menestrina G, Monteil H, Prevost G, Mourey L (2004b) Crystal structure of leucotoxin S component: new insight into the Staphylococcal beta-barrel pore-forming toxins. J Biol Chem 279:41028–41037

Hildebrand A, Pohl M, Bhakdi S (1991) *Staphylococcus aureus* alpha-toxin. Dual mechanism of binding to target cells. J Biol Chem 266:17195–17200

Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, Bubeck Wardenburg J (2011) A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med 17:1310–1314

Jayasinghe L, Bayley H (2005) The leukocidin pore: evidence for an octamer with four LukF subunits and four LukS subunits alternating around a central axis. Protein Sci 14:2550–2561

Joubert O, Voegelin J, Guillet V, Tranier S, Werner S, Colin DA, Serra MD, Keller D, Monteil H, Mourey L et al (2007) Distinction between pore assembly by Staphylococcal alpha-Toxin versus Leukotoxins. J Biomed Biotechnol 2007:25935

Julianelle LA (1922) Studies of hemolytic staphylococci—Hemolytic activity—Biochemical reactions—Serologic reactions. J Infect Dis 31:256–284

Labandeira-Rey M, Couzon F, Boisset S, Brown EL, Bes M, Benito Y, Barbu EM, Vazquez V, Hook M, Etienne J et al (2007) *Staphylococcus aureus* panton-valentine leukocidin causes necrotizing pneumonia. Science 315:1130–1133

Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128–1132

Loffler B, Hussain M, Grundmeier M, Bruck M, Holzinger D, Varga G, Roth J, Kahl BC, Proctor RA, Peters G (2010) *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6:e1000715

Loffler B, Niemann S, Ehrhardt C, Horn D, Lanckohr C, Lina G, Ludwig S, Peters G (2013) Pathogenesis of *Staphylococcus aureus* necrotizing pneumonia: the role of PVL and an influenza coinfection. Expert Rev Anti Infect Ther 11:1041–1051

Lubkin A, Torres VJ (2015) The ever-emerging complexity of alpha-toxin's interaction with host cells. Proc Natl Acad Sci USA 112:14123–14124

Malachowa N, Kobayashi SD, Braughton KR, Whitney AR, Parnell MJ, Gardner DJ, Deleo FR (2012) *Staphylococcus aureus* Leukotoxin GH promotes inflammation. J Infect Dis 206:1185–1193

Malachowa N, Kobayashi SD, Freedman B, Dorward DW, DeLeo FR (2013) *Staphylococcus aureus* leukotoxin GH promotes formation of neutrophil extracellular traps. J Immunol 191:6022–6029

Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, Braughton KR, Shabb DW, Diep BA, Chambers HF, Otto M et al (2011) Global changes in *Staphylococcus aureus* gene expression in human blood. PLoS One 6:e18617

Maurer K, Reyes-Robles T, Alonzo F III, Durbin J, Torres VJ, Cadwell K (2015) Autophagy mediates tolerance to *Staphylococcus aureus* alpha-toxin. Cell Host Microbe 17:429–440

McCarthy AJ, Lindsay JA (2013) *Staphylococcus aureus* innate immune evasion is lineage-specific: a bioinfomatics study. Infect Genet Evol 19C:7–14

Melehani JH, James DB, DuMont AL, Torres VJ, Duncan JA (2015) *Staphylococcus aureus* Leukocidin A/B (LukAB) kills human monocytes via Host NLRP3 and ASC when extracellular, but not intracellular. PLoS Pathog 11:e1004970

Menestrina G, Dalla Serra M, Comai M, Coraiola M, Viero G, Werner S, Colin DA, Monteil H, Prevost G (2003) Ion channels and bacterial infection: the case of beta-barrel pore-forming protein toxins of *Staphylococcus aureus*. FEBS Lett 552:54–60

Miles G, Movileanu L, Bayley H (2002) Subunit composition of a bicomponent toxin: staphylococcal leukocidin forms an octameric transmembrane pore. Protein Sci 11:894–902

Monma N, Nguyen VT, Kaneko J, Higuchi H, Kamio Y (2004) Essential residues, W177 and R198, of LukF for phosphatidylcholine-binding and pore-formation by staphylococcal gamma-hemolysin on human erythrocyte membranes. J Biochem 136:427–431

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

Mueller EA, Merriman JA, Schlievert PA (2015) Toxic shock syndrome toxin-1, not alpha toxin, mediated Bundaberg fatalities. Microbiology 161:2361–2368

Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C et al (2003) Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290:2976–2984

Neisser M, Wechsberg F (1901) Ueber das staphylotoxin. Med Microbiol Immunol 36:299–349

Nilsson IM, Hartford O, Foster T, Tarkowski A (1999) Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. Infect Immun 67:1045–1049

Noda M, Kato I, Hirayama T, Matsuda F (1980) Fixation and inactivation of staphylococcal leukocidin by phosphatidylcholine and ganglioside GM1 in rabbit polymorphonuclear leukocytes. Infect Immun 29:678–684

Novick RP, Subedi A (2007) The SaPIs: mobile pathogenicity islands of Staphylococcus. Chem Immunol Allergy 93:42–57

Nygaard TK, Pallister KB, Dumont AL, Dewald M, Watkins RL, Pallister EQ, Malone C, Griffith S, Horswill AR, Torres VJ et al (2012) Alpha-toxin induces programmed cell death of human T cells, B cells, and monocytes during USA300 infection. PLoS One 7:e36532

Olson R, Nariya H, Yokota K, Kamio Y, Gouaux E (1999) Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel. Nat Struct Biol 6:134–140

Ozawa T, Kaneko J, Kamio Y (1995) Essential binding of LukF of staphylococcal gamma-hemolysin followed by the binding of H gamma II for the hemolysis of human erythrocytes. Biosci Biotechnol Biochem 59:1181–1183

Panton PN, Valentine FCO (1932) Staphylococcal toxin. Lancet 1:506–508

Pedelacq JD, Maveyraud L, Prevost G, Baba-Moussa L, Gonzalez A, Courcelle E, Shepard W, Monteil H, Samama JP, Mourey L (1999) The structure of a *Staphylococcus aureus* leucocidin component (LukF-PV) reveals the fold of the water-soluble species of a family of transmembrane pore-forming toxins. Structure 7:277–287

Perret M, Badiou C, Lina G, Burbaud S, Benito Y, Bes M, Cottin V, Couzon F, Juruj C, Dauwalder O et al (2012) Cross-talk between *S. aureus* leukocidins-intoxicated macrophages and lung epithelial cells triggers chemokine secretion in an inflammasome-dependent manner. Cell Microbiol 14:1019–1036

Popov LM, Marceau CD, Starkl PM, Lumb JH, Shah J, Guerrera D, Cooper RL, Merakou C, Bouley DM, Meng W et al (2015) The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. Proc Natl Acad Sci USA 112:14337–14342

Potrich C, Bastiani H, Colin DA, Huck S, Prevost G, Dalla Serra M (2009) The influence of membrane lipids in *Staphylococcus aureus* gamma-hemolysins pore formation. J Membr Biol 227:13–24

Powers ME, Becker RE, Sailer A, Turner JR, Bubeck Wardenburg J (2015) Synergistic action of *Staphylococcus aureus* alpha-toxin on platelets and myeloid lineage cells contributes to lethal sepsis. Cell Host Microbe 17:775–787

Powers ME, Kim HK, Wang Y, Bubeck Wardenburg J (2012) ADAM10 mediates vascular injury induced by *Staphylococcus aureus* alpha-hemolysin. J Infect Dis 206:352–356

Prevost G, Mourey L, Colin DA, Menestrina G (2001) Staphylococcal pore-forming toxins. Curr Top Microbiol Immunol 257:53–83

Reyes-Robles T, Alonzo F III, Kozhaya L, Lacy DB, Unutmaz D, Torres VJ (2013) *Staphylococcus aureus* leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. Cell Host Microbe 14:453–459

Rouha H, Badarau A, Visram ZC, Battles MB, Prinz B, Magyarics Z, Nagy G, Mirkina I, Stulik L, Zerbs M et al (2015) Five birds, one stone: neutralization of alpha-hemolysin and 4 bi-component leukocidins of *Staphylococcus aureus* with a single human monoclonal antibody. MAbs 7:243–254

Schaible UE, Kaufmann SH (2004) Iron and microbial infection. Nat Rev Microbiol 2:946–953

Scherr TD, Hanke ML, Huang O, James DB, Horswill AR, Bayles KW, Fey PD, Torres VJ, Kielian T (2015) *Staphylococcus aureus* biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. MBio 6:

Schwiering M, Brack A, Stork R, Hellmann N (2013) Lipid and phase specificity of alpha-toxin from *S. aureus*. Biochim Biophys Acta 1828:1962–1972

Siqueira JA, Speeg-Schatz C, Freitas FI, Sahel J, Monteil H, Prevost G (1997) Channel-forming leucotoxins from *Staphylococcus aureus* cause severe inflammatory reactions in a rabbit eye model. J Med Microbiol 46:486–494

Skaar EP, Raffatellu M (2015) Metals in infectious diseases and nutritional immunity. Metallomics 7:926–928

Skaar EP, Schneewind O (2004) Iron-regulated surface determinants (Isd) of *Staphylococcus aureus*: stealing iron from heme. Microbes Infect 6:390–397

Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866

Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, Kemmink J, de Haas CJ, van Kessel KP, Vandenesch F et al (2013) The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. Cell Host Microbe 13:584–594

Spaan AN, Reyes-Robles T, Badiou C, Cochet S, Yoong P, Day CJ, de Haas CJC, Boguslawski K, van Kessel KPM, Vandenesh F et al (2015a). *Staphylococcus aureus* targets the Duffy antigen receptor for chemokines (DARC) to lyse erythrocytes. Cell Host Microbe (in press)

Spaan AN, Schiepers A, de Haas CJ, van Hooijdonk DD, Badiou C, Contamin H, Vandenesch F, Lina G, Gerard NP, Gerard C et al. (2015b) Differential interaction of the staphylococcal toxins panton-valentine leukocidin and gamma-hemolysin CB with human C5a Receptors. J Immunol

Spaan AN, Vrieling M, Wallet P, Badiou C, Reyes-Robles T, Ohneck EA, Benito Y, de Haas CJ, Day CJ, Jennings MP et al (2014) The staphylococcal toxins gamma-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. Nat Commun 5:5438

Sugawara T, Yamashita D, Kato K, Peng Z, Ueda J, Kaneko J, Kamio Y, Tanaka Y, Yao M (2015) Structural basis for pore-forming mechanism of staphylococcal alpha-hemolysin. Toxicon 108:226–231

Supersac G, Piemont Y, Kubina M, Prevost G, Foster TJ (1998) Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of *Staphylococcus aureus*. Microb Pathog 24:241–251

Szmigielski S, Jeljaszewicz J, Kobus M, Luczak M, Ludwicka A, Mollby R, Wadstrom T (1976) Cytotoxic effects of staphylococcal alpha-hemolysins, beta-hemolysins and gamma-hemolysins. Zbl Bakt-Int J Med M 691–705

Thomsen IP, Dumont AL, James DB, Yoong P, Saville BR, Soper N, Torres VJ, Creech CB (2014) Children with invasive *Staphylococcus aureus* disease exhibit a potently neutralizing antibody response to the cytotoxin LukAB. Infect Immun 82:1234–1242

Tomita N, Abe K, Kamio Y, Ohta M (2011) Cluster-forming property correlated with hemolytic activity by staphylococcal gamma-hemolysin transmembrane pores. FEBS Lett 585:3452–3456

Tomita T, Watanabe M, Yarita Y (1993) Assembly and channel-forming activity of a naturally-occurring nicked molecule of *Staphylococcus aureus* alpha-toxin. Biochim Biophys Acta 1145:51–57

Valeva A, Hellmann N, Walev I, Strand D, Plate M, Boukhallouk F, Brack A, Hanada K, Decker H, Bhakdi S (2006) Evidence that clustered phosphocholine head groups serve as sites for binding and assembly of an oligomeric protein pore. J Biol Chem 281:26014–26021

Van de Velde H (1894) Etude sur le mécanisme de la virulence du staphylocoque pyogène. La Cellule, 403–460

Vandenesch F, Couzon F, Boisset S, Benito Y, Brown EL, Lina G, Etienne J, Bowden MG (2010) The Panton-Valentine leukocidin is a virulence factor in a murine model of necrotizing pneumonia. J Infect Dis 201, 967–969 (author reply 969–970)

Vandenesch F, Lina G, Henry T (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, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME et al (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984

Ventura CL, Malachowa N, Hammer CH, Nardone GA, Robinson MA, Kobayashi SD, DeLeo FR (2010) Identification of a novel *Staphylococcus aureus* two-component leukotoxin using cell surface proteomics. PLoS One 5:e11634

Walker B, Krishnasastry M, Zorn L, Bayley H (1992) Assembly of the oligomeric membrane pore formed by Staphylococcal alpha-hemolysin examined by truncation mutagenesis. J Biol Chem 267:21782–21786

Watanabe M, Tomita T, Yasuda T (1987) Membrane-damaging action of staphylococcal alpha-toxin on phospholipid-cholesterol liposomes. Biochim Biophys Acta 898:257–265

Weinberg ED (1975) Nutritional immunity. Host's attempt to withold iron from microbial invaders. JAMA 231:39–41

Weld JT, Gunther A (1931) Differentiation between certain toxic properties of filtrates of hemolytic *Staphylococcus aureus*. J Exp Med 54:315–322

Wilke GA, Bubeck Wardenburg J (2010) Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci USA 107:13473–13478

Woodin AM (1960) Purification of the two components of leucocidin from *Staphylococcus aureus*. Biochem J 75:158–165

Wright J (1936) Staphylococcal leucocidin (Neisser-Wechsberg type) and antileucocidin. Lancet 1:1002–1004

Yamashita D, Sugawara T, Takeshita M, Kaneko J, Kamio Y, Tanaka I, Tanaka Y, Yao M (2014) Molecular basis of transmembrane beta-barrel formation of staphylococcal pore-forming toxins. Nat Commun 5:4897

Yamashita K, Kawai Y, Tanaka Y, Hirano N, Kaneko J, Tomita N, Ohta M, Kamio Y, Yao M, Tanaka I (2011) Crystal structure of the octameric pore of staphylococcal gamma-hemolysin reveals the beta-barrel pore formation mechanism by two components. Proc Natl Acad Sci USA 108:17314–17319

Yanai M, Rocha MA, Matolek AZ, Chintalacharuvu A, Taira Y, Chintalacharuvu K, Beenhouwer DO (2014) Separately or combined, LukG/LukH is functionally unique compared to other staphylococcal bicomponent leukotoxins. PLoS One 9:e89308

Yokota K, Kamio Y (2000) Tyrosine72 residue at the bottom of rim domain in LukF crucial for the sequential binding of the staphylococcal gamma-hemolysin to human erythrocytes. Biosci Biotechnol Biochem 64:2744–2747

Yoong P, Pier GB (2012) Immune-activating properties of Panton-Valentine leukocidin improve the outcome in a model of methicillin-resistant *Staphylococcus aureus* pneumonia. Infect Immun 80:2894–2904

Yoong P, Torres VJ (2013) The effects of *Staphylococcus aureus* leukotoxins on the host: cell lysis and beyond. Curr Opin Microbiol 16:63–69

Yoong P, Torres VJ (2015) Counter inhibition between leukotoxins attenuates *Staphylococcus aureus* virulence. Nat Commun (in press)

Zivkovic A, Sharif O, Stich K, Doninger B, Biaggio M, Colinge J, Bilban M, Mesteri I, Hazemi P, Lemmens-Gruber R et al (2011) TLR 2 and CD14 mediate innate immunity and lung inflammation to staphylococcal Panton-Valentine leukocidin in vivo. J Immunol 186:1608–1617

The Role of Two-Component Signal Transduction Systems in *Staphylococcus aureus* Virulence Regulation

Andreas F. Haag and Fabio Bagnoli

Abstract *Staphylococcus aureus* is a versatile, opportunistic human pathogen that can asymptomatically colonize a human host but can also cause a variety of cutaneous and systemic infections. The ability of *S. aureus* to adapt to such diverse environments is reflected in the presence of complex regulatory networks fine-tuning metabolic and virulence gene expression. One of the most widely distributed mechanisms is the two-component signal transduction system (TCS) which allows a pathogen to alter its gene expression profile in response to environmental stimuli. The simpler TCSs consist of only a transmembrane histidine kinase (HK) and a cytosolic response regulator. *S. aureus* encodes a total of 16 conserved pairs of TCSs that are involved in diverse signalling cascades ranging from global virulence gene regulation (e.g. quorum sensing by the Agr system), the bacterial response to antimicrobial agents, cell wall metabolism, respiration and nutrient sensing. These regulatory circuits are often interconnected and affect each other's expression, thus fine-tuning staphylococcal gene regulation. This manuscript gives an overview of the current knowledge of staphylococcal environmental sensing by TCS and its influence on virulence gene expression and virulence itself. Understanding bacterial gene regulation by TCS can give major insights into staphylococcal pathogenicity and has important implications for knowledge-based drug design and vaccine formulation.

Abbreviations

AMP	Antimicrobial peptide
CAMP	Cationic antimicrobial peptide
CA-MRSA	Community-associated methicillin-resistant *S. aureus*
HA-MRSA	Healthcare-associated methicillin-resistant *S. aureus*
HDP	Host defence cationic antimicrobial peptides

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HK	Histidine kinase
MSSA	Methicillin-sensitive *S. aureus*
PMNs	Polymorphonuclear leucocytes
SSL	*Staphylococcus* superantigen-like
TCS	Two-component signal transduction system
VISA	Vancomycin-intermediate *S. aureus*
VRSA	Vancomycin-resistant *S. aureus*
VSSA	Vancomycin-susceptible *S. aureus*

Contents

1 Introduction

Staphylococcus aureus is a remarkably versatile and resilient organism and is the most commonly isolated human pathogen. *S. aureus* is a commensal that colonizes the host without causing disease; however, it can cause a variety of cutaneous and systemic infections (Iwatsuki et al. 2006; Foster 2009). The capacity of *S. aureus* to cause a diverse spectrum of human diseases reflects its ability to adapt to distinct microenvironments in the human body and suggests that the pathogenesis of *S. aureus* infections is a complex process involving diverse arrays of secreted and

surface-associated virulence determinants that are coordinately expressed at different stages of infection. Bacterial pathogenicity results from a complex interplay with regulatory systems that respond to multiple external signals from the host environment and the bacterial population. *S. aureus* uses these signals to modulate the expression of a large number of genes, including virulence factors, in order to adapt to changing environmental conditions.

"Bacterial survival in the environment is as delicate a balancing act as swordplay on a tightrope, where the slightest misstep is fatal" (Dubrac and Msadek 2008). This quotation accurately describes the struggle that bacteria face in an often constantly changing environment in which they have to continuously adapt. Bacteria need to sense and relay environmental signals, to successfully adapt to environmental changes. One of the most widespread and efficient strategies to do so is two-component signal transduction systems (TCS). The term TCS is somewhat misleading and it is only true when referring to the most basic form of this signal transduction machinery and often auxiliary proteins are involved in signalling. In the aforementioned case, sensing, transducing and transcriptional activation modules are combined in merely two proteins thus coupling an external signal to genetic adaptation. Such a system normally consists of a membrane-bound histidine kinase (HK) (although in rare cases, this kinase can be cytosolic) and a cytosolic response regulator protein required for inducing transcriptional adaptation. Signal acquisition results in the autophosphorylation of the sensor HK at a conserved histidine residue and is then transduced by transfer of the phosphoryl group to an aspartic acid residue of its cognate response regulator. Phosphorylation of the response regulator generally triggers conformational changes thereby modulating the affinity of the effector domain for its target DNA (Casino et al. 2010).

Most *S. aureus* strains encode 16 TCSs with an additional TCS present in the staphylococcal cassette chromosome *mec* of MRSA, which is linked to induction of methicillin resistance (Table 1) (Kuroda et al. 2001). These TCSs are involved in sensing a diverse array of environmental stimuli such as nutrient concentration, cell density, pH, ionic strength and membrane stresses and the amount of TCS present contributes to the ability of *S. aureus* to adapt to the diverse microenvironments it encounters during its life cycle. Several TCS have been shown to be major regulators of virulence gene expression (AgrCA, SaeRS) (Giraudo et al. 1999; Novick and Jiang 2003), antibiotic resistance (VraSR, GraXSR and BraRS) (Gardete et al. 2006; Meehl et al. 2007; Hiron et al. 2011), cell wall metabolism (Martin et al. 1999; Fournier and Hooper 2000; Brunskill and Bayles 1996a), bacterial respiration (Pragman et al. 2004; Fedtke et al. 2002; Kamps et al. 2004; Sun et al. 2012) and nutrient sensing (Stauff et al. 2007).

Even though our understanding of staphylococcal gene regulation by TCS has made significant progress in the last decade, much still remains to be understood. We understand the genes affected by most of the TCS by having studied mutants in the respective sensor kinase, response regulator and auxiliary genes. Nevertheless, we do not know the signal(s) that are sensed and their relevance to bacterial physiology for most *S. aureus* TCS. Furthermore, two of the 16 TCS of *S. aureus* have not been characterized so far as their mutants did not show an evident in vitro

Table 1 TCSs of *S. aureus*

TSC #	TCS	Alternative names	Gene name (N315) RR/HK	Family	Major function	Localization RR/HK[a]
1	WalRK	YycFG, VicRK, MicAB	SA0017/SA0018	OmpR	Bacterial cell envelope composition	C/M
2	TCS2[b]		SA0066/SA0067	OmpR	Kdp-like, potassium transport	C/M
3	TCS3		SA0215/SA0216	AraC	Unknown function	C/M
4	LytRS		SA0251/SA0250	LytTR	Murein hydrolase activity	C/M
5	GraRS	ApsRS	SA0614/SA0615	OmpR	CAMP sensing	C/M
6	SaeRS		SA0661/SA0660	OmpR	Secreted factors mostly involved in immune evasion	C/M
7	TCS7		SA1159/SA1158	LuxR	Specific to staphylococci, unknown function	C/M
8	ArlRS		SA1247/SA1246	OmpR	Adhesion, autolysis, multidrug resistance and virulence genes, *arlR* truncated in N315	C/M
9	SrrAB	SrhSR, ResED	SA1323/SA1322	OmpR	Aerobic and anaerobic respiration	C/M
10	PhoPR		SA1516/SA1515	OmpR	Phosphate assimilation	C/M
11	AirSR	YhcSR	SA1666/SA1667	LuxR	Oxygen sensing	C/C
12	VraSR		SA1700/SA1701	LuxR	Cell wall biosynthesis	C/M
13	AgrAC		SA1844/SA1843	LytTR	Exo- and cell wall protein synthesis, quorum sensing	C/M
14	KdpED		SA1883/SA1882	OmpR	Potassium transport	C/M
15	HssRS		SA2151/SA2152	OmpR	heme sensing	C/M
16	NreCBA		SA2179/SA2180	LuxR	Nitrogen assimilation/oxygen regulatory protein NreC	C/C
17	BraRS	NsaRS, BceRS	SA2418/SA2417	OmpR	Bacitracin efflux/influx/sensing	C/M

[a]C = cytoplasm, M = membrane, *RR* = response regulator, HK = histidine kinase
[b]Not present in all strains

phenotype. Understanding expressional changes, the key regulators and stimuli for the induction of virulence factor expression is paramount to understanding disease development and will help in combatting infections caused by *S. aureus*.

In this article, we will be discussing the current knowledge of *S. aureus* gene regulation by TCS and we will focus on their implication for bacterial virulence as well as for drug and vaccine design.

2 Global Regulators of Virulence Expression

2.1 AgrCA

2.1.1 Molecular Basis of Agr Regulation

The *agr* system is one of the main global gene regulators controlling the expression of *S. aureus* virulence genes (Table 2) and is considered to be one of the best studied models of quorum sensing. Agr was identified as a Tn551 transposon mutant showing reduced expression of, amongst others, haemolysins and increased expression of protein A (Recsei et al. 1986). The *agr* locus encodes two divergent RNA transcripts driven by promoters P2 and P3 encoding the two-component Agr system transcript RNAII and the regulatory RNAIII, respectively (Novick et al. 1993; Morfeldt et al. 1995). RNAII comprises the four genes of the quorum-sensing module *agrBDCA*. AgrB is a transmembrane endopeptidase playing a central role in processing and secreting the AgrD propeptide into the active pheromone called autoinducing peptide (AIP) containing a unique thiolactone ring structure (Fig. 1a) (Novick et al. 1993; Ji et al. 1995; Novick et al. 1995; Novick and Geisinger 2008; Ji et al. 1997; Qiu et al. 2005; Saenz et al. 2000; Zhang et al. 2002). Currently, 4 different types of AIP are known to be produced by *S. aureus.* The strains can be organized into several groups according to their response towards the different AIP, since each of these pheromones will only activate the *agr* response in strains belonging to the same group. Conversely, AIP belonging to one group of *S. aureus* can inhibit activation of the *agr* response in other groups (Ji et al. 1997; Novick and Muir 1999). This specificity can also be derived by sequence analysis. While the N-terminal one third of AgrB and the cytoplasmic, C-terminal HK domain of AgrC are highly conserved, the extracellular sensor domain of AgrC and C-terminal region of AgrB are highly divergent (Novick and Geisinger 2008). It has therefore been suggested that AIPs I to III have evolved in concert with the divergent regions of AgrB and AgrC from a common ancestor (Wright et al. 2005). AIP IV can weakly activate *agr* expression in group I AIP strains and therefore is likely to have developed from group I AIP strains at a later stage during evolution (Wright et al. 2005).

AgrC and AgrA form the sensor kinase and response regulator of a TCS that relays the signal of the quorum-sensing stimulon once an AIP threshold concentration is reached (Fig. 1a) (Ji et al. 1995). AgrC is a dimeric, membrane-bound protein that undergoes transient autophosphorylation of its kinase domain upon AIP binding to its extracellular sensor domain (George Cisar et al. 2009). The phosphate is then transferred to the response regulator AgrA and in vitro phosphorylation

Table 2 Influence on gene regulation of selected TCSs on selected genes

TCS	Positive regulation	Negative regulation	References
AgrCA	*eta, etb, hla, hlb, hld, hlgA/hlgCB, lukED, luk-PV, seb, sec, tst, capH, atl, aur, geh, hysA, nuc, sak, scpA, scpB, splA, splB, splC, splD, splE, splF, sspA, agrA, RNAIII, arlRS, saePQRS*	*spa, ssl5, ssl7, ssl8, ssl9, fnbA, fnbB, coa*	Kato et al. (2011), Sheehan et al. (1992), Arvidson and Tegmark (2001), Giraudo et al. (1996), Morfeldt et al. (1995, Novick and Jiang (2003), Dunman et al. (2001), Bronner et al. (2004), Zhang and Stewart (2000), Recsei et al. (1986), Dassy et al. (1993), Luong et al. (2002), Vandenesch et al. (1991), Benson et al. (2011, 2012), Blevins et al. (2002), Wolz et al. (2000), Ravcheev et al. (2011), Kiedrowski et al. (2011), Olson et al. (2013), Reed et al. (2001), Cheung et al. (1997), Thoendel et al. (2011)
AirRS	*sbi, spa*[a] , *eap*[a] , *efb, fnbA, fnbB, sdrC, sdrD, sdrE, coa, sspA, sspB*	*spa*[a] , *eap*[a] , *agrA, arlRS, hlb*	Sun et al. (2012), Hall et al. (2015)
ArlRS	*sdrC, sdrD, sdrE, rot, agrA, mgrA, capH*	*spa, coa, geh, splA, splB, splC, splD, splE, splF, sspA, isdA, isdB*	Fournier and Hooper (2000), Fournier et al. (2001), Fournier and Klier (2004), Liang et al. (2005), Luong and Lee (2006), Luong et al. (2006), Meier et al. (2007)
BraRS	*spa, isdA, isdB*	*hlb, capH, fnbA, hysA, splA, splB, splC, splD, splE, splF, sspA, sspB*	Kolar et al. (2011)
GraRS	*hlb, hld, hlgA/hlgCB, lukM, lukSF, capH, chp, sbi, clfB, efb, icaABCD, sdrC, sdrD, sdrE, atl, coa, geh, mntABC, agrA, lytSR, mgrA, perR, rot, sarA, sarS, sarX*		Falord et al. (2011), Herbert et al. (2007)
KpdDE	*hlgA/hlgCB, capH, spa, geh*	*aur*	Xue et al. (2011), Freeman et al. (2013), Moscoso et al. (2015)
SaeRS	*eta, etb, hla, hlb, hld, hlgA/hlgCB, lukED, lukM, lukSF, seb, tst, chp, sbi, scn, spa, ssl5, ssl7, ssl8, ssl9, eap, efb, embp, fnbA, fnbB, coa, geh, nuc, sak, splA, splB, splC, splD, splE, splF, isdA, isdB, fur*	*capH, aur*	Giraudo et al. (1994, 1997, 1999), Johnson et al. (2011), Goerke et al. (2005), Mainiero et al. (2010), Rogasch et al. (2006), Ravcheev et al. (2011), Harraghy et al. (2005)

(continued)

Table 2 (continued)

TCS	Positive regulation	Negative regulation	References
WalRK	*hla, hlb, hlgA/hlgCB, lukSF, chp, sbi, scn, eap, efb, embp, fnbA, fnbB, atl, coa, vWfbp, splA, splB, splC, splD, splE, splF, saePQRS*	*spa, sarS, sarT*	Delaune et al. (2011, 2012)

[a]Growth phase dependent

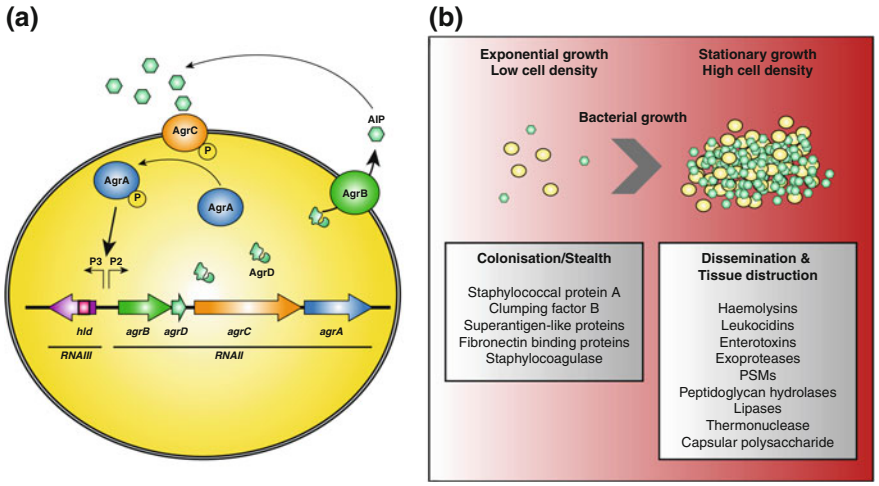

Fig. 1 Regulation of gene expression through the quorum-sensing TCS AgrCA. *S. aureus* employs a quorum-sensing mechanism to trigger expression of a huge number of virulence factors. **a** *S. aureus* synthesises a small, linear peptide encoded in *agrD*. AgrD interacts with the transmembrane endopeptidase AgrB that plays a central role in processing and secreting the AgrD propeptide into the active pheromone called autoinducing peptide (AIP) containing a unique thiolactone ring structure. At low cell densities, the concentration of AIP is not sufficient to activate the sensor histidine kinase AgrC. Once the bacteria reach sufficient numbers and therefore sufficient AIP concentrations, AgrC is activated, autophosphorylates and then transfers its phosphoryl group to the response regulator AgrA. AgrA can then bind to the P2 and P3 promoters of the *agr* locus resulting in a strong induction of its transcription and self-amplification of its signal. Activation of the quorum-signalling cascade results in rapid amplification of RNAIII, which is the main effector of the quorum-sensing response and can either induce or repress expression of virulence factors. The only other target genes known to be directly induced by AgrA are phenol-soluble modulins. **b** At low cell densities, the quorum-sensing signalling is silent and expression of colonization factors is favoured. Once the quorum-sensing response is triggered, rapid RNAIII expression is triggered and colonization factors become repressed. In contrast, dissemination and tissue destruction mechanisms are induced

using acetyl phosphate showed that AgrA phosphorylation triggered conformational changes in the protein resulting in the formation of dimers (Srivastava et al. 2014; Koenig et al. 2004). Phosphorylated AgrA is the main regulator of the *agr*

autoinduction cycle and binds to direct repeats within the *agr* P2 and P3 promoters with high, yet different affinities. AgrA binds more strongly to the P2 promoter than the P3 implying that autoactivation of the P2 operon would precede expression from P3 (Fig. 1a) (Koenig et al. 2004; Cheung et al. 2004). The difference in binding affinities has been attributed to a difference of two nucleotides within the direct repeats of the two promoters and substitution of these nucleotides within the P3 promoter with the nucleotide found in the P2 repeats increased AgrA affinity (Koenig et al. 2004). A bioinformatics search for similar repeat units initially suggested that *agr* was the only target for AgrA-dependent activation (Koenig et al. 2004). However, it was found that AgrA induced the expression of α- and β-phenol-soluble modulins (PSM) by direct interaction with their respective promoters (Peschel and Otto 2013; Queck et al. 2008).

The expression of *agr*is further controlled by binding of additional transcription factors to the P2 promoter. AgrA binds as a dimer to tandem AgrA boxes within the P2 promoter inducing ≈80° bending. SarA binds to the promoter region between the tandem AgrA boxes further bending the DNA and allowing the two bound AgrA dimers to interact. This was proposed to result in the more efficient recruitment of RNA polymerase augmenting P2 expression (Reyes et al. 2011). SarR can bind to the same site as SarA, but with higher affinity. In contrast to SarA, SarR does not induce DNA bending and therefore would result in less efficient recruitment of RNA polymerase to the P2 promoter and thus result in downregulation of transcription from P2. Given that P3 transcriptional activation appears to be solely dependent on AgrA, it would follow that SarA and SarR could contribute to modulating P3 expression via their role in P2 promoter expression and thus *agrA* transcription (Reyes et al. 2011).

Apart from *agr* autoactivation, various environmental stimuli such as glucose and pH changes are known to affect *agr* expression (Regassa et al. 1992; Regassa and Betley 1992). The DEAD-box-protein CshA (an RNA helicase) was recently shown to affect *agrA* mRNA stability by targeting of this RNA to a proposed RNA degradosome (Oun et al. 2013). It was recently shown by RNA sequencing and microarray analysis, that CshA was involved in the degradation of bulk mRNA and that a subset of mRNAs was significantly stabilized in the absence of *cshA* (Giraud et al. 2015). The RNAIII-activating protein (RAP) and its target TRAP have been implicated in the regulation and transcriptional activation of *agrBDCA*. Analogous to AIP, RAP was proposed to accumulate in a cell density-dependent manner and once its threshold was reached to activate TRAP via histidine phosphorylation. TRAP would then positively affect cell adhesion and induce the *agr* locus, a process possibly involving the staphylococcal virulence regulator (Svr) (Yarwood and Schlievert 2003; Balaban et al. 2001; Bronner et al. 2004; Gov et al. 2004; Korem et al. 2003). However, more recent studies suggest that neither TRAP nor SvrA is involved in regulation of *agr* but rather that TRAP acted by protecting DNA from oxidative stress, thereby preventing spontaneous and adaptive mutations (Kiran and Balaban 2009; Chen and Novick 2007).

The main effector of the *agr* stimulon is the 517 nucleotide RNAIII transcribed from the *agr* P3 promoter (Novick et al. 1993; Novick and Geisinger 2008; Boisset

et al. 2007; Vandenesch et al. 1991). Its interaction with target mRNAs controls the switch between colonization and invasion via changes in expression of surface proteins, excreted toxins and proteases (Fig. 1). RNAIII expression depends on AgrA as it is not expressed in an *agrA* mutant and depending on growth phase and strain background its half-life can range from 11 to >45 min (Novick and Geisinger 2008; Janzon et al. 1989). Transcription of RNAII increases throughout growth usually reaching highest expression in post-exponential phase (Vandenesch et al. 1991). The RNAIII transcript contains a short open reading frame encoding the 26 amino acid haemolysin, Hld, yet deletion of *hld* did not result in an agr^- phenotype (Janzon and Arvidson 1990; Novick et al. 1993). Introduction of the first 188 nucleotides of the RNAIII transcript prior to the open reading frame of *hld* on a plasmid was sufficient to restore an agr^- strain to an agr^+ phenotype, whereas introduction of a RNAII-containing plasmid did not complement the agr^- phenotype (Novick et al. 1993).

RNAIII consists of 14 stem-loop structures and two long helices formed by long-rage base pairings that segregate independent structural domains (Benito et al. 2000). Different domains are involved in the regulation of specific targets either at translational or transcriptional levels. The 5′ end of RNAIII competes with the intramolecular secondary RNA structure blocking the ribosomal binding site (RBS) of the *hla* transcript, thereby facilitating its translation (Novick et al. 1993; Morfeldt et al. 1995). In contrast, the two 3′ hairpins H13 and H14 and the central hairpin H7 act as repressors for the expression of early virulence factors such as staphylococcal protein A (*spa*) (Huntzinger et al. 2005), coagulase (*coa*) (Chevalier et al. 2010) or the repressor of toxins (*rot*) (Boisset et al. 2007), by binding in an antisense manner to the respective mRNAs and inducing RNaseIII-dependent mRNA degradation [for a more detailed review on regulatory RNAs refer to (Felden et al. 2011)].

2.1.2 Agr and Virulence Regulation

The *agr* quorum-sensing system is one of the key regulators of staphylococcal virulence and impacts the expression levels of more than 100 genes. In fact, Dunman et al. (2001) showed by microarray analysis that *agr* appeared to upregulate the expression of 104 and downregulate the expression of 34 genes in a cell density-dependent manner (Table 2). Agr controls the progression of *S. aureus* from a colonizing to an invasive phenotype by inducing the expression of secreted virulence factors while reducing the expression of cell surface proteins involved in adhesion and aggregation (Yarwood and Schlievert 2003; Novick and Geisinger 2008; Bronner et al. 2004; Singh and Ray 2014; Batzilla et al. 2006; Recsei et al. 1986). *S. aureus* produces capsular polysaccharide as a means to evade immune response during bacteraemia by preventing opsonophagocytosis (Nanra et al. 2012). Capsular polysaccharide biosynthesis is positively regulated by *agr* (Dassy et al. 1993; Luong et al. 2002; van Wamel et al. 2002; Nanra et al. 2012), yet strains have been isolated in the clinic that were deficient in capsule production and

concomitantly were found to not produce *agr* (Fischer et al. 2014), suggesting that capsule expression is not required for prolonged *S. aureus* infections and loss of expression might be an adaptation for persistence in the host (Tuchscherr et al. 2010). In addition to capsule biosynthesis, the expression of secreted proteins and toxins (*i.e.* lipases, protease, nucleases, hyalouronate lyase, PSMs, α, β, γ and δ haemolysins, leukocidin, toxic shock syndrome toxins and exfoliative toxins) is upregulated by *agr* (Yarwood and Schlievert 2003; Novick et al. 1993, 1995; Ji et al. 1995; Dassy et al. 1993; Luong et al. 2002; van Wamel et al. 2002; Recsei et al. 1986; Dunman et al. 2001). In contrast, expression of protein A and fibronectin binding proteins is repressed (Fig. 1b).

It has been reported that between 15 and 60 % of clinical isolates (depending on the study) from HA *S. aureus* infections appear to be *agr* negative and that they are associated with persistent bacteraemia and poorer patient outcomes. This association does not appear to stem from alterations in virulence or infectivity per se but is rather the result of hospital-related risk factors such as increased risk of bloodstream infections after surgical interventions and antibiotic selective pressure (Park et al. 2013; Paulander et al. 2013; Smyth et al. 2012). In fact, hospitalized patients carrying agr^+ or agr^- *S. aureus* strains in their nares are just as likely to develop bloodstream infections (Smyth et al. 2012; Painter et al. 2014). The Agr system induces the expression of a large number of virulence factors primarily involved in host invasion, thus resulting in a large metabolic burden to the bacterium. Hospitalized patients are often immunocompromised or have undergone invasive surgery, which render them more at risk to *S. aureus* infection. Expression of invasive factors such as toxins becomes thus less crucial to the bacterium for establishing infection and a trade-off between metabolic burden and the expression of *agr*-induced virulence factors would favour the selection of agr^- strains within hospitals. Acquisition of methicillin resistance cassettes also appears to influence *agr* expression levels. In particular, strains expressing high levels of penicillin-binding protein 2a (PBP2a) show lower expression of *agr* and deletion of PBP2a or the resistance cassette encoding this protein (*mecA* and type II SCCmec cassette, respectively) restored *agr* activity (Pozzi et al. 2012; Rudkin et al. 2012).

Differences in *agr* regulation have also been observed between isolates from HA and CA *S. aureus* infections. While expression of *agr* did not differ between the HA- and CA strains tested, regulation of genes downstream in the signalling cascade diverged significantly (Cheung et al. 2011). Overall, expression of secreted, degradative enzymes, PSMs and Panton-Valentine leukocidin was higher in the CA strain LAC, while genes that were downregulated in the HA strain 252 maintained their expression levels in strain LAC (Cheung et al. 2011). Interestingly, this study also showed that the generally accepted negative effect of *agr* on the expression of surface proteins might be considerably affected by the strain background. In particular, fibrinogen-binding protein genes *clfA* and *efb* were positively affected by *agr* and not repressed as predicted (Cheung et al. 2011).

The *agr* quorum-sensing system is a model for signal transduction and one of the major regulators of staphylococcal virulence gene expression. Even though the TCS has been studied extensively and our understanding in how it contributes to

different *S. aureus* disease manifestations has increased significantly, many questions remain to be addressed. Why does the *agr* TCS seem to be detrimental to the bacterium within a hospital environment? Are the immunocompromised nature of and/or infection facilitating wounds of hospital/community patients the sole factors driving the observed fitness burden associated with the Agr TCS?

2.2 SaeRS

2.2.1 Molecular Basis of SaeRS

The staphylococcal accessory element (*sae*) TCS system is a key regulator of many secreted *S. aureus* toxins, exoenzymes and immunomodulatory proteins know to be important for *S. aureus* pathogenesis (Table 2). The *sae* locus was identified as a Tn551 insertional mutant that showed alterations in supernatant exoprotein production but was phenotypically distinct from other regulatory mutants (Giraudo et al. 1994). Similar to the *agr* locus, the *sae* locus was later confirmed to primarily regulate gene expression at the transcriptional level (Giraudo et al. 1999). The locus encodes a two-component signal transduction system containing four genes. SaeR and SaeS are the response regulator and HK of the system, respectively (Giraudo et al. 1999), while the other two gene products, SaeP and SaeQ, form a protein complex with SaeS to regulate the sensor kinase phosphatase activity (Jeong et al. 2012). Thus, SaeP and SaeQ are involved in dephosphorylating activated SarR, thereby affecting expression levels of SarR-induced genes (Jeong et al. 2012). Two promoters drive the expression of the four genes in the *sae* locus. The response regulator *saeR* and the HK *saeS* are transcribed at a fairly constitutive rate from the P3 promoter located within the *saeQ* coding sequence, while the P1 promoter upstream of *saeP* has a 2–30 times higher activity relative to the P3 promoter and can drive transcription of all four genes of the locus (Fig. 2). The P1 promoter furthermore contains two SaeR binding sites resulting in its autoinduction by the SaeRS TCS (Geiger et al. 2008; Nygaard et al. 2010; Sun et al. 2010). Transcription from solely promoter P3 is sufficient for target gene activation (Jeong et al. 2011), and two classes of target genes have been described. Class I targets such as *coa*, *sae* P1 promoter, *fnbA* and *eap* require high levels of phosphorylated SaeR for activation, while class II targets such as *hla* and *hlb* only necessitated lower basal phosphorylation levels of SaeR for activation (Fig. 2) (Mainiero et al. 2010). This then implies that the level of phosphorylated SaeR affinity varies between SaeR targets. The repressor of toxins Rot and SigB have been shown to negatively regulate expression from the *sae* P1 promoter, thus indirectly repressing activation of *hla* expression (Li and Cheung 2008; Goerke et al. 2005).

In contrast to the HK of the *agr* system, SaeS contains only two transmembrane domains that are separated by 9 extracellular amino acid residues (Adhikari and Novick 2008), which initially were thought to be too few to bind a ligand (Mascher 2006). SaeS has therefore been classified as an intramembrane-sensing HK relying

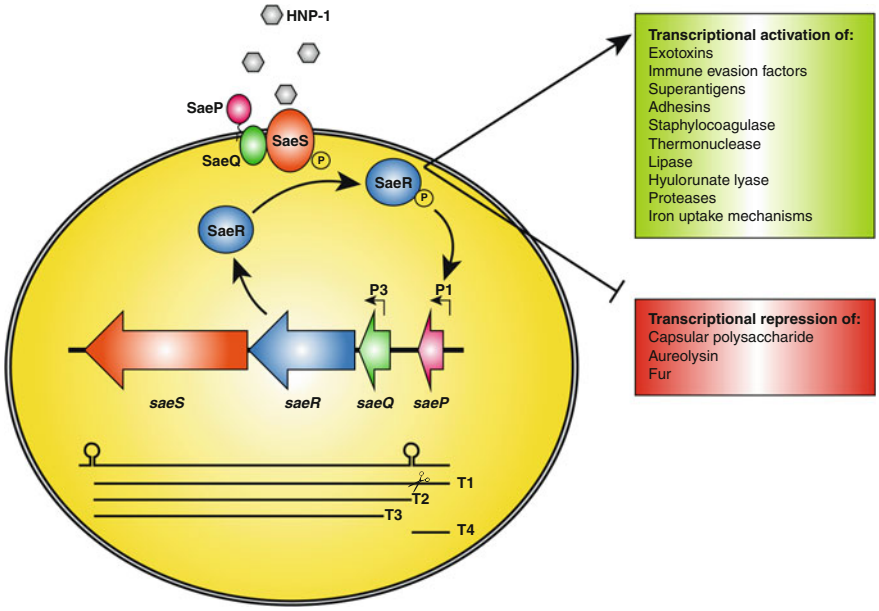

Fig. 2 Regulation of gene expression through the staphylococcal accessory element TCS SaeRS. SaeRS is an autoregulated TCS system and its expression can be triggered by host stimuli such as the human neutrophil peptide 1. Two promoters drive expression of the *sae* locus. While the response regulator SaeR and the histidine kinase SaeS are transcribed at a fairly constitutive rate from P3 located within *saeQ*, P1 contains two SaeR binding sites and is inducible upon signal sensing. Induction of P1 results in 2–30 times higher *sae* expression than from P3 alone. The T1 transcript can be further processed by RNaseY resulting in a total of 4 transcripts from the locus. SaeP and SaeQ form a protein complex with SaeS to activate the sensor kinase's phosphatase activity and are thus involved in dephosphorylating activated SarR. SaeRS activates transcription of exotoxins, immune evasion factors, several proteases and lipases and downregulates expression of capsule, aureolysin and the ferric uptake regulator, Fur

on an additional protein to transduce the signal to SaeS. Activation of the SaeRS TCS depends on external stimuli resulting in membrane perturbation and was shown to be repressed by low pH and high NaCl concentrations, while it was induced in the presence of H_2O_2 and α-defensins (Geiger et al. 2012). A more recent study investigated the mechanism by which SaeS was able to specifically detect α-defensin 1 (HNP-1) and human polymorphonuclear leucocytes (PMNs) (Fig. 2). The study showed that the substitution of M31A in the extracellular 9 amino acid loop was sufficient to abolish Sae-dependent gene activation, while two aromatic anchors of the loop were required for basal SaeS signalling (Flack et al. 2014). Interestingly, SaeS in *S. aureus* strain Newman contains the amino acids substitution L18P within the first transmembrane domain resulting in constitutive kinase activity and thus high-level expression of SaeRS target genes (Schafer et al. 2009; Adhikari and Novick 2008; Mainiero et al. 2010).

The *sae* regulatory circuit seems to be influenced by other global regulatory mechanism such as *agr* and the ferric iron uptake regulator Fur. Johnson et al. (Johnson et al. 2011) showed that both Sae and Fur were required for the induction of *eap* and *emp* as well as for the full induction of oxidative stress response and expression of non-covalently bound surface proteins during iron starvation. Thus, it is possible that genes that were seen to be regulated by Fur using microarray analysis but were lacking a conserved Fur binding motif were influenced by its effect on the transcription of global regulators such as Sae (Johnson et al. 2011). A link between Sae and Fur seems plausible as iron availability is one of the major environmental signals for *S. aureus* to sense host exposure and thus subjecting the regulation of exoprotein and immune evasion factor production by Sae under the control of Fur could contribute to its virulence and/or energy conservation.

2.2.2 SaeRS and Virulence Regulation

Several virulence genes are under the control of the SaeRS TCS (Table 2), and additional regulatory roles have been attributed to the TCS in vivo. The *sae* locus is essential for the transcription of *hla*, *hlb* and *coa* (Giraudo et al. 1997), and *sae* mutant strains show significantly reduced *hla* transcript levels under both in vitro and in vivo conditions (Fig. 2) (Goerke et al. 2001). In the guinea pig model used to study implant device-originated infections, *hla* was found to be activated and secreted in an *sae*-dependent but *agr*- and *sarA*-independent manner suggesting an important role for the SaeRS for in vivo regulation of *hla* expression. Likewise, *hla* expression was considerably lower in an *sae* mutant relative to the wild-type strain when tested in a rabbit endocarditis model (Xiong et al. 2006). SaeRS positively influences the expression levels of a variety of genes involved in immune evasion and adhesion to host molecules such as *seb*, *efb*, *eap*, *lukF*, *lukM*, *hlgACB*, *chp, scn*, *sspA*, *fnbA* and *fnbB* (Fig. 2) (Rogasch et al. 2006; Harraghy et al. 2005; Rooijakkers et al. 2006; Kuroda et al. 2007). The Sae TCS acts together with Rot to positively regulate the expression of *Staphylococcus* superantigen-like (SSL) proteins in *S. aureus* strain Newman, while *ssl gene* expression was repressed by *agr* (Benson et al. 2012; Pantrangi et al. 2010). These Ssls have several immunomodulatory properties such as inhibition of complement activation and neutrophil recruitment, as well as blocking opsonization by IgG and IgA. Benson et al. (2012) speculated that Rot could be involved in the recruitment of SaeR to the *ssl* promoters, resulting in RNA polymerase recruitment and activation of gene expression. It is interesting to note that strain background differences have been shown to alter regulatory behaviours of *ssl* induction. For instance, in strain RN6390 Rot appeared to act as a repressor of *ssl8* expression (Pantrangi et al. 2015). While Sae generally facilitates the expression of immunomodulatory components, it represses the expression of capsular polysaccharides (Rogasch et al. 2006; Mainiero et al. 2010; Thakker et al. 1998).

Affecting the expression of secreted and immunomodulatory proteins, it is not surprising that the SaeRS TCS also plays an important role in *S. aureus* virulence

and survival during infection. Mutants in *sae* have been found to show a significantly reduced virulence in a *Caenorhabditis elegans* model of staphylococcal infection (Bae et al. 2004). Compared to wild-type *S. aureus* strains, *sae* mutants resulted in lower mortality in intraperitoneal mouse infection models (Rampone et al. 1996; Giraudo et al. 1996), while in murine models of systemic infection or abscess formation, fewer bacteria were recovered from spleens or abscess lesions infected with the *sae* mutant strain (Benton et al. 2004). An *sae* deletion mutant showed a reduced ability to adhere to lung epithelial cells and showed decreased levels of staphylococcal induced apoptosis (Liang et al. 2006).

Sae signalling is crucial in activating *S. aureus* responses to PMNs, by inducing the transcription of toxins of the leukocidin family involved in PMN-lysis (Dumont et al. 2011; Voyich et al. 2005, 2009). Spent media supernatants of wild-type *S. aureus* were shown to induce membrane permeabilization of 85 % of PMN, while *sae*-defective strains were unable to induce significant membrane permeabilization (Flack et al. 2014). In particular, the extracellular loop amino acid M31 appeared to be essential for the activation of the *sae* stimulon by either HNP-1 or PMNs, while mutants in amino acids W32 and F33 showed reduced expression of *sae*-controlled genes in vitro but were still able to respond once exposed to PMNs (Flack et al. 2014). This therefore led to the conclusion that, while certain key residues in the extracellular loop of SaeS were important for *sae*-dependent gene expression, stimulation by host-derived signals, amongst others H_2O_2 and α-defensins, was essential to trigger virulence in *S. aureus* (Geiger et al. 2008, 2012; Flack et al. 2014). Calprotectin, a major component of neutrophils, sequesters the divalent cations Mn^{2+} and Zn^{2+}, thus limiting staphylococcal growth due to nutrient limitation. Excess of Zn^{2+}, Fe^{2+} and Cu^{2+} was shown to reduce the expression of *saeQ* (Cho et al. 2015). The absence of iron was demonstrated to induce the SaeRS system via Fur (Johnson et al. 2011), yet Cho et al. (Cho et al. 2015) did not observe an upregulation of *saeQ* in their *fur* mutant strain. Zn^{2+}-bound calprotectin in the presence of excess Zn^{2+}, however, was seen to induce SaeRS-dependent gene expression resulting in increases proinflammatory immune response and mouse mortality (Cho et al. 2015). A USA300 *sae*-deficient mutant was found to be unable to cause dermonecrotic lesions in a mouse model for skin and soft tissue infections (Zhao et al. 2015). Following a proteomic analysis of the antibody-repertoire of mice exposed to either the wild-type strain or its *sae* mutant, the authors concluded that a broad polyclonal immune response was triggered in both strains but that no antibodies were observed for several SaeRS-dependent virulence factors in the *sae* mutant. Therefore, lack of protective immunity was associated with the lack of antibodies targeting proteins of the SaeRS-regulon (Zhao et al. 2015).

The SaeRS TCS is also involved in the formation of biofilms. *S. aureus* strain Newman is incapable of robust biofilm formation due to an amino acid substitution, which leaves the SaeS HK constitutively active (Schafer et al. 2009). Biofilm formation could be restored by replacing the Newman $saeS^P$ allele with the $saeS^L$ allele found in other strains or by deleting *saeRS* (Cue et al. 2015). Conversely, biofilm formation could be inhibited by introducing the $saeS^P$ allele into the biofilm-proficient strain USA300 FPR3757 (Cue et al. 2015). Culture supernatants

of strain Newman were able to inhibit biofilm formation, while Newman supernatants carrying the *saeS*^{L} allele no longer prevented this phenotype. Conversely, supernatants of FPR3757 containing the *saeS*^{P} allele were unable to inhibit biofilm formation (Cue et al. 2015). This phenotype could be partially attributed to increased levels of the staphylococcal thermonuclease, Nuc, in Newman supernatants, yet it remains unclear as to why supernatants from FPR3757 containing the *saeS*^{P} allele were unable to prevent biofilm formation (Cue et al. 2015). Elucidation of the full role of SaeRS in biofilm formation will require further investigation.

3 Response to AMPs and Cell Wall Damage

Antimicrobial peptides (AMPs) are ribosomally synthesized natural antibiotics produced by nearly all organisms, from bacteria to plants and animals. The largest group of AMPs is that of the cysteine-containing peptides that can be both anionic and cationic and are stabilized by disulphide bonds. They are complemented by proline-, arginine-, histidine-, phenylalanine-, glycine- and tryptophane-rich AMPs that are mostly cationic. AMPs interact with microbial membranes, resulting in two possible modes of action, depending on the peptide and the microbial species. The peptides can be membrane-disruptive resulting in cell lysis, or alternatively membrane interaction can lead to the formation of transient pores and the transport of peptides inside the cell, bringing them into contact with intracellular targets (Maroti et al. 2011). AMPs are part of the host's innate immune system and constitute a generic defence response of animals and plants to fend off invading microbes. AMPs in animals are produced by epithelial cells, which come in direct contact with the environment, but they can also be secreted into circulating fluids (e.g. the bloodstream or the haemolymph) which deliver AMPs to infection sites. Many bacteria can sense AMPs directly using TCS such as the *Salmonella* PhoPQ sensor which can recognize cathelicidin LL-37 (Maroti et al. 2011). *S. aureus* employs a total of three TCS that are involved in its response to AMP exposure (Fig. 3). In this section, we are giving an overview of these TCS systems, their regulation and their function in *S. aureus* survival and downstream gene regulation.

3.1 VraSR

The vancomycin-resistance-associated sensor/regulator (VraSR) TCS was first identified to be upregulated in hetero-vancomycin-resistant and vancomycin-resistant *S. aureus* (VRSA) compared to vancomycin-susceptible (VSSA) strains (Kuroda et al. 2000). VraS is the sensor HK, while VraR is its response regulator (Fig. 3). The VraS HK consists of an N-terminal transmembrane domain and a C-terminal conserved HK core. The N-terminal transmembrane domain of VraS consists of two membrane-spanning regions that are connected

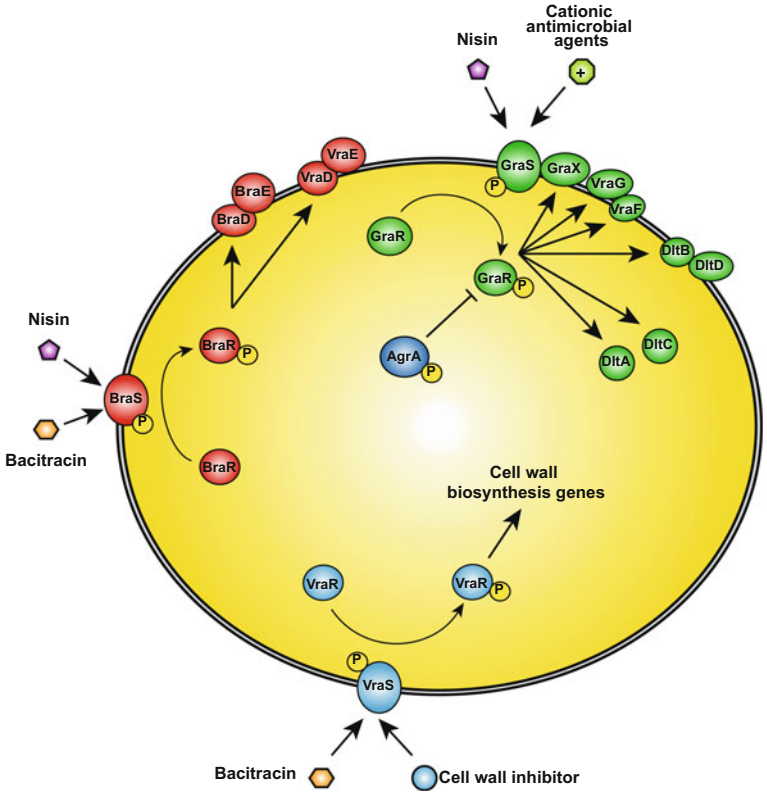

Fig. 3 *S. aureus* response to antimicrobial agents. The histidine kinase VraS can sense bacitracin and respond to general cell wall perturbation by cell wall inhibitory compounds. Upon activation, VraS phosphorylates its response regulator VraR and triggers changes in the expression of cell wall biosynthesis genes. The GraXSR TCS is involved in the *S. aureus* response to glycol and cationic peptides. GraXSR regulates the expression of the adjacent VraFG pump, which plays an essential role in the signalling cascade by sensing the presence of CAMPs and signalling through GraS to activate GraR-dependent transcription. GraXRS does not regulate its own expression but affects the expression of major regulators of virulence gene expression, colonization factors and exotoxin-encoding genes as well as the *ica* and *dlt* operons. Disruption of GraXRS signalling results in major changes to the bacterial cell surface such as D-alanylation of teichoic acids. BraRS activates the transcription of two operons, *braDE* and *vraDE*, both encoding ABC transporters. Induction of *braDE* and *vraDE* in the presence of bacitracin or nisin is dependent only on BraRS and not on VraSR or GraXSR. BraDE is involved in bacitracin sensing and signalling through BraSR, whereas VraDE acts specifically as a detoxification module and is sufficient to confer bacitracin and nisin resistance when produced on its own

through a periplasmic linker. This domain varies widely amongst HKs (Mascher 2006). The conserved HK core of VraS contains the dimerization domain, which harbours the conserved His residue, and the ATP-binding domain.

Overexpression of VraR in the VSSA strain N315 reduced vancomycin susceptibility. The VraSR system is induced by exposure of *S. aureus* cell to

cell-wall-affecting antibiotics such as glycopeptides, β-lactams, bacitracin, D-cycloserine and cationic antimicrobial peptides (CAMPs); mutants in *vraSR* are significantly more susceptible to these antibiotics (Levinger et al. 2012; Kuroda et al. 2000; Gardete et al. 2006; Su et al. 2015; Pietiainen et al. 2009). Northern blot analysis determined that *vraS* and *vraR* are cotranscribed showing two specific transcripts of 2.7-kb and 3.0-kb (Kuroda et al. 2003; Yin et al. 2006). The *vraSR* operon itself consists of only 1.7 kb and was indeed transcribed together with the two upstream open reading frames [*orf1* and *vraT* (*yvqF*)], resulting in the 2.7-kb and 3.0-kb mRNA fragments (Yin et al. 2006). Microarray analysis comparing the transcriptional response of the N315 wild-type strain and its isogenic *vraSR* mutant after vancomycin treatment revealed that out of a total of 139 vancomycin-inducible genes in the wild-type strain 46 were not induced to the same extent in the *vraSR* mutant (Kuroda et al. 2003). Several of the *vraSR*-specific genes, such as *pbp2*, *sgtB*, UDP-N-acetylglucosamine 1-carboxylvinyl transferase 2 murein monomer precursor synthesis (*murZ*), methicillin-resistance-related protein (*fmtA*) and teicoplanin-resistance-related proteins (*tcaA*/*tcaB*) were associated with cell-wall peptidoglycan synthesis. The VraSR system therefore appears to coordinate the important steps of cell wall biosynthesis: MurZ and polymerization of peptidoglycan (Fig. 3) (Pbp2 and SgtB). This profound effect on cell wall biogenesis correlates with the observed increase in susceptibility of the *vraSR* mutant to cell-wall-affecting antibiotics (Kuroda et al. 2003). VraSR also plays a central role in controlling methicillin resistance of *S. aureus* by means other than the penicillin-binding proteins (PBP) (Boyle-Vavra et al. 2006). Methicillin resistance in *S. aureus* is mediated by the acquired PBP (PBP2a), which is encoded by *mecA*. Together with the native PBP2 protein, PBP2a mediates oxacillin/methicillin resistance via their respective transglycosylase and transpeptidase activities. Even though expression of *pbp2* was unaltered and *mecA* expression increased in the *vraSR* mutant relative to the wild-type strain, the mutant was found to be more sensitive to oxacillin. This suggested that *vraSR* exerts a regulatory effect on an alternate target, thus accounting for the susceptibility of the mutant (Boyle-Vavra et al. 2006). Induction of the VraSR TCS was connected to general perturbations in cell wall biogenesis by placing the essential cell-wall synthesis gene *pbpB* under the control of an inducible promoter, thus generating changes to the *S. aureus* cell wall (Gardete et al. 2006). Changes in the expression levels of *pbpB* were rapidly mirrored by *vraSR* signalling followed by changes in the *vraSR* regulon. It was therefore suggested that VraSR acts as a sentinel system capable of sensing and responding to perturbations of cell wall biosynthesis and triggering an appropriate response (Gardete et al. 2006).

The *vraSR* operon is cotranscribed with the two upstream genes *orf1* and *vraT*. Interestingly, many clinical strains show an association with mutations in either *vraSR* or *vraT* and increased resistance to cell-wall-damaging antibiotics (Kato et al. 2010). This therefore indicates that *vraT* plays a role in resistance development and might influence *vraSR* regulation. McCallum et al. (2011) confirmed the role of *vraT* in activating *vraSR* signalling by constructing markerless deletions of each of the four genes in the operon. This study showed that *orf1* played no observable role

on resistance phenotypes for any of the cell envelope stress-inducing agents tested. The remaining three genes were all essential for the induction of the cell wall stress stimulon, and mutants showed various degrees of increased susceptibility to cell-wall-active antibiotics. In fact, bacterial two-hybrid analysis suggested that the integral membrane protein VraT interacted directly with VraS but not VraR, suggesting that it could be involved in sensing the trigger of VraSR signalling (McCallum et al. 2011).

VraS is capable of undergoing autophosphorylation of a histidine residue (His156) in vitro and its phosphoryl group is rapidly transferred to an aspartic acid residue (D55) of VraR. In addition, phosphorylated VraR undergoes rapid dephosphorylation by VraS (Belcheva and Golemi-Kotra 2008; Galbusera et al. 2011). Only once VraR is phosphorylated can it form an active dimer able to bind DNA (Belcheva and Golemi-Kotra 2008). Structural analysis of VraR revealed that unphosphorylated VraR exists in a closed conformation that inhibits dimer formation. Phosphorylation at the active site promotes conformational changes that are propagated throughout the receiver domain, promoting the opening of a hydrophobic pocket that is essential for homodimer formation and enhanced DNA-binding activity (Leonard et al. 2013). A recent study identified that VraR can be phosphorylated at four distinct threonine residues by Stk1, a staphylococcal eukaryotic-type serine/threonine kinase (Canova et al. 2014). These phosphorylations were shown to negatively affect VraR DNA-binding capacity and therefore led to dysregulation of VraSR signalling (Canova et al. 2014).

The role of VraSR in staphylococcal response to cell-wall-damaging/cell-wall-acting antibiotics has important implications as to its development as a potential drug target. Inhibiting VraSR signalling might result in increased or restored effectiveness of now ineffective antibiotics such as methicillin in infections with MRSA strains. Using a murine model of *S. aureus* necrotizing pneumonia, a wild-type MRSA strain and its isogenic *vraSR* deletion mutant were compared. Oxacillin treatment significantly improved survival and reduced the bacterial burden in *vraSR* mutant-infected mice, while no difference was observed in mice infected with the wild-type strain (Jo et al. 2011). Similarly, in a murine skin infection model oxacillin treatment eliminated the development of dermonecrosis amongst *vraSR* mutant-infected mice and decreased the bacterial burden within lesions, oxacillin treatment did not affect the wild-type strain (Jo et al. 2011).

Taken together, the VraSR TCS is a major regulator of the staphylococcal response to cell-wall-damaging or cell-wall-perturbing agents. Stimulation of VraSR signalling results in the expression of cell-wall biosynthesis enzymes leading to the thickening of the bacterial cell wall and to increased resistance to cell-wall-acting antibiotics. VraSR is therefore of key interest for developing drug targets inhibiting the stimulation of cell wall biogenesis and thereby rendering *S. aureus* susceptible to cell-wall-targeted antibiotics.

3.2 GraXSR

The glycopeptide-resistance-associated (GraXRS) (also known as antimicrobial peptide sensor ApsRS) five-component system is one of the three (GraXRS, VraSR and NsaRS) main regulatory pathways in staphylococci for controlling CAMP resistance (Fig. 3) (Falord et al. 2011; Mensa et al. 2014). The GraXSR system was identified from a microarray transcriptomic screen comparing the expression levels of *S. aureus* strains showing different vancomycin resistance. The *graS* gene was found to be expressed to a higher degree in strains showing increased resistance to the antibiotic and overexpression of *graS* in a vancomycin-sensitive strain increased its resistance to the drug (Cui et al. 2005).

The *graXRS* genes are located immediately upstream of the ABC transporter genes *vraF* and *vraG*, which had already previously been found to be expressed more in vancomycin-intermediate *S. aureus* (VISA) and were identified as associated with vancomycin resistance (Kuroda et al. 2000). The *graXRS* genes encode one of four TCS system loci that are in proximity to ABC transporter genes. Interestingly, this close relationship between TCS and ABC transporters was only observed in firmicutes (Meehl et al. 2007). The GraXRS system shows high similarity to the BceRS TCS system of *Bacillus subtilis*. Similar to its *S. aureus* homolog, the *bceRS* genes are located immediately upstream of the *bceAB* genes, an ABC transporter system, and control their expression by sensing extracellular bacitracin, affecting bacitracin susceptibility in *B. subtilis* (Ohki et al. 2003). The CAMP-response mechanism mediated by GraSR depends on the activity of VraFG, encoded by the *vraFG* operon (Fig. 3). This operon is under the direct regulation of GraR. In fact, the ABC transporter is considered to be a/the sensor for CAMPs. Notably, GraR does not regulate its own promoter (*graXSR*) (Falord et al. 2012) and expression levels of *graR* are not altered by cell-wall-active antibiotics (Falord et al. 2011).

The association of the GraXRS TCS and an ABC transport system in *S. aureus* were also shown to be involved in the resistance of the bacterium to CAMPs and antibiotics by regulating the expression of the adjacent VraFG pump (Cui et al. 2005; Li et al. 2007; Meehl et al. 2007). Mutants in either *graXRS* or *vraFG* are more sensitive to vancomycin, CAMPs such as the human lysozyme-derived peptide $_{107}$R-A-W-V-A-W-R-N-R$_{115}$ (LP9), polymyxin B, colistin or gallidermin suggesting that the VraFG transporter might play a role in the detoxification of AMPs (Herbert et al. 2007; Meehl et al. 2007; Falord et al. 2011). However, the VraFG transporter does not act as a detoxification module, as it cannot confer resistance when produced on its own, but instead plays an essential role by sensing the presence of CAMPs and signalling through GraS to activate GraR-dependent transcription (Fig. 3) (Falord et al. 2012).

Unlike similar HKs, GraS lacks autophosphorylation activity, and the interaction between GraS and GraR appears to be very weak in comparison to the stronger interaction observed between BceS and its conjugated response regulator, BceR. Muzamal et al. (2014) therefore suggested that that CAMP signalling may not flow

directly from GraS to GraR. Bacterial two-hybrid assays confirmed that the GraS kinase and both GraX and the VraG permease interacted (Falord et al. 2012). The auxiliary protein GraX interacts with VraF and GraR and requires the histidine phosphotransfer and dimerization domain of GraS for this interaction. Further, VraF requires the GraS region that connects the membrane-bound domain with the cytoplasmic domain of VraF for interaction with GraS. The interactions of GraX with GraRS and VraF indicate that GraX may serve as a scaffold to bring these proteins in close proximity to GraS and to facilitate the activation of the GraRS signalling cascade (Fig. 3) (Muzamal et al. 2014).

Like VraR, GraR is a target of the Stk1 kinase. Phosphorylation of GraR by Stk1 depends entirely on its intact tertiary structure and occurs at the N-terminus of its DNA-binding domain. Unlike Stk1-dependent phosphorylation of VraR, phosphorylation of GraR results in increased DNA-binding activity and therefore increased activation of GraR-regulated genes (Fridman et al. 2013).

GraXRS does not regulated its own expression (Falord et al. 2011) but affects the expression levels of 248 genes, some of which are major regulators of virulence gene expression, colonization factors and exotoxin-encoding genes as well as the *ica* and *dlt* operons (Fig. 3, Table 2) (Herbert et al. 2007; Meehl et al. 2007; Falord et al. 2011). Decreased expression of the later operons in the *graRS* mutant could also account for its biofilm-deficient phenotype (Boles et al. 2010). A highly conserved ten-base-pair palindromic sequence (5′ ACAAA TTTGT 3′) located upstream from GraR-regulated genes (*mprF*, *dlt* and *vraFG* operons) was shown to be essential for transcriptional regulation and induction by GraR in response to CAMPs, suggesting that this could be a likely GraR binding site (Falord et al. 2011).

GraRS positively regulates the expression of *rot, sarS* and *mgrA* (Table 2), thus GraRS may have an indirect role in regulating staphylococcal virulence (Herbert et al. 2007). Disruption of GraXRS signalling results in major changes to the bacterial cell surface, including D-alanylation of teichoic acids, the incorporation of lysylphosphatidylglycerol into the bacterial membrane and a concomitant increase in lysine biosynthesis, and the induction of VraFG transporter expression (Li et al. 2007; Meehl et al. 2007). Changes in cell surface charge are thought to be one of the key mechanisms in *S. aureus* CAMP resistance (Cheung et al. 2014). Interestingly, the GraXRS response in *S. aureus* was induced by a more restricted set of AMPs than in *Staphylococcus epidermidis.* This dissimilarity was due to structural differences in the AMP binding on the highly negatively charged loop of the GraS sensor protein (Li et al. 2007). Cheung et al. (2014) later showed that the extracellular loop of the HK was important for antimicrobial peptide recognition and GraR signalling.

The GraXRS TCS plays an important role in the development of VRSA strains as a single point mutation in either *graS* or *graR* can make a previously sensitive strain resistant to the antibiotic (Howden et al. 2008; Neoh et al. 2008). These mutations only raised the vancomycin resistance level of the strain between levels observed for susceptible and VISA isolates. Therefore, these data showed that the TCS *graRS* is a key mediator of this resistance and that low-level resistance could

be acquired by a single point mutation (Howden et al. 2008; Neoh et al. 2008). Intriguingly, stepwise evolution of vancomycin resistance from VSSA to VISA was observed after the mutated *vraS* and *graR* genes of the VISA Mu50 were introduced into the VSSA Mu50Ω, suggesting that a combination of two point mutations was sufficient to create a VISA strain (Cui et al. 2009).

The observation that *graRS* mutants were more susceptible to AMPs prompted researchers to investigate whether this alteration could influence staphylococcal survival within the host. Deletion of *graRS* considerably altered bacterial surface charge, increased susceptibility to killing by human neutrophils or the defence peptide LL-37 and attenuated bacterial virulence in mouse infection models (Kraus et al. 2008; Li et al. 2007). In a silkworm larvae model of pathogenicity, which is used to identify mutants that are altered in their ability to survive when interacting with the host's innate immune response, a mutant in the *graXRS* genes was shown to be less virulent than its isogenic parent strain (Kurokawa et al. 2007). A *graS* deletion mutant and mutants in the cationic peptide sensing loop of the kinase were significantly attenuated in a rabbit invasive endocarditis model of infection (Cheung et al. 2014).

3.3 *BraRS*

The BraRS (bacitracin resistance associated) TCS system, also known as NsaSR (nisin susceptibility associated) or BceSR TCS, was independently identified by various groups as being essential for *S. aureus* resistance to bacitracin and nisin (Hiron et al. 2011; Yoshida et al. 2011; Blake et al. 2011; Matsuo et al. 2010; Kawada-Matsuo et al. 2011; Kolar et al. 2011). The BraSR and GraSR TCSs share some common features: they both have an intramembrane sensor kinase family and control the synthesis of closely related ABC transporters (VraDE and VraFG), and they are both essential for nisin resistance (Hiron et al. 2011). BraRS is one of three TCS (GraSR and VraSR being the other two) associated with the staphylococcal response to antimicrobial agents. The two genes encoding the TCS, *braR* and *braS*, are located immediately upstream (107 bp) of genes encoding the ABC transporter BraDE (NsaDE), which is also involved in resistance. The *braRS* genes are cotranscribed with a small gene 25-bp upstream of the two genes, while *braD* and *braE* are transcribed as a separate operon (Hiron et al. 2011). In the presence of low antibiotic concentrations, BraRS activates the transcription of two operons, *braDE* and *vraDE*, both encoding ABC transporters (Fig. 3). Various mutants showed that the induction of *braDE* and *vraDE* in the presence of bacitracin or nisin is dependent only on BraRS and not on VraSR or GraSR. Translational fusions of the *braRS* promoter to *lacZ* revealed that expression levels of *braRS* in the presence or absence of bacitracin did not change and was therefore not autoregulated (Hiron et al. 2011; Yoshida et al. 2011; Kolar et al. 2011).

A highly conserved 14-base-pair imperfect palindromic motif (CTTTCAA NN T/CTGTAAG) upstream from the *braDE* and *vraDE* genes is essential for

BraSR-mediated transcriptional initiation, suggesting the likely BraR binding site (Hiron et al. 2011). Interestingly, the two ABC transporters play distinct and original roles in antibiotic resistance: BraDE is involved in bacitracin sensing and signalling through BraSR, whereas VraDE acts specifically as a detoxification module and is sufficient to confer bacitracin and nisin resistance when produced on its own (Hiron et al. 2011; Kawada-Matsuo et al. 2011). The transcription of 245 genes, including genes involved in transport, drug resistance, cell envelope synthesis, transcriptional regulation, amino acid metabolism and virulence, is altered in a *braS* mutant (Table 2) (Kolar et al. 2011).

Two distinct pathways contribute to bacitracin resistance in *S. aureus*: the highly efficient, sensitive and specific BraSR/BraDE/VraDE multicomponent system and the less effective damage-sensing VraSR system which responds to general envelope stress conditions (Hiron et al. 2011; Yoshida et al. 2011).

Despite the extensive initial studies on BraRS and its role in conferring antimicrobial resistance to *S. aureus,* several questions still remain. Firstly, it remains to be determined how the BraDE ABC transporter senses bacitracin. The *B. subtilis* BraE homolog BceB contains a large extracellular loop, which has been suggested to be essential for bacitracin detection (Rietkotter et al. 2008; Coumes-Florens et al. 2011). Conversely, replacing the extracellular loop of BraE with that of VraE prevented bacitracin sensing by the chimeric BraDE*VraE ABC transporter (Hiron et al. 2011). Furthermore, the mode of interaction between BraDE and BraSR remains unclear and it remains to be determined if conformational changes in the ABC transporter upon bacitracin binding or transport can promote such an interaction. It is also unclear how the VraDE ABC transporter confers bacitracin resistance and the direction of bacitracin transport by VraDE remains unknown.

4 Cell Wall Metabolism, Autolysis and Cell Death

Bacterial growth and replication requires the exquisite control not only of its DNA replication machinery but also of its cell wall biosynthesis and remodelling. The *S. aureus* replication cycle necessitates the synthesis and remodelling of its peptidoglycan layer while maintaining turgor pressure in order to retain its coccal shape (Monteiro et al. 2015). The bacterium divides asynchronously by placing a septum and then expressing a defined set of peptidoglycan hydrolases in order to initiate separation of daughter and mother cell. After initial hydrolysis of the peripheral septum ring, turgor pressure rapidly realizes mechanical separation of the two cells resulting in a 61/39 % ratio of maternal (old) to newly formed peptidoglycan cell wall. In order to prevent maternal cell wall thinning and perforation by hydrolase activity, *S. aureus* undergoes an extensive remodelling of its entire peptidoglycan surface by the concerted action of penicillin-binding protein 4 and several cell wall hydrolases (Monteiro et al. 2015). This machinery needs to be tightly controlled as excess hydrolase activity could easily result in cell death, while excess

peptidoglycan biosynthesis could negatively affect the viability and fitness of the bacterium. *S. aureus* employs three TCS to regulate cell wall metabolism and one of these plays a central and crucial role as master regulator.

4.1 WalRK

The WalRK TCS (also known as YycFG, VicRK and MicAB TCS) is the only essential signal transduction system required for *S. aureus* growth. This TCS is very highly conserved and appears to be specific to low-G+C Gram-positive bacteria. WalK constitutes the system's HK, while WalR is its cognate response regulator. The first insights into its functional role were gained when a conditionally lethal mutant was isolated from a screen for temperature-sensitive *S. aureus* mutants and was unable to grow on agar plates at 43 °C (Martin et al. 1999; Dubrac and Msadek 2004). This *walR* mutation caused hypersensitivity to macrolides and unsaturated long-chain fatty acids. Furthermore, the conditional lethal phenotype could be rescued by increasing the osmolarity of the growth medium by the addition of NaCl or sucrose, which indicated a potential role of the TCS in the proper regulation of bacterial cell wall or membrane composition (Martin et al. 1999). The *walKR* genes in *B. subtilis* (*yycFG*) are expressed during exponential growth and rapidly shut off at entry into stationary phase, suggesting that this system is active, and also necessary, during active replication (Fabret and Hoch 1998). In *B. subtilis*, YycFH activity is regulated by the gene products encoded immediately downstream of *yycG*, *yycH* and *yycI*. Deletion of *yycH* or *yycI* in *B. subtilis* gives identical phenotypes, with a 10-fold upregulation of the YycFG system and YycG, YycH, and YycI have been shown to form a ternary complex. Both YycH and YycI localize to the periplasm and are anchored to the membrane via a single N-terminal transmembrane helix. Therefore, YycH and YycI appear to act together to negatively regulate the YycG HK (Szurmant et al. 2005, 2006, 2007).

WalR binds to a consensus recognition sequence consisting of two hexanucleotide direct repeats, separated by five nucleotides [5′-TGTWAH-N_5-TGTWAH-3′]. The motif was found upstream of 13 *S. aureus* genes of which five are involved in virulence and binding of WalR to the promoters of *ssaA*, *isaA* and *lytM* (Dubrac and Msadek 2004). WalRK positively controls autolytic activity, in particular that of the two major *S. aureus* autolysins, Atl and LytM. The transcription of 13 genes involved in cell wall metabolism and degradation was activated through the WalRK TCS and reduction of WalRK levels in the bacterial cell resulted in increased resistance to Triton X-100 and lysostaphin-induced cell lysis (Fig. 4, Table 2) (Delaune et al. 2011; Dubrac et al. 2007). Conversely, lowered levels of WalRK lead to a significant decrease in peptidoglycan biosynthesis, turnover and to cell wall modifications, which included increased peptidoglycan crosslinking and glycan chain length (Dubrac et al. 2007). The reduction in cell-wall-degrading proteins in the WalRK-depleted strain could also explain why these bacteria die but do not lyse. Of note, *walRK* expression levels in an inducible

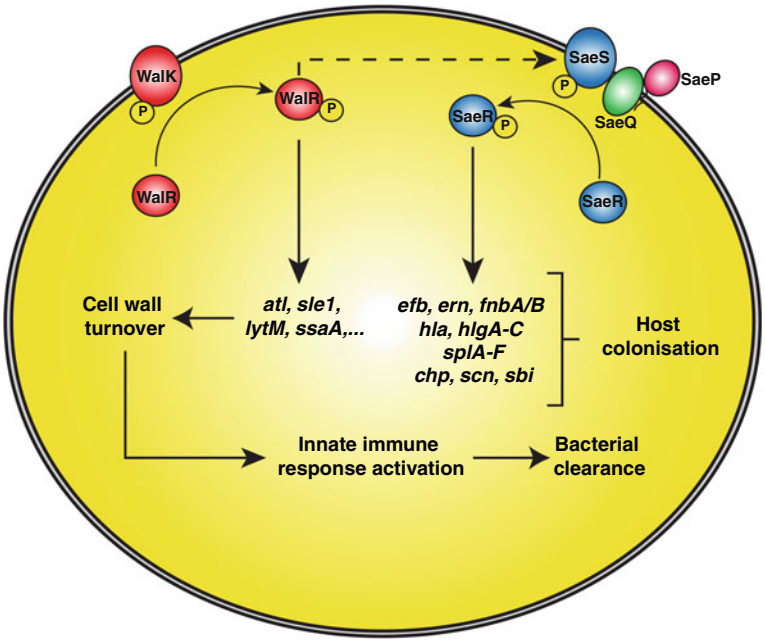

Fig. 4 Impact of the WalKR two-component system (TCS) on *S. aureus* virulence. The WalKR TCS is activated through phosphorylation of the WalR response regulator by the WalK histidine kinase, leading to increased expression of several genes involved in cell wall degradation and turnover. Higher release rates of cell wall degradation products after infection can then trigger the host innate immune response through activation of the NF–κB pathway, resulting in more efficient bacterial clearance and decreased virulence. Increased WalKR activity also leads to stimulation of the SaeSR TCS (*dotted arrow*), higher expression of virulence genes involved in host–pathogen interactions and innate immune system evasion. Fine-tuning of WalKR activity must therefore play an important role in the switch between *S. aureus* commensal and pathogenic lifestyles

strain positively correlated with the strains capacity for biofilm formation, suggesting that the regulatory role of WalRK on cell wall turnover and autolysis positively affects biofilm formation (Dubrac et al. 2007).

For more than a decade, it was unclear why the WalRK TCS was essential for bacterial growth, since none of the genes that are regulated by the WalRK TCS are lethal per se. Transmission electron micrographs showed cell wall thickening and aberrant division septa in a WalRK mutant, suggesting its requirement may be linked to its role in coordinating cell wall metabolism and cell division (Delaune et al. 2011). By uncoupling expression of WalRK-regulated genes involved in cell wall metabolism from direct WalRK regulation and overexpressing them on a plasmid, two proteins (LytM and SsaA) were shown to be able to restore cell viability in the absence of WalRK. LytM is a glycyl–glycyl endopeptidase, hydrolysing the pentaglycine interpeptide crossbridge, and SsaA belongs to the

CHAP amidase family, members of which such as LysK and LytA have been shown to have D-alanyl-glycyl endopeptidase activity, cleaving between the crossbridge and the stem peptide. These data added further support that peptidoglycan crosslinking relaxation through crossbridge hydrolysis plays a crucial role in the essential requirement of the WalRK TCS for cell viability (Delaune et al. 2011).

The observed thickening of the *S. aureus* cell wall in the WalRK-depleted strain already indicated that this TCS might play a role in the development of vancomycin resistance phenotypes, as cell wall thickening is a common characteristic of vancomycin resistant strains. As such it was not at all surprising that WalRK mutations were identified between VSSA and VISA strains. Single-nucleotide substitutions within either WalK or WalR lead to coresistance to vancomycin and daptomycin and caused the typical cell wall thickening observed in resistant clinical isolates (Howden et al. 2011; Shoji et al. 2011; McEvoy et al. 2013; Hu et al. 2015). This is yet another example as to how easy it is for *S. aureus* to develop vancomycin resistance by simply introducing single point mutations into the genes of a TCS (Sect. 3). Combinations of single-nucleotide mutations in these TCS and other genes might further augment resistance.

The essential role of the WalKR TCS and its restriction to low-G+C Gram-positive bacteria proffered the development of specific drugs inhibiting WalKR signalling. WalK autophosphorylation in *S. aureus* and *B. subtilis* can be inhibited by aranorosinol B, a known antibacterial agent against Gram-positive bacteria, and other structure-based inhibitor design approaches have identified further molecules inhibiting WalK/YycG in several members of the firmicutes family (Watanabe et al. 2003; Qin et al. 2006, Qin et al. 2007).

When gene expression levels of a constitutively active WalR mutant strain and the strain expressing the wild-type allele were compared, 24 major virulence genes were found to be upregulated by WalRK (Delaune et al. 2012). These genes encoded for host matrix interaction and degradation proteins (*efb*, *emp*, *fnbA*, *fnbB* and *spl* operon), cytolytic toxins (*hlgACB*, *hla* and *hlb*) and innate immune response evasion molecules (*scn*, *chp* and *sbi*) (Fig. 4). Interestingly, the WalRK system does not effectuate this upregulation directly but rather by altering/inducing the expression of the SaeRS TCS, which is one of the major regulatory hubs of virulence factor expression (Delaune et al. 2012) (refer to Sect. 2). WalR does not act directly on *saeRS* to regulate their expression but appears to be acting through the Sae signal transduction pathway, as there are no conserved binding sites in the *sae* operon promoter region and activation of *saeP* expression was also lost in the Δ*saeRS* mutant (Fig. 4) (Delaune et al. 2012).

Given the profound impact on one of the major *S. aureus* virulence regulator systems, it was not surprising that producing constitutively active WalR strongly diminished bacterial virulence using a murine infection model. This was largely caused by the early triggering of the host inflammatory response associated with higher levels of released peptidoglycan fragments resulting in increased neutrophil recruitment and proinflammatory cytokine production leading to enhanced bacterial clearance (Delaune et al. 2012).

4.2 ArlRS

The *arlRS* locus (autolysis-related locus) constitutes a TCS regulatory system with the HK ArlS and the response regulator ArlR, showing strong similarity to the response regulator of the PhoB-OmpR family (Fournier et al. 2000). The locus was first identified from a Tn*917*LTV1 transposon library screen for mutants with altered expression of the gene encoding for the multidrug efflux pump, *norA* (Fournier et al. 2000; Fournier and Hooper 2000). The locus itself is composed of two overlapping open reading frames that are transcribed as two major mRNA transcripts of 1.5 and 2.7 kbp from the strain RN6390, but several smaller transcripts have been reported in other strains. The environmental signal involved in activating the ArlRS TCS remains unclear, although it has been shown to be involved in the regulation of DNA supercoiling in response to high osmotic culture conditions (Fournier and Klier 2004).

Initial reports showed that an *arlS* mutant exhibited dramatic autolysis as a result of increased peptidoglycan hydrolase activity and formed biofilms on polystyrene surfaces (Fournier and Hooper 2000). The increased capacity of *arlRS*-deficient mutants to form a biofilm was corroborated in a later study, showing that *arlRS* mutants demonstrated increased initial attachment as well as increased accumulation of poly-N-acetylglucosamine (PNAG) (Toledo-Arana et al. 2005). Accumulation of PNAG was shown to be non-essential for this phenotype, while addition of a mutation in the staphylococcal accessory regulator *sarA* abolished and introduction of an *agr* mutation augmented it (Toledo-Arana et al. 2005). Microarray analysis confirmed that ArlR positively regulates the expression of the *lytSR* TCS involved in autolysis as well as *lrgA* and *lrgB*, two genes encoding holing-like proteins involved in murein hydrolase transport and inhibition, respectively (Liang et al. 2005). However, inactivation of *arlRS* does not play a role in autolysis of methicillin-resistant *S. aureus* (MRSA) strains, such as community-associated (CA-) MRSA strains USA300 and MW2 or the healthcare-associated (HA-)MRSA strain COL (Memmi et al. 2012). These differences were not related to the presence or absence of the methicillin resistance determinant *mecA*. In both CA- and HA-MRSA strains *arlRS* represses *lytN*, while expression of *atl*, *lytM* and *lytH* remained unaltered. The *agr* transcripts, RNAII and RNAIII, were significantly more downregulated in the *arlRS* mutant of MW2 than in the MSSA strain Newman. Therefore, autolysis regulation mediated by the ArlRS TCS seems to be regulated/affected differently in MSSA and MRSA strains (Memmi et al. 2012).

Inactivation of *arlR* and *arlS* resulted in different expression patterns of extracellular proteins and increased production of Spa, Hla and SspA (Table 2). The ArlRS TCS modifies the synthesis of virulence factors by altering the levels of RNA II and RNA III transcription from the *agr* locus and transcription of *sarA* from the *sar* locus (Fournier et al. 2001). Conversely, expression of the *arl* locus is growth phase dependent increasing slightly from exponential to post-exponential phase. Unlike the *sae* and *agr* loci, the *arl* locus is not autoregulated as its transcription was unaltered in *arlR* and *arlS* mutants. Agr and SarA activate the

transcription of *arl,* as inactivation of *sarA* and *agrA* decreased expression of the *arl* locus. Indeed, the effect of ArlRS on the expression of virulence factors is indirect and a consequence of ArlRS's impact on the transcription of regulatory factors such as Agr, SarA, SigmaB and SarR as well (Fournier et al. 2001). Transcription of *ArlRS* is also induced by NorG, a GntR-like protein that has been shown to affect *S. aureus* genes involved in resistance to quinolones and β-lactams, such as those encoding the NorB and AbcA transporters (Truong-Bolduc et al. 2011). The regulatory effects of ArlRS on *spa* expression have also been attributed to a role of ArlRS in DNA supercoiling. High osmolarity of the culture medium increases DNA supercoiling in an ArlRS-dependent manner, thus making it less accessible to RNA polymerases and decreasing *spa* expression. In the absence of *arlRS*, the effect of DNA supercoiling modulators on *spa* expression was decreased, suggesting that active Arl proteins are necessary for the full effect of DNA gyrase inhibitors (Fournier and Klier 2004; Schroder et al. 2014). Capsule biosynthesis is affected indirectly by the ArlRS TCS as it activates the transcription of the transcriptional regulator *mgrA*, which in turn activates capsule biosynthesis (Luong and Lee 2006).

Recently, ArlRS has been shown to be essential for agglutination of *S. aureus* in the presence of human plasma or fibrinogen. The effect was mediated by the upregulation of *ebh* expression in an *arlRS* mutant. Agglutination could be restored in the *arlRS* mutant by deleting *ebh*. Expression of *ehb* from a constitutive promoter also prevented agglutination (Walker et al. 2013). This alteration in *S. aureus* agglutination also translates into a pathogenic phenotype. In a rabbit model of sepsis and endocarditis, the *arlRS* mutant displayed a large defect in vegetation formation and pathogenesis. This phenotype was partially restored by removing *ebh* (Walker et al. 2013).

Considering the substantial impact of the ArlRS TCS on the expression of virulence factors and regulators in vitro and in vivo, it is not surprising that *arlRS*-deficient strains were also attenuated in several other virulence models. Fewer *arl*-deficient mutant bacteria were recovered in two mouse models of systemic infection and abscess formation (Benton et al. 2004) and in a murine model of hematogenous pyelonephritis (Liang et al. 2005). Furthermore, the *arlR* and *arlS* mutants were significantly attenuated in a silk worm model of infection (Miyazaki et al. 2012).

Taken together the ArlRS, TCS plays a significant role in *S. aureus* virulence gene regulation by affecting the expression of several key virulence factors either by directly modifying their transcription or indirectly by altering the levels of key global virulence regulators. There is a strong strain dependency in the observed phenotypes for *arlRS* mutants and the precise mechanisms of regulation and the signals to which this system responds will require further elucidation.

4.3 LytSR

The LytSR TCS was identified as being involved in autolysis of *S. aureus*. An insertional mutant in *lytS* formed more aggregates, showed altered cell surface

morphology and an increased murein hydrolase activity (Brunskill and Bayles 1996a). LytS is the TCS's sensor HK and contains six N-terminal transmembrane domains, while LytR is the system's cognate response regulator (Brunskill and Bayles 1996b). The *lytS* and *lytR* genes are separated by 5 nucleotides and are cotranscribed resulting in an approximately 2.5-kb mRNA transcript (Brunskill and Bayles 1996a). The transcriptional start site of *lytSR* is located 25 bp upstream of the *lytS* start codon and the promoter contains a putative σ^A-like consensus sequence (Brunskill and Bayles 1996a). LytS is autophosphorylated at His390, while Asn394 is important for its phosphatase activity. LytS then phosphorylates LytR at a conserved aspartate residue, upon which the regulators ability to bind to the target promoter(s) is enhanced resulting in transcription of the downstream gene (Lehman et al. 2015). Interestingly, LytR can also be phosphorylated independently of LytS during planktonic growth with acetyl phosphate acting as phosphor donor (Lehman et al. 2015). LytSR positively regulates the expression of two genes immediately downstream, *lrgA* and *lrgB*. LrgA shares characteristics with the bacteriophage-encoded holin proteins involved in murein hydrolase transport (Brunskill and Bayles 1996b). Mutants in *lrgAB* had increased extracellular murein hydrolase activity compared to the wild-type strain and were more resistant to penicillin-induced lysis (Groicher et al. 2000). Murein hydrolases catalyse the cleavage of specific structural components of the bacterial cell wall. β-lactam antibiotics block the enlargement process of the bacterial cell wall and the bacteria are unable to grow if these autolysins are inhibited. Increased expression/activity of murein hydrolases could thus render strains penicillin-tolerant (Bronner et al. 2004). LytSR appears to be controlling *lrgAB* expression by monitoring changes in the proton motif force associated with the cytoplasmic membrane. Patton et al. (2006) further proposed a model in which the LytSR regulatory system responds to a collapse in membrane potential by inducing the transcription of the *lrgAB* operon. The presence of six potential transmembrane domains in LytS was suggested to be ideally suited to sense membrane-associated signals such as membrane potential. Loss of membrane potential could result in the autophosphorylation of LytS, which in turn phosphorylates LytR. LytR could then bind to the promoter region and induce *lrgAB* transcription. It has been speculated that LytSR signalling is required for assessing the overall health of the bacterial cell, and ultimately in making the commitment to cell death.

The LytSR TCS system plays an important role in the development of *S. aureus* biofilms. This role can be mostly attributed to its effects on the regulation of *lrgAB* and thus autolysis of the bacteria leading to the release of genomic DNA, which then becomes an important structural component of the biofilm matrix. As a consequence, a *lytS* mutant was found to form a more adherent biofilm than the wild-type and complemented strains and showed an increase in matrix-associated DNA.

Transcriptomic analysis of a *lytS* mutant compared to its wild-type strain showed that a total of 460 genes were downregulated at least 2-fold, while only 7 genes were expressed at least 2-fold higher. Most of the genes downregulated in the *lytS* mutation were involved in carbohydrate, energy, or nucleotide metabolism as well as replication, transcription and translation (Sharma-Kuinkel et al. 2009).

Sensing of changes in the transmembrane potential is of particular interest when considering *S. aureus's* interactions with its host. Many host defence cationic AMPs (CAMPs) perturb the staphylococcal cell membrane and alter transmembrane potential as a core function of their lethality. The *S. aureus* LytSR TCRS has been proposed to serve as a membrane potential sensor (Patton et al. 2006). A *lytS* mutant strain displayed significantly increased in vitro susceptibilities to an ample range of CAMPs including neutrophil-derived human neutrophil peptide 1, platelet-derived thrombin-induced platelet microbicidal proteins, its mimetic peptide RP-1, as well as to calcium-daptomycin (Yang et al. 2013). Of note, an *lrgAB* mutant was unaltered in its susceptibility to cationic peptides. In contrast to cationic peptide resistance conferred by GraRS which is achieved by altering bacterial surface charge, the *lytS* mutant did not display similar changes. These data were further corroborated in an endocarditis model during daptomycin treatment. In this case, the *lytS* mutant strain but not the *lrgAB* mutant was significantly attenuated compared to the wild-type strain (Yang et al. 2013).

5 Respiration, Fermentation and Nitrate Metabolism

S. aureus is a facultative anaerobe that can grow without oxygen using either anaerobic respiration with nitrate as the terminal electron acceptor or by fermenting carbohydrates. The bacterium colonizes various niches that have different oxygen availability. In particular during deeper infections (*i.e.* in abscesses), oxygen can rapidly become a limiting resource requiring the bacterium to switch from aerobic respiration to an anaerobic form of energy generation (Throup et al. 2001). While aerobic growth is generally favourable in terms of resources spent for a certain amount of energy obtained, the huge diversity of environments that *S. aureus* inhabits exposes the bacterium to conditions that do not permit it. *S. aureus* employs three TCS that respond to environmental oxygen levels in order to fine-tune respiratory activity and divert energy fluxes into different metabolic pathways.

5.1 SrrAB

The SrrAB (staphylococcus respirator response) TCS (also known as SrhSR TCS) shows significant similarity to the *B. subtilis* ResDE TCS, which controls the induction of genes required for anaerobic growth in this organism. SrrA is the cytoplasmic response regulator and SrrB the membrane-bound HK of the signal transduction system (Pragman et al. 2004). *srrAB* is transcribed from a single transcriptional start site resulting in either a 762-bp *srrA* or a 1752-bp *srrAB* transcript. The N-terminal domain of SrrA appears to be crucial for the regulatory effect of SrrA under oxygen limitation as natural strains lacking portions of the SrrA N-terminus (with and without phosphorylation domain) were no longer able to

repress the expression of several virulence factors (Pragman et al. 2007a). ArcR, a member of the Crp/Fnr family of bacterial transcriptional regulators, binds to the upstream regions of the genes encoding the two-component systems SrrAB and WalKR. The *arcR* gene encoding ArcR forms an operon with the arginine deiminase pathway genes *arcABDC*. This operon allows *S. aureus* to utilize arginine as a source of energy for growth under anaerobic conditions (Makhlin et al. 2007).

SrrAB positively regulates fermentative enzymes such as lactate dehydrogenase and alcohol dehydrogenase and seems to be a negative regulator of some TCA cycle enzymes (Throup et al. 2001). SrrA binds to the *agr*, *spa*, *tst, srr* and *icaR* promoters and represses their transcription, particularly under low-oxygen conditions (Pragman et al. 2004; Yarwood et al. 2001). Conversely it appears to be enhancing the levels of *tst*, *spa*, and *icaR* under aerobic conditions (Pragman et al. 2007b). Overexpression of SrrAB resulted in decreased expression of these virulence factors and in reduced virulence in a rabbit model of bacterial endocarditis (Pragman et al. 2004). SrrAB-deficient strains were found to be attenuated in a mouse pyelonephritis infection model (Throup et al. 2001), a *C. elegans* model of virulence (Bae et al. 2004) and a murine sepsis model (Richardson et al. 2006). A transposon mutagenesis screen for mutants inhibited in their biofilm forming capacity identified the SrrAB TCS as being involved in biofilm formation. In contrast to all other mutants identified in this screen, the *srrA* mutant produced more of the polysaccharide intercellular adhesin (PIA) but showed less biofilm formation (Tu Quoc et al. 2007). Since biofilm communities are thought to contain anaerobic microenvironments, the authors proposed that the *srrA* mutant was unable to develop biofilms because of a defect linked to oxygen sensing rather than PIA production. SrrAB is important for *S. aureus* survival in neutrophils and is a major activator of *ica* expression and PIA production in anaerobic environments, where it contributes to the protection of *S. aureus* against non-oxidative defence mechanisms (Ulrich et al. 2007).

A recent study determined that SrrA was the predominant transcriptional activator of *plc* encoding a phosphatidylinositol (PI)-specific phospholipase C (PI-PLC) capable of hydrolysing PI and cleaving glycosyl-PI (GPI)-linked proteins from cell surfaces (White et al. 2014). Regulation of *plc* in vitro and in vivo was linked to oxidative stress in an SrrAB-dependent manner. Even though a *plc* mutant in a CA-MRSA USA300 background exhibited a survival defect in human whole blood and in isolated neutrophils, it displayed no survival defect in murine models of infection or murine whole blood (White et al. 2014). These observations indicate once again the inherent problems of using model systems for studying the role of virulence factors (Salgado-Pabon and Schlievert 2014) and could suggest that Plc plays a role in human staphylococcal infection, while it is not essential in the murine model used.

SrrAB has been shown to be involved in the regulation of many genes induced by nitric oxide (NO•) and inactivation of *srrAB* resulted in heightened NO· sensitivity (Richardson et al. 2006). Although the environmental signal for SrrAB

activation has not been fully confirmed yet, Fuchs et al. (2007) suggested that the oxygen concentration per se might not be crucial for the regulation of genes involved in fermentation processes. Instead, the reduced state of component(s) of the respiratory chain, the membrane potential, and/or the increased level of NADH might be a signal for anaerobic gene regulation in *S. aureus*. Some regulatory roles might also be attributed to the interaction of the SrrAB regulon with other TCS and regulators. The *nreCBA* operon encodes a TCS which is involved in the regulation of nitrate/nitrite reduction in *S. aureus* in response to oxygen levels (Kamps et al. 2004), while a third thus far uncharacterized TCS (SACOL0202/SACOL0203) was induced under low-oxygen conditions (Fuchs et al. 2007). An example of this coregulation is *ldh1* whose expression is controlled by the oxygen responsive regulators SrrAB, NreB, and the redox regulator, Rex. Rex senses NAD+/NADH balance and is upregulated by CcpA in the presence of a carbohydrate carbon source (Chaffin et al. 2012). A more recent study showed that *S. aureus* can both sense and respond to NO• stress and hypoxia via SrrAB (Kinkel et al. 2013). This role is of key importance as *S. aureus* lacks the NO•-sensing transcriptional regulator NsrR, which is used in many bacteria to sense NO• stress. The SrrAB regulon comprises genes required for cytochrome biosynthesis and assembly (*qoxABCD*, *cydAB*, *hemABCX*), anaerobic metabolism (*pflAB*, *adhE*, *nrdDG*), iron–sulphur cluster repair (*scdA*), and NO· detoxification (*hmp*). Kinkel et al. (2013) showed by using targeted mutations in SrrAB-regulated loci that *hmp* and *qoxABCD* were required for NO· resistance, whereas *nrdDG* was specifically required for anaerobic growth. This is in contrast to NsrR which directly senses NO• SrrAB activation by hypoxia, NO·, or a *qoxABCD* quinol oxidase mutation suggesting that the SrrAB TCS senses impaired electron flow in the electron transport chain rather than directly interacting with NO· (Kinkel et al. 2013).

In 37 % of analysed *S. aureus* genomes, nitrosative stress resistance can also be aided by the presence of *nor*, encoding a predicted quinol-type nitric oxide reductase. Expression of *nor* was upregulated during low-oxygen growth in an SrrAB-dependent manner (Lewis et al. 2015). The authors proposed that Nor contributes to NO-dependent respiration during nitrosative stress by receiving its electrons directly from reduced quinones in the electron transport chain when catalysing NO reduction. Because *S. aureus* only uses menaquinone in the respiratory chain, Nor could potentially contribute to metabolic adaptation to nitrosative stress by both directly oxidising the quinone pool (and thereby allowing increased flow of electrons through the respiratory chain) and by utilizing NO. as an alternative electron acceptor (Lewis et al. 2015).

Taken together, SrrAB contributes to *S. aureus* virulence by interacting both directly with virulence genes and regulators and by fine-tuning the bacterial metabolism for aerobic and anaerobic respiration. With oxygen availability being one of the main characteristics in different host niches, the SrrAB TCS takes a central role in *S. aureus* virulence regulation.

5.2 *NreCBA*

Nitrate and nitrite metabolism in many bacteria involves the Fnr protein (fumarate and nitrate reductase). Fnr specifically binds to target DNA sites as a dimer containing one $[4Fe–4S]^{2+}$ iron sulphur (FeS) cluster coordinated by four cysteine residues. Upon exposure to air, Fnr is inactivated and the $[4Fe–4S]^{2+}$ disassembled, resulting in Fnr monomers that do not bind target DNA (Fedtke et al. 2002). An Fnr protein as described in other bacteria is neither encoded in the *Staphylococcus carnosus* nor in the *S. aureus* genome (Fedtke et al. 2002). The NreCB (nitrogen regulation) TCS was first described in *S. carnosus* when a mutant in the *nreCBA* operon was shown to be significantly affected in nitrate and nitrite reduction as well as in its growth (Fedtke et al. 2002). Transcription of genes involved in nitrate and nitrite metabolism and transport (*narT*, *narGHJI* and the nitrite reductase (*nir*) operon) was severely reduced in the *nreCBA* mutant strain even when cells where cultured under aerobic or anaerobic conditions in the absence of nitrate or nitrite (Fedtke et al. 2002). Interestingly, transcription of the *nreCBA* operon was not altered under these conditions. NreC is phosphorylated by NreB and phospho-NreC specifically binds to a GC-rich palindromic sequence (5′-TAGGGN_4CCCTA-3′) to enhance transcription initiation (Fedtke et al. 2002).

NreB is different to other TCS sensors as it is not membrane bound but found in the cytosol. Similar to Fnr, NreB contains four N-terminal cysteine residues, which in conjunction with iron was essential for NreB function (Kamps et al. 2004; Mullner et al. 2008). Isolated NreB could be activated by incubation with cysteine desulphurase, ferrous iron and cysteine, a characteristic typical for FeS-containing proteins such as Fnr. Activation was reversible by oxygen suggesting that the regulatory activities of NreB involved direct sensing of oxygen concentrations (Kamps et al. 2004). Reinhardt et al. (2010) confirmed that in aerobically grown *S. carnosus* the apo-form of NreB (Fe–S-less) is the predominant form of NreB present, while in anaerobically grown bacteria NreB containing a coordinated $[4Fe\text{-}4S]^{2+}$-cluster is predominant. In the absence of oxygen, the autokinase of NreB is activated, and the phosphoryl group is transferred to the response regulator NreC. Unlike FNR, NreB does not act directly as transcriptional activator but instead transfers the phosphoryl group to the response regulator NreC (Kamps et al. 2004).

Comparative DNA microarray and proteomic analyses between the *S. aureus* wild-type and *nreCBA* mutant strains under anoxic conditions in the absence and presence of nitrate showed no direct influence of the NreCBA system on the expression of staphylococcal virulence factors, yet genes and proteins involved in nitrate and nitrate reduction were significantly reduced in the *nreCBA* mutant compared to the wild-type strain. Therefore, the *nreCBA* mutant was unable to utilize nitrate as a respiratory oxidant and was forced into fermentative growth under anoxia (Schlag et al. 2008). Even though the expression of virulence factors was not affected in the *nreCBA* mutant, the ability of *S. aureus* to use nitrate as a terminal electron acceptor in nitrate respiration could confer a fitness advantage to

the bacterium during infection. Recently, NreA has been identified as a new type of nitrate receptor in *S. carnosus*. Nitrate induction of a *narG-lip* reporter gene required presence of NreBC, yet when *nreA* was deleted, nitrate was no longer required for maximal induction, suggesting that NreA is a nitrate-regulated inhibitor of NreBC (Nilkens et al. 2014). The interaction of NreA with nitrate was also confirmed by cocrystallization (Niemann et al. 2014). In vitro, NreA decreased NreB phosphorylation by direct interaction inhibiting its autophosphorylating activity. Therefore, NreA interacts with NreB and controls its phosphorylation level in a nitrate-dependent manner. By this mechanism, *S. aureus* uses nitrate and NreA to modulate the function of the oxygen sensor NreB, resulting in nitrate/oxygen cosensing by an NreBA sensor unit as part of the NreCBA system (Nilkens et al. 2014).

Even though little is known as to the virulence properties of *nreCBA* mutants of *S. aureus*, the TCS plays a crucial role in facilitating anaerobic nitrate respiration in *S. carnosus*. In vitro experiments in *S. aureus* have shown that the regulation of the tested virulence factors was not affected in an *nreCBA* mutant, yet it is still unclear as to what phenotype this would translate to during infection. One could expect that the mutant might be severely affected in its ability to infect animals given that nitrate respiration was shown to confer a fitness advantage to the bacterium. Other regulatory mechanisms might interact with the NreCBA TCS under invasive conditions and thereby alter the staphylococcal virulence profile. It remains to be determined how NreCBA will affect virulence/infectivity in vivo.

5.3 AirRS

The AirRS (anaerobic iron–sulphur cluster-containing redox sensor regulator, previously known as YhcRS) TCS has been found to be essential for the growth of some *S. aureus* strains. Downregulation of *airRS* expression by induction of *airS* antisense RNA was shown to effectively inhibit and terminate bacterial growth (Sun et al. 2005). However, transposon mutants of both *airS* and *airR* were isolated in the Phoenix transposon mutant library (Bae et al. 2004), indicating that the AirSR system might be non-essential in at least some strain backgrounds. This observation was further corroborated by repeating the antisense *airS* experiment in the Newman strain background, where no effect on bacterial growth could be observed (Sun et al. 2012). Yan et al. (2012) later demonstrated that *airR* binds to the promoters of the *opuCABCD* operon (encoding components of a glycine betaine/carnitine/choline ABC transporter) and that expression of this operon in trans could partially recover the conditional *airRS* mutant's lethal phenotype in this strain background. Conversely, this operon was affected in a similar way in the Newman strain background, whose viability was not affected (Sun et al. 2012) suggesting that changes in betaine/carnitine/choline transport could not account for the observed lethality of the *airRS* mutant (Yan et al. 2012). The genes of the *airRS* operon are cotranscribed as a ≈1.6-kb mRNA (Sun et al. 2005). The AirRS TCS was shown to

be involved in regulating nitrate respiration under anaerobic conditions and AirR was found to directly bind to the promoter regions of *narG* and *nreCBA,* thereby also altering NreCBA regulon (see above) (Yan et al. 2011). The AirS protein is a sensor HK consisting of two domains, the N-terminal sensory domain and C-terminal HK domain. The N-terminal sensory domain contains an Fe–S cluster-binding motif composed of four conserved cysteine residues (Sun et al. 2012). AirSR responds to oxidation signals such as O_2, H_2O_2 or NO. by using a redox-active [2Fe–2S] cluster in the sensor kinase AirS. In fact, the oxidised AirS with a $[2Fe–2S]^{2+}$ cluster shows the highest kinase activity and both reduced or overoxidised AirS lose this activity. *S. aureus* AirSR represents the first TCS known to utilize [2Fe–2S] as a sensory motif to respond to oxygen and various ROSs/RNSs (Sun et al. 2012).

Microarray analysis of a transposon insertion mutant of *airR* compared to the Newman wild-type strain revealed that the *airR* mutant showed no significant difference when both strains were grown under aerobic conditions. However, AirSR considerably alters *S. aureus* gene regulation under anaerobic growth by affecting the expression and regulation of more than 350 genes including: global regulatory systems such as the quorum sensing (RNAIII, AgrA, and AgrD), the TCS SaeRS and stress-associated factors (RsbU and RsbW) (Table 2). As a consequence, the expression levels of key virulence factors such as capsular polysaccharide biosynthesis protein (Cap5A), Spa and γ-haemolysin (HlgC) were altered in the *airR* mutant (Sun et al. 2012). In general, the *airR* mutant had altered expression of a number of genes ranging from virulence, transcriptional regulation, stress response, protein synthesis, DNA replication, metabolism, cell wall synthesis to a number of uncharacterized functions (Sun et al. 2012). In order to determine a more detailed picture of AirRS-dependent gene regulation in pathogenesis a combination of antisense RNA interference technology, an inducible overexpression system and gene deletions were employed (Hall et al. 2015). Depletion of AirSR by antisense RNA expression or deletion of the genes significantly decreased bacterial survival in human blood. Conversely, overexpression of AirR significantly promoted survival of *S. aureus* in blood. This observation was connected to the positive effect of AirR on the secretion of virulence factors that inhibit opsonin-based phagocytosis and AirRS-dependent transcriptional regulation of the *sspABC* operon which is responsible for encoding V8 protease (SspA), staphopain B (SspB) and staphostatin B (SspC) (Hall et al. 2015). SspA and SspB are known virulence factors which proteolytically digest opsonins and inhibit killing of *S. aureus* by professional phagocytes.

The *airR* mutant strain showed increased resistance to H_2O_2, vancomycin, ciprofloxacin and norfloxacin when grown under anaerobic conditions (Sun et al. 2012). The authors proposed that the AirSR may respond to these antibiotics through sensing of the generated ROS, although the detailed mechanism of how AirSR affects the *S. aureus* antibiotic susceptibility remains to be determined (Sun et al. 2012).

Taken together, the AirRS TCS has a major impact on global staphylococcal gene regulation affecting major regulatory pathways in virulence gene expression. AirRS-dependent regulation is active under anaerobiosis and more than 350 gene

are differentially expressed between and *airR* mutant and its parental strain. Given the global impact of AirRS on gene regulation, it is not at all surprising that mutants in this TCS were affected in virulence.

6 Nutrient Sensing and Metabolism

The availability of nutrients and micronutrients (such as iron, potassium and phosphate) is a key determinant of the microenvironment in which a bacterium resides and is essential for bacterial metabolism and survival. Eukaryotic hosts utilize nutritional immunity in order to abrogate or limit bacterial growth by chelating iron to high-affinity host factors such as transferrin, rendering them virtually absent in solution. The majority of iron in humans is stored in haemoglobin and *S. aureus* has developed mechanisms by which it can liberate haem iron (Cassat and Skaar 2013). Iron serves as a redox catalyst, accepting or donating electrons in biological processes. Nevertheless, the redox potential of iron also generates cellular toxicity under conditions of iron overload and *S. aureus* has to fine-tune intracellular iron concentration in order to survive (Cassat and Skaar 2013). In this section, we are going to focus on TCS that are involved in nutrient acquisition and regulation of gene expression in response to nutrient availability.

6.1 HssSR

Host haem (a metalloporphyrin) provides an accessible and vital source of iron during infection. Haem acquisition is accomplished through cell-wall-anchored, membrane-bound and cytoplasmic proteins of the Isd and Hts loci. At high concentrations, haem is toxic and capable of killing *S. aureus*. *S. aureus* maintains cellular haem homeostasis through the coordinated actions of the haem-sensing TCS (HssRS) and the haem-regulated transporter efflux pump (HrtAB). HssRS-dependent expression of HrtAB results in the alleviation of haem toxicity and tempered staphylococcal virulence (Stauff et al. 2007). Signalling between the sensor HK HssS and the response regulator HssR is necessary for growth of *S. aureus* in high concentrations of haem. Phosphorylated HssR binds to a direct repeat DNA sequence (5′-GTTCATATTN$_2$GTTCATATT-3′) within the *hrtAB* promoter upon exposure of *S. aureus* to high concentrations of haem, thereby inducing expression of the *hrtAB* operon (Stauff et al. 2007; Friedman et al. 2006).

The HssRS/HrtAB haem detoxification system is conserved in a number of mammalian pathogens and underscores the potential role this system plays in the survival of bacteria in haem-rich environments (Wakeman et al. 2014). When it was tested whether metalloporphyrins other than haem could induce the *S. aureus* haem detoxification system, it was found that only toxic metalloporphyrins were able to maximally activate its expression. This suggested that the sensing mechanism of

HssRS might require a component of the associated toxicity rather than or in addition to the metalloporphyrin itself (Wakeman et al. 2014). A significant component of haem toxicity is oxidative damage. Only a subset of toxic metalloporphyrins elicits oxidative damage, whereas all toxic non-iron metalloporphyrins tested inhibited bacterial respiration (Wakeman et al. 2014). Interestingly, despite the fact that toxic metalloporphyrin treatment induces the expression of *S. aureus* haem detoxification machinery, the HrtAB haem export pump was unable to detoxify most of these molecules. The ineffectiveness of HrtAB against toxic haem analogues therefore also provides an explanation for their increased antimicrobial activity relative to haem (Wakeman et al. 2014).

Interestingly, inactivation of the HssSR or HrtAB systems leads to enhanced liver-specific *S. aureus* virulence, which correlates with a reduced innate immune response to infection in a vertebrate infection model (Torres et al. 2007). *S. aureus* might exploit the association of haem with the major protein constituents of blood and muscle as a molecular marker to distinguish internal host tissue from surface colonization sites and by tempering its virulence the bacterium could avoid generating excessive host tissue damage (Torres et al. 2007).

S. aureus mutants with an insertion in *hssR* were 2 to 4-fold more resistant to plectasin and eurocin, two host defence peptides, as compared to the wild-type. Addition of plectasin did not influence the expression of *hssR* or *hrtA*. The lack of plectasin regulation of the systems implies that the TCS does not sense the defensins and the ABC transporter system HrtAB is not involved in exporting the peptides (Thomsen et al. 2010). This therefore indicated that the effect by which peptide sensitivity was conferred to *S. aureus* via HssR was mediated by one or more of the potential 14 genes/operon sharing the HssR binding site in their promoter (Thomsen et al. 2010; Stauff et al. 2008).

Researchers have developed molecules that can exploit the HssRS system for use as combinatorial antibiotic agents. Endogenous haem biosynthesis in *S. aureus* was stimulated by small molecule activators leading to increased intracellular haem levels and thereby activating HssRS (Mike et al. 2013). The metabolic alterations induced by these small molecules are toxic to fermenting *S. aureus*. A combination with known respiratory inhibitors was found to be highly antimicrobial against fermenting bacteria including small colony variants and virtually eliminated the development of resistance to aminoglycoside antibiotics. Notably, treatment with one of these small molecules also reduced bacterial burdens in a systemic model of staphylococcal infection (Mike et al. 2013).

6.2 KdpDE

The KdpDE TCS was first characterized in *Escherichia coli*, in which the HK KdpD and its cognate response regulator KdpE regulate the production of the high-affinity K^+ transporter Kdp-ATPase (Ballal et al. 2007). In *E. coli,* Kdp-ATPase is an efficient K^+-scavenging system that is expressed when cells are subjected to extreme

K^+ limitation or osmotic upshock and other low-affinity K^+ transporters cannot meet the cellular requirements for K^+. The HK KdpD autophosphorylates and transfers its phosphoryl group to the response regulator KpdE, increasing its affinity to a 23-bp region in the promoter of the *kdpFABC* operon and inducing transcription (Xue et al. 2011). The *kdpFABC* operon in *S. aureus* is organized similarly to that of *E. coli*, but the *kdpDE* genes are oriented divergently to the rest of the operon in *S. aureus* upstream of the *kdpA* gene, yet its function in this bacterium seems to be diverse (Xue et al. 2011). DNaseI footprinting analysis in *S. aureus* showed that KdpE binds a 23-bp region (5′-GCATACACATCTTAATGATTTCT-3′) in the *kdpF* promoter and likely influences *kdpFABC* expression by competitively binding to this 23-bp sequence and inhibiting the binding of transcriptional factors, such as σ-factor and RNA polymerase (Xue et al. 2011).

KdpD and KdpE are cotranscribed (Zhao et al. 2010) and their transcripts were shown to change their expression in response to several environmental stimuli such as growth under biofilm conditions, neutrophil microbicides or during human neutrophil phagocytosis (Palazzolo-Ballance et al. 2008; Beenken et al. 2004; Voyich et al. 2005). In *S. aureus* NCTC8325, KdpDE displays a repression effect on the transcription of *kdpFABC* even under strict K^+ limitation suggesting that KdpFABC is not a major K^+ transporter in this bacterium (Xue et al. 2011). KdpD activity is influenced by binding c-di-AMP through its universal stress protein domain. c-di-AMP binding to KdpD inhibits the upregulation of the *kdpFABC* operon under salt stress, thus indicating that c-di-AMP is a negative regulator of *kdpFABC* and potentially potassium uptake in *S. aureus* (Moscoso et al. 2015).

Expression of *kdpDE* is stimulated by the Agr quorum-sensing system in an RNAIII and possibly Rot-dependent manner connecting the TCS to one of the major regulatory networks controlling expression of virulence factors (Table 2) (Xue et al. 2011). The KdpDE TCS is also intertwined with a secondary quorum-sensing mechanism that is shared by both Gram-positive and Gram-negative bacteria. This system produces an autoinducing peptide via the LuxS enzyme in a metabolic pathway known as the activated methyl cycle and is mainly involved in regulating bacterial metabolism (Winzer et al. 2002; Vendeville et al. 2005; Schauder et al. 2001). In contrast to other autoinducers that are usually involved in intraspecies communication, AI-2 is widely present in bacteria, leading to the suggestion that it is a universal language for interspecies communication. The TCS genes *kdpDE* were upregulated by *luxS* deletion and their repression could be restored by the addition of exogenous AI-2 (Zhao et al. 2010). KdpDE itself is able to regulate capsular polysaccharide production via the phosphorylation of KdpE and its subsequent binding to the *cap* promoter (Zhao et al. 2010).

The KdpDE system appears to be involved in virulence in some bacteria. For instance, in *Mycobacterium tuberculosis*, deletion of *kdpDE* resulted in increased virulence. Mice infected with the *M. tuberculosis kdpDE* mutant died more rapidly than those infected with wild-type bacteria (Parish et al. 2003). The absence of *luxS* increased *S. aureus* survival in human blood and human U937 monocytic cells, and the absence of *kdpDE* attenuated *S. aureus* survival (Zhao et al. 2010; Xue et al. 2011). Interestingly, *kdpDE* mutants show altered transcriptional levels of several

virulence factors. Expression of *hla*, *aur*, *geh* and *hlgB* was repressed, while expression of *spa* and *cap* was stimulated by KdpDE. Despite different regulatory outcomes, the response regulator KdpE binds to the promoter regions of all of these genes apart from *hla* (Xue et al. 2011). Even though it is not one of the major K^+ transporters in *S. aureus*, the transcript levels of *kdpDE* are repressed by increasing external K^+ concentrations, indicating that *S. aureus* might modulate its infectious status by sensing specific external K^+ stimuli in different environments. This repression is also mirrored in the transcript levels of *spa* (Xue et al. 2011). Repression of *kdpDE* by increased K^+ availability could play an important role in its regulation of virulence determinants. K^+ concentrations similar to those found in host blood and tissue fluid (≈4 mM) or within host cells (≈100 mM) repressed *kdpD* transcription (Xue et al. 2011).

The KdpDE TCS might therefore act as an additional sensor for *S. aureus* signalling the transition from a colonizing to an invasive lifestyle. In the natural environment, a high level of KdpDE transcription helps to activate the expression of cell wall proteins and polysaccharides, which are beneficial to colonization. However, in transition from the natural environment to the host, which has a higher K^+ concentration, transcript levels of *kdpDE* decrease, causing a low expression of cell wall proteins but a high production of extracellular toxins and enzymes which facilitate local invasion (Xue et al. 2011).

The KdpDE TCS and its regulatory network are highly interconnected with major regulators of virulence gene expression. It is possible that it acts as an additional mechanism for fine-tuning the expression of several virulence factors in response to host stimuli such as K^+ availability. The observed attenuated phenotypes of *kdpDE* mutants in several models of virulence and the capacity of KdpDE in regulating alterations in the gene expression pattern indicates that KdpDE plays an important role in the pathogenesis of *S. aureus*.

6.3 PhoRP

Not much is known about this TCS and to date only one study in *S. aureus* has shown that the PhoRP TCS might be involved in the regulation of virulence factors. A random transposon library screen for *S. aureus* mutants that were altered in their haemolysin expression compared to the wild-type strain identified that mutations the PhoRP TCS increased expression of haemolysin (Burnside et al. 2010).

In *B. subtilis*, the PhoPR TCS system controls one of the major responses to phosphate limitation, directs the expression of phosphate scavenging enzymes, lowers the synthesis of phosphate-rich wall teichoic acid and, in replacement, initiates the synthesis of non-phosphate-containing teichuronic acid (Botella et al. 2014). One of the main functions of the PhoRP TCS is to monitor cell wall teichoic acid metabolism, as PhoR autokinase activity is controlled by the expression level of an intermediate used in its synthesis. Under phosphate-rich conditions, autophosphorylation of PhoR is inhibited by this intermediate. Upon phosphate

limitation, PhoR is phosphorylated and then transfers its phosphate group to its cognate response regulator PhoP, thereby triggering transcriptional changes in downstream targets (Botella et al. 2014).

7 Conclusions

TCSs enable *S. aureus* to adapt to a plethora of environmental stimuli and are essential for its ability to create such a various range of disease manifestations. Virtually, all characterized TCSs contribute to some extent to the regulation of virulence-associated gene expression and often more than one TCS act in concert with other staphylococcal regulators to fine-tune virulence gene expression. Despite our knowledge of the genes that are affected by the specific TCS, little is known about the signals that are relayed. Identification of these signals could provide valuable mechanistic insights into how *S. aureus* senses its environment and may potentially provide targets for use in designing inhibitory compounds to be used in conjunction with classic antibiotic treatment. Given the propensity of *S. aureus* to quickly develop new resistance mechanism, development of multicomponent vaccines which target genes controlled by different regulatory systems or with different expression profiles could provide a suitable strategy for combating staphylococcal infections. For example, *spa* is highly expressed early on during in vitro growth, while *hla* is induced towards stationary phase. The asynchronous expression of virulence factors and *spA* has also been attributed to differential development of humoral response against *S. aureus* antigens upon infection (Pozzi et al. 2015). Indeed, the B-cell killing activity of SpA prevents development of antibodies against many but not all *S. aureus* antigens (Kim et al. 2010). Targeting SpA as a vaccine candidate would therefore unleash antibody responses against those antigens expressed during early exponential growth, while targeting Hla would cover stationary-phase bacteria. A combination of antigens covering sets of diversely regulated genes would therefore increase protective efficacy. A multicomponent *S. aureus* vaccine has recently shown promising protection in murine models of infection (Bagnoli et al. 2015).

References

Adhikari RP, Novick RP (2008) Regulatory organization of the staphylococcal *sae* locus. Microbiology 154(Pt 3):949–959. doi:10.1099/mic.0.2007/012245-0

Arvidson S, Tegmark K (2001) Regulation of virulence determinants in *Staphylococcus aureus*. Int J Med Microbiol 291(2):159–170. doi:10.1078/1438-4221-00112

Bae T, Banger AK, Wallace A, Glass EM, Aslund F, Schneewind O, Missiakas DM (2004) *Staphylococcus aureus* virulence genes identified by bursa aurealis mutagenesis and nematode killing. Proc Natl Acad Sci USA 101(33):12312–12317. doi:10.1073/pnas.0404728101

Bagnoli F, Fontana MR, Soldaini E, Mishra RP, Fiaschi L, Cartocci E, Nardi-Dei V, Ruggiero P, Nosari S, De Falco MG, Lofano G, Marchi S, Galletti B, Mariotti P, Bacconi M, Torre A, Maccari S, Scarselli M, Rinaudo CD, Inoshima N, Savino S, Mori E, Rossi-Paccani S, Baudner B, Pallaoro M, Swennen E, Petracca R, Brettoni C, Liberatori S, Norais N, Monaci E, Bubeck Wardenburg J, Schneewind O, O'Hagan DT, Valiante NM, Bensi G, Bertholet S, De Gregorio E, Rappuoli R, Grandi G (2015) Vaccine composition formulated with a novel TLR7-dependent adjuvant induces high and broad protection against *Staphylococcus aureus*. Proc Natl Acad Sci USA. doi:10.1073/pnas.1424924112

Balaban N, Goldkorn T, Gov Y, Hirshberg M, Koyfman N, Matthews HR, Nhan RT, Singh B, Uziel O (2001) Regulation of *Staphylococcus aureus* pathogenesis via target of RNAIII-activating protein (TRAP). J Biol Chem 276(4):2658–2667

Ballal A, Basu B, Apte SK (2007) The Kdp-ATPase system and its regulation. J Biosci 32 (3):559–568

Batzilla CF, Rachid S, Engelmann S, Hecker M, Hacker J, Ziebuhr W (2006) Impact of the accessory gene regulatory system (Agr) on extracellular proteins, *codY* expression and amino acid metabolism in *Staphylococcus epidermidis*. Proteomics 6(12):3602–3613. doi:10.1002/pmic.200500732

Beenken KE, Dunman PM, McAleese F, Macapagal D, Murphy E, Projan SJ, Blevins JS, Smeltzer MS (2004) Global gene expression in *Staphylococcus aureus* biofilms. J Bacteriol 186(14):4665–4684. doi:10.1128/JB.186.14.4665-4684.2004

Belcheva A, Golemi-Kotra D (2008) A close-up view of the VraSR two-component system. A mediator of *Staphylococcus aureus* response to cell wall damage. J Biol Chem 283 (18):12354–12364. doi:10.1074/jbc.M710010200

Benito Y, Kolb FA, Romby P, Lina G, Etienne J, Vandenesch F (2000) Probing the structure of RNAIII, the *Staphylococcus aureus agr* regulatory RNA, and identification of the RNA domain involved in repression of protein A expression. RNA 6(5):668–679

Benson MA, Lilo S, Wasserman GA, Thoendel M, Smith A, Horswill AR, Fraser J, Novick RP, Shopsin B, Torres VJ (2011) *Staphylococcus aureus* regulates the expression and production of the staphylococcal superantigen-like secreted proteins in a Rot-dependent manner. Mol Microbiol 81(3):659–675. doi:10.1111/j.1365-2958.2011.07720.x

Benson MA, Lilo S, Nygaard T, Voyich JM, Torres VJ (2012) Rot and SaeRS cooperate to activate expression of the staphylococcal superantigen-like exoproteins. J Bacteriol 194 (16):4355–4365. doi:10.1128/JB.00706-12

Benton BM, Zhang JP, Bond S, Pope C, Christian T, Lee L, Winterberg KM, Schmid MB, Buysse JM (2004) Large-scale identification of genes required for full virulence of *Staphylococcus aureus*. J Bacteriol 186(24):8478–8489. doi:10.1128/JB.186.24.8478-8489.2004

Blake KL, Randall CP, O'Neill AJ (2011) *In vitro* studies indicate a high resistance potential for the lantibiotic nisin in *Staphylococcus aureus* and define a genetic basis for nisin resistance. Antimicrob Agents Chemother 55(5):2362–2368. doi:10.1128/AAC.01077-10

Blevins JS, Beenken KE, Elasri MO, Hurlburt BK, Smeltzer MS (2002) Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus*. Infect Immun 70 (2):470–480

Boisset S, Geissmann T, Huntzinger E, Fechter P, Bendridi N, Possedko M, Chevalier C, Helfer AC, Benito Y, Jacquier A, Gaspin C, Vandenesch F, Romby P (2007) *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. Genes Dev 21(11):1353–1366. doi:10.1101/gad.423507

Boles BR, Thoendel M, Roth AJ, Horswill AR (2010) Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation. PLoS ONE 5(4): e10146. doi:10.1371/journal.pone.0010146

Botella E, Devine SK, Hubner S, Salzberg LI, Gale RT, Brown ED, Link H, Sauer U, Codee JD, Noone D, Devine KM (2014) PhoR autokinase activity is controlled by an intermediate in wall teichoic acid metabolism that is sensed by the intracellular PAS domain during the

PhoPR-mediated phosphate limitation response of *Bacillus subtilis*. Mol Microbiol 94 (6):1242–1259. doi:10.1111/mmi.12833

Boyle-Vavra S, Yin S, Daum RS (2006) The VraS/VraR two-component regulatory system required for oxacillin resistance in community-acquired methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol Lett 262(2):163–171. doi:10.1111/j.1574-6968.2006.00384.x

Bronner S, Monteil H, Prevost G (2004) Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. FEMS Microbiol Rev 28(2):183–200. doi:10.1016/j.femsre.2003.09.003

Brunskill EW, Bayles KW (1996a) Identification and molecular characterization of a putative regulatory locus that affects autolysis in *Staphylococcus aureus*. J Bacteriol 178(3):611–618

Brunskill EW, Bayles KW (1996b) Identification of LytSR-regulated genes from *Staphylococcus aureus*. J Bacteriol 178(19):5810–5812

Burnside K, Lembo A, de Los Reyes M, Iliuk A, Binhtran NT, Connelly JE, Lin WJ, Schmidt BZ, Richardson AR, Fang FC, Tao WA, Rajagopal L (2010) Regulation of hemolysin expression and virulence of *Staphylococcus aureus* by a serine/threonine kinase and phosphatase. PLoS ONE 5(6):e11071. doi:10.1371/journal.pone.0011071

Canova MJ, Baronian G, Brelle S, Cohen-Gonsaud M, Bischoff M, Molle V (2014) A novel mode of regulation of the *Staphylococcus aureus* vancomycin-resistance-associated response regulator VraR mediated by Stk1 protein phosphorylation. Biochem Biophys Res Commun 447(1):165–171. doi:10.1016/j.bbrc.2014.03.128

Casino P, Rubio V, Marina A (2010) The mechanism of signal transduction by two-component systems. Curr Opin Struct Biol 20(6):763–771. doi:10.1016/j.sbi.2010.09.010

Cassat JE, Skaar EP (2013) Iron in infection and immunity. Cell Host Microbe 13(5):509–519. doi:10.1016/j.chom.2013.04.010

Chaffin DO, Taylor D, Skerrett SJ, Rubens CE (2012) Changes in the *Staphylococcus aureus* transcriptome during early adaptation to the lung. PLoS ONE 7(8):e41329. doi:10.1371/journal.pone.0041329

Chen J, Novick RP (2007) *svrA*, a multi-drug exporter, does not control *agr*. Microbiology 153(Pt 5):1604–1608. doi:10.1099/mic.0.2007/006247-0

Cheung AL, Eberhardt K, Heinrichs JH (1997) Regulation of protein A synthesis by the *sar* and *agr* loci of *Staphylococcus aureus*. Infect Immun 65(6):2243–2249

Cheung AL, Bayer AS, Zhang G, Gresham H, Xiong YQ (2004) Regulation of virulence determinants in vitro and in vivo in *Staphylococcus aureus*. FEMS Immunol Med Microbiol 40 (1):1–9

Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M (2011) Role of the accessory gene regulator agr in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. Infect Immun 79(5):1927–1935. doi:10.1128/IAI.00046-11

Cheung AL, Bayer AS, Yeaman MR, Xiong YQ, Waring AJ, Memmi G, Donegan N, Chaili S, Yang SJ (2014) Site-specific mutation of the sensor kinase GraS in *Staphylococcus aureus* alters the adaptive response to distinct cationic antimicrobial peptides. Infect Immun 82 (12):5336–5345. doi:10.1128/IAI.02480-14

Chevalier C, Boisset S, Romilly C, Masquida B, Fechter P, Geissmann T, Vandenesch F, Romby P (2010) *Staphylococcus aureus* RNAIII binds to two distant regions of *coa* mRNA to arrest translation and promote mRNA degradation. PLoS Pathog 6(3):e1000809. doi:10.1371/journal.ppat.1000809

Cho H, Jeong DW, Liu Q, Yeo WS, Vogl T, Skaar EP, Chazin WJ, Bae T (2015) Calprotectin increases the activity of the SaeRS two component system and murine mortality during *Staphylococcus aureus* infections. PLoS Pathog 11(7):e1005026. doi:10.1371/journal.ppat.1005026

Coumes-Florens S, Brochier-Armanet C, Guiseppi A, Denizot F, Foglino M (2011) A new highly conserved antibiotic sensing/resistance pathway in firmicutes involves an ABC transporter interplaying with a signal transduction system. PLoS ONE 6(1):e15951. doi:10.1371/journal.pone.0015951

Cue D, Junecko JM, Lei MG, Blevins JS, Smeltzer MS, Lee CY (2015) SaeRS-dependent inhibition of biofilm formation in *Staphylococcus aureus* Newman. PLoS ONE 10(4): e0123027. doi:10.1371/journal.pone.0123027

Cui L, Lian JQ, Neoh HM, Reyes E, Hiramatsu K (2005) DNA microarray-based identification of genes associated with glycopeptide resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 49(8):3404–3413. doi:10.1128/AAC.49.8.3404-3413.2005

Cui L, Neoh HM, Shoji M, Hiramatsu K (2009) Contribution of *vraSR* and *graSR* point mutations to vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 53(3):1231–1234. doi:10.1128/AAC.01173-08

Dassy B, Hogan T, Foster TJ, Fournier JM (1993) Involvement of the accessory gene regulator (*agr*) in expression of type 5 capsular polysaccharide by *Staphylococcus aureus*. J Gen Microbiol 139(Pt 6):1301–1306

Delaune A, Poupel O, Mallet A, Coic YM, Msadek T, Dubrac S (2011) Peptidoglycan crosslinking relaxation plays an important role in *Staphylococcus aureus* WalKR-dependent cell viability. PLoS ONE 6(2):e17054. doi:10.1371/journal.pone.0017054

Delaune A, Dubrac S, Blanchet C, Poupel O, Mader U, Hiron A, Leduc A, Fitting C, Nicolas P, Cavaillon JM, Adib-Conquy M, Msadek T (2012) The WalKR system controls major staphylococcal virulence genes and is involved in triggering the host inflammatory response. Infect Immun 80(10):3438–3453. doi:10.1128/iai.00195-12

Dubrac S, Msadek T (2004) Identification of genes controlled by the essential YycG/YycF two-component system of *Staphylococcus aureus*. J Bacteriol 186(4):1175–1181

Dubrac S, Msadek T (2008) Tearing down the wall: peptidoglycan metabolism and the WalK/WalR (YycG/YycF) essential two-component system. Adv Exp Med Biol 631:214–228. doi:10.1007/978-0-387-78885-2_15

Dubrac S, Boneca IG, Poupel O, Msadek T (2007) New insights into the WalK/WalR (YycG/YycF) essential signal transduction pathway reveal a major role in controlling cell wall metabolism and biofilm formation in *Staphylococcus aureus*. J Bacteriol 189(22):8257–8269. doi:10.1128/JB.00645-07

Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, Shopsin B, Unutmaz D, Voyich JM, Torres VJ (2011) Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. Mol Microbiol 79(3):814–825. doi:10.1111/j.1365-2958.2010.07490.x

Dunman PM, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, Brown EL, Zagursky RJ, Shlaes D, Projan SJ (2001) Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci. J Bacteriol 183(24):7341–7353. doi:10.1128/JB.183.24.7341-7353.2001

Fabret C, Hoch JA (1998) A two-component signal transduction system essential for growth of *Bacillus subtilis*: implications for anti-infective therapy. J Bacteriol 180(23):6375–6383

Falord M, Mader U, Hiron A, Debarbouille M, Msadek T (2011) Investigation of the *Staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell wall signal transduction pathways. PLoS ONE 6(7):e21323. doi:10.1371/journal.pone.0021323

Falord M, Karimova G, Hiron A, Msadek T (2012) GraXSR proteins interact with the VraFG ABC transporter to form a five-component system required for cationic antimicrobial peptide sensing and resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 56(2):1047–1058. doi:10.1128/AAC.05054-11

Fedtke I, Kamps A, Krismer B, Gotz F (2002) The nitrate reductase and nitrite reductase operons and the *narT* gene of *Staphylococcus carnosus* are positively controlled by the novel two-component system NreBC. J Bacteriol 184(23):6624–6634

Felden B, Vandenesch F, Bouloc P, Romby P (2011) The *Staphylococcus aureus* RNome and its commitment to virulence. PLoS Pathog 7(3):e1002006. doi:10.1371/journal.ppat.1002006

Fischer J, Lee JC, Peters G, Kahl BC (2014) Acapsular clinical *Staphylococcus aureus* isolates lack *agr* function. Clin Microbiol Infect 20(7):O414–417. doi:10.1111/1469-0691.12429

Flack CE, Zurek OW, Meishery DD, Pallister KB, Malone CL, Horswill AR, Voyich JM (2014) Differential regulation of staphylococcal virulence by the sensor kinase SaeS in response to neutrophil-derived stimuli. Proc Natl Acad Sci U S A 111(19):E2037–2045. doi:10.1073/pnas.1322125111

Foster TJ (2009) Colonization and infection of the human host by staphylococci: adhesion, survival and immune evasion. Vet Dermatol 20(5–6):456–470. doi:10.1111/j.1365-3164.2009.00825.x

Fournier B, Hooper DC (2000) A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. J Bacteriol 182 (14):3955–3964

Fournier B, Klier A (2004) Protein A gene expression is regulated by DNA supercoiling which is modified by the ArlS-ArlR two-component system of *Staphylococcus aureus*. Microbiology 150(Pt 11):3807–3819. doi:10.1099/mic.0.27194-0

Fournier B, Aras R, Hooper DC (2000) Expression of the multidrug resistance transporter NorA from *Staphylococcus aureus* is modified by a two-component regulatory system. J Bacteriol 182(3):664–671

Fournier B, Klier A, Rapoport G (2001) The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. Mol Microbiol 41(1):247–261

Freeman ZN, Dorus S, Waterfield NR (2013) The KdpD/KdpE two-component system: integrating K(+) homeostasis and virulence. PLoS Pathog 9(3):e1003201. doi:10.1371/journal.ppat.1003201

Fridman M, Williams GD, Muzamal U, Hunter H, Siu KW, Golemi-Kotra D (2013) Two unique phosphorylation-driven signaling pathways crosstalk in *Staphylococcus aureus* to modulate the cell-wall charge: Stk1/Stp1 meets GraSR. Biochemistry 52(45):7975–7986. doi:10.1021/bi401177n

Friedman DB, Stauff DL, Pishchany G, Whitwell CW, Torres VJ, Skaar EP (2006) *Staphylococcus aureus* redirects central metabolism to increase iron availability. PLoS Pathog 2(8):e87. doi:10.1371/journal.ppat.0020087

Fuchs S, Pane-Farre J, Kohler C, Hecker M, Engelmann S (2007) Anaerobic gene expression in *Staphylococcus aureus*. J Bacteriol 189(11):4275–4289. doi:10.1128/JB.00081-07

Galbusera E, Renzoni A, Andrey DO, Monod A, Barras C, Tortora P, Polissi A, Kelley WL (2011) Site-specific mutation of *Staphylococcus aureus* VraS reveals a crucial role for the VraR-VraS sensor in the emergence of glycopeptide resistance. Antimicrob Agents Chemother 55 (3):1008–1020. doi:10.1128/AAC.00720-10

Gardete S, Wu SW, Gill S, Tomasz A (2006) Role of VraSR in antibiotic resistance and antibiotic-induced stress response in *Staphylococcus aureus*. Antimicrob Agents Chemother 50 (10):3424–3434. doi:10.1128/AAC.00356-06

Geiger T, Goerke C, Mainiero M, Kraus D, Wolz C (2008) The virulence regulator Sae of *Staphylococcus aureus*: promoter activities and response to phagocytosis-related signals. J Bacteriol 190(10):3419–3428. doi:10.1128/JB.01927-07

Geiger T, Francois P, Liebeke M, Fraunholz M, Goerke C, Krismer B, Schrenzel J, Lalk M, Wolz C (2012) The stringent response of *Staphylococcus aureus* and its impact on survival after phagocytosis through the induction of intracellular PSMs expression. PLoS Pathog 8(11): e1003016. doi:10.1371/journal.ppat.1003016

George Cisar EA, Geisinger E, Muir TW, Novick RP (2009) Symmetric signalling within asymmetric dimers of the *Staphylococcus aureus* receptor histidine kinase AgrC. Mol Microbiol 74(1):44–57. doi:10.1111/j.1365-2958.2009.06849.x

Giraud C, Hausmann S, Lemeille S, Prados J, Redder P, Linder P (2015) The C-terminal region of the RNA helicase CshA is required for the interaction with the degradosome and turnover of bulk RNA in the opportunistic pathogen *Staphylococcus aureus*. RNA Biol:0. doi:10.1080/15476286.2015.1035505

Giraudo AT, Raspanti CG, Calzolari A, Nagel R (1994) Characterization of a Tn551-mutant of *Staphylococcus aureus* defective in the production of several exoproteins. Can J Microbiol 40 (8):677–681

Giraudo AT, Rampone H, Calzolari A, Nagel R (1996) Phenotypic characterization and virulence of a *sae- agr*-mutant of *Staphylococcus aureus*. Can J Microbiol 42(2):120–123

Giraudo AT, Cheung AL, Nagel R (1997) The *sae* locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. Arch Microbiol 168(1):53–58

Giraudo AT, Calzolari A, Cataldi AA, Bogni C, Nagel R (1999) The *sae* locus of *Staphylococcus aureus* encodes a two-component regulatory system. FEMS Microbiol Lett 177(1):15–22

Goerke C, Fluckiger U, Steinhuber A, Zimmerli W, Wolz C (2001) Impact of the regulatory loci *agr*, *sarA* and *sae* of *Staphylococcus aureus* on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis. Mol Microbiol 40 (6):1439–1447

Goerke C, Fluckiger U, Steinhuber A, Bisanzio V, Ulrich M, Bischoff M, Patti JM, Wolz C (2005) Role of *Staphylococcus aureus* global regulators sae and sigmaB in virulence gene expression during device-related infection. Infect Immun 73(6):3415–3421. doi:10.1128/IAI.73.6.3415-3421.2005

Gov Y, Borovok I, Korem M, Singh VK, Jayaswal RK, Wilkinson BJ, Rich SM, Balaban N (2004) Quorum sensing in Staphylococci is regulated via phosphorylation of three conserved histidine residues. J Biol Chem 279(15):14665–14672. doi:10.1074/jbc.M311106200

Groicher KH, Firek BA, Fujimoto DF, Bayles KW (2000) The *Staphylococcus aureus lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. J Bacteriol 182(7):1794–1801

Hall JW, Yang J, Guo H, Ji Y (2015) The AirSR two-component system contributes to *Staphylococcus aureus* survival in human blood and transcriptionally regulates *sspABC* operon. Front Microbiol 6:682. doi:10.3389/fmicb.2015.00682

Harraghy N, Kormanec J, Wolz C, Homerova D, Goerke C, Ohlsen K, Qazi S, Hill P, Herrmann M (2005) *sae* is essential for expression of the staphylococcal adhesins Eap and Emp. Microbiology 151(Pt 6):1789–1800. doi:10.1099/mic.0.27902-0

Herbert S, Bera A, Nerz C, Kraus D, Peschel A, Goerke C, Meehl M, Cheung A, Gotz F (2007) Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. PLoS Pathog 3(7):e102. doi:10.1371/journal.ppat.0030102

Hiron A, Falord M, Valle J, Debarbouille M, Msadek T (2011) Bacitracin and nisin resistance in *Staphylococcus aureus*: a novel pathway involving the BraS/BraR two-component system (SA2417/SA2418) and both the BraD/BraE and VraD/VraE ABC transporters. Mol Microbiol 81(3):602–622. doi:10.1111/j.1365-2958.2011.07735.x

Howden BP, Stinear TP, Allen DL, Johnson PD, Ward PB, Davies JK (2008) Genomic analysis reveals a point mutation in the two-component sensor gene *graS* that leads to intermediate vancomycin resistance in clinical *Staphylococcus aureus*. Antimicrob Agents Chemother 52 (10):3755–3762. doi:10.1128/AAC.01613-07

Howden BP, McEvoy CR, Allen DL, Chua K, Gao W, Harrison PF, Bell J, Coombs G, Bennett-Wood V, Porter JL, Robins-Browne R, Davies JK, Seemann T, Stinear TP (2011) Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR. PLoS Pathog 7(11):e1002359. doi:10.1371/journal.ppat.1002359

Hu J, Zhang X, Liu X, Chen C, Sun B (2015) Mechanism of reduced vancomycin susceptibility conferred by *walK* mutation in community-acquired methicillin-resistant *Staphylococcus aureus* strain MW2. Antimicrob Agents Chemother 59(2):1352–1355. doi:10.1128/AAC.04290-14

Huntzinger E, Boisset S, Saveanu C, Benito Y, Geissmann T, Namane A, Lina G, Etienne J, Ehresmann B, Ehresmann C, Jacquier A, Vandenesch F, Romby P (2005) *Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate *spa* gene expression. EMBO J 24(4):824–835. doi:10.1038/sj.emboj.7600572

Iwatsuki K, Yamasaki O, Morizane S, Oono T (2006) *Staphylococcal cutaneous* infections: invasion, evasion and aggression. J Dermatol Sci 42(3):203–214. doi:10.1016/j.jdermsci.2006.03.011

Janzon L, Arvidson S (1990) The role of the delta-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. EMBO J 9(5):1391–1399

Janzon L, Lofdahl S, Arvidson S (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

Jeong DW, Cho H, Lee H, Li C, Garza J, Fried M, Bae T (2011) Identification of the P3 promoter and distinct roles of the two promoters of the SaeRS two-component system in *Staphylococcus aureus*. J Bacteriol 193(18):4672–4684. doi:10.1128/JB.00353-11

Jeong DW, Cho H, Jones MB, Shatzkes K, Sun F, Ji Q, Liu Q, Peterson SN, He C, Bae T (2012) The auxiliary protein complex SaePQ activates the phosphatase activity of sensor kinase SaeS in the SaeRS two-component system of *Staphylococcus aureus*. Mol Microbiol 86(2):331–348. doi:10.1111/j.1365-2958.2012.08198.x

Ji G, Beavis RC, Novick RP (1995) Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci USA 92(26):12055–12059

Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276(5321):2027–2030

Jo DS, Montgomery CP, Yin S, Boyle-Vavra S, Daum RS (2011) Improved oxacillin treatment outcomes in experimental skin and lung infection by a methicillin-resistant *Staphylococcus aureus* isolate with a *vraSR* operon deletion. Antimicrob Agents Chemother 55(6):2818–2823. doi:10.1128/AAC.01704-10

Johnson M, Sengupta M, Purves J, Tarrant E, Williams PH, Cockayne A, Muthaiyan A, Stephenson R, Ledala N, Wilkinson BJ, Jayaswal RK, Morrissey JA (2011) Fur is required for the activation of virulence gene expression through the induction of the Sae regulatory system in *Staphylococcus aureus*. Int J Med Microbiol 301(1):44–52. doi:10.1016/j.ijmm.2010.05.003

Kamps A, Achebach S, Fedtke I, Unden G, Gotz F (2004) Staphylococcal NreB: an O(2)-sensing histidine protein kinase with an O(2)-labile iron–sulphur cluster of the FNR type. Mol Microbiol 52(3):713–723. doi:10.1111/j.1365-2958.2004.04024.x

Kato Y, Suzuki T, Ida T, Maebashi K (2010) Genetic changes associated with glycopeptide resistance in *Staphylococcus aureus*: predominance of amino acid substitutions in YvqF/VraSR. J Antimicrob Chemother 65(1):37–45. doi:10.1093/jac/dkp394

Kato F, Kadomoto N, Iwamoto Y, Bunai K, Komatsuzawa H, Sugai M (2011) Regulatory mechanism for exfoliative toxin production in *Staphylococcus aureus*. Infect Immun 79(4):1660–1670. doi:10.1128/IAI.00872-10

Kawada-Matsuo M, Yoshida Y, Nakamura N, Komatsuzawa H (2011) Role of two-component systems in the resistance of *Staphylococcus aureus* to antibacterial agents. Virulence 2(5):427–430. doi:10.4161/viru.2.5.17711

Kiedrowski MR, Kavanaugh JS, Malone CL, Mootz JM, Voyich JM, Smeltzer MS, Bayles KW, Horswill AR (2011) Nuclease modulates biofilm formation in community-associated methicillin-resistant *Staphylococcus aureus*. PLoS ONE 6(11):e26714. doi:10.1371/journal.pone.0026714

Kim HK, Cheng AG, Kim HY, Missiakas DM, Schneewind O (2010) Nontoxigenic protein A vaccine for methicillin-resistant *Staphylococcus aureus* infections in mice. J Exp Med 207(9):1863–1870. doi:10.1084/jem.20092514

Kinkel TL, Roux CM, Dunman PM, Fang FC (2013) The *Staphylococcus aureus* SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. MBio 4(6):e00696–00613. doi:10.1128/mBio.00696-13

Kiran MD, Balaban N (2009) TRAP plays a role in stress response in *Staphylococcus aureus*. Int J Artif Organs 32(9):592–599

Koenig RL, Ray JL, Maleki SJ, Smeltzer MS, Hurlburt BK (2004) *Staphylococcus aureus* AgrA binding to the RNAIII-*agr* regulatory region. J Bacteriol 186(22):7549–7555. doi:10.1128/JB.186.22.7549-7555.2004

Kolar SL, Nagarajan V, Oszmiana A, Rivera FE, Miller HK, Davenport JE, Riordan JT, Potempa J, Barber DS, Koziel J, Elasri MO, Shaw LN (2011) NsaRS is a cell-envelope-stress-sensing two-component system of *Staphylococcus aureus*. Microbiology 157(Pt 8):2206–2219. doi:10.1099/mic.0.049692-0

Korem M, Sheoran AS, Gov Y, Tzipori S, Borovok I, Balaban N (2003) Characterization of RAP, a quorum sensing activator of *Staphylococcus aureus*. FEMS Microbiol Lett 223(2):167–175

Kraus D, Herbert S, Kristian SA, Khosravi A, Nizet V, Gotz F, Peschel A (2008) The GraRS regulatory system controls *Staphylococcus aureus* susceptibility to antimicrobial host defenses. BMC Microbiol 8:85. doi:10.1186/1471-2180-8-85

Kuroda M, Kuwahara-Arai K, Hiramatsu K (2000) Identification of the up- and down-regulated genes in vancomycin-resistant *Staphylococcus aureus* strains Mu3 and Mu50 by cDNA differential hybridization method. Biochem Biophys Res Commun 269(2):485–490. doi:10.1006/bbrc.2000.2277

Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K (2001) Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet 357(9264):1225–1240

Kuroda M, Kuroda H, Oshima T, Takeuchi F, Mori H, Hiramatsu K (2003) Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol Microbiol 49(3):807–821

Kuroda H, Kuroda M, Cui L, Hiramatsu K (2007) Subinhibitory concentrations of beta-lactam induce haemolytic activity in *Staphylococcus aureus* through the SaeRS two-component system. FEMS Microbiol Lett 268(1):98–105. doi:10.1111/j.1574-6968.2006.00568.x

Kurokawa K, Kaito C, Sekimizu K (2007) Two-component signaling in the virulence of *Staphylococcus aureus*: a silkworm larvae-pathogenic agent infection model of virulence. Methods Enzymol 422:233–244. doi:10.1016/S0076-6879(06)22011-1

Lehman MK, Bose JL, Sharma-Kuinkel BK, Moormeier DE, Endres JL, Sadykov MR, Biswas I, Bayles KW (2015) Identification of the amino acids essential for LytSR-mediated signal transduction in *Staphylococcus aureus* and their roles in biofilm-specific gene expression. Mol Microbiol 95(4):723–737. doi:10.1111/mmi.12902

Leonard PG, Golemi-Kotra D, Stock AM (2013) Phosphorylation-dependent conformational changes and domain rearrangements in *Staphylococcus aureus* VraR activation. Proc Natl Acad Sci USA 110(21):8525–8530. doi:10.1073/pnas.1302819110

Levinger O, Bikels-Goshen T, Landau E, Fichman M, Shapira R (2012) Epigallocatechin gallate induces upregulation of the two-component VraSR system by evoking a cell wall stress response in *Staphylococcus aureus*. Appl Environ Microbiol 78(22):7954–7959. doi:10.1128/AEM.02253-12

Lewis AM, Matzdorf SS, Endres JL, Windham IH, Bayles KW, Rice KC (2015) Examination of the *Staphylococcus aureus* nitric oxide reductase (saNOR) reveals its contribution to modulating intracellular NO levels and cellular respiration. Mol Microbiol 96(3):651–669. doi:10.1111/mmi.12962

Li D, Cheung A (2008) Repression of *hla* by *rot* is dependent on *sae* in *Staphylococcus aureus*. Infect Immun 76(3):1068–1075. doi:10.1128/IAI.01069-07

Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M (2007) The antimicrobial peptide-sensing system *aps* of *Staphylococcus aureus*. Mol Microbiol 66(5):1136–1147. doi:10.1111/j.1365-2958.2007.05986.x

Liang X, Zheng L, Landwehr C, Lunsford D, Holmes D, Ji Y (2005) Global regulation of gene expression by ArlRS, a two-component signal transduction regulatory system of *Staphylococcus aureus*. J Bacteriol 187(15):5486–5492. doi:10.1128/JB.187.15.5486-5492.2005

Liang X, Yu C, Sun J, Liu H, Landwehr C, Holmes D, Ji Y (2006) Inactivation of a two-component signal transduction system, SaeRS, eliminates adherence and attenuates virulence of *Staphylococcus aureus*. Infect Immun 74(8):4655–4665. doi:10.1128/IAI.00322-06

Luong TT, Lee CY (2006) The *arl* locus positively regulates *Staphylococcus aureus* type 5 capsule via an *mgrA*-dependent pathway. Microbiology 152(Pt 10):3123–3131. doi:10.1099/mic.0.29177-0

Luong T, Sau S, Gomez M, Lee JC, Lee CY (2002) Regulation of *Staphylococcus aureus* capsular polysaccharide expression by *agr* and *sarA*. Infect Immun 70(2):444–450

Luong TT, Dunman PM, Murphy E, Projan SJ, Lee CY (2006) Transcription profiling of the mgrA regulon in *Staphylococcus aureus*. J Bacteriol 188(5):1899–1910. doi:10.1128/JB.188.5.1899-1910.2006

Mainiero M, Goerke C, Geiger T, Gonser C, Herbert S, Wolz C (2010) Differential target gene activation by the *Staphylococcus aureus* two-component system *saeRS*. J Bacteriol 192 (3):613–623. doi:10.1128/JB.01242-09

Makhlin J, Kofman T, Borovok I, Kohler C, Engelmann S, Cohen G, Aharonowitz Y (2007) *Staphylococcus aureus* ArcR controls expression of the arginine deiminase operon. J Bacteriol 189(16):5976–5986. doi:10.1128/JB.00592-07

Maroti G, Kereszt A, Kondorosi E, Mergaert P (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol 162(4):363–374. doi:10.1016/j.resmic.2011.02.005

Martin PK, Li T, Sun D, Biek DP, Schmid MB (1999) Role in cell permeability of an essential two-component system in *Staphylococcus aureus*. J Bacteriol 181(12):3666–3673

Mascher T (2006) Intramembrane-sensing histidine kinases: a new family of cell envelope stress sensors in Firmicutes bacteria. FEMS Microbiol Lett 264(2):133–144. doi:10.1111/j.1574-6968.2006.00444.x

Matsuo M, Kato F, Oogai Y, Kawai T, Sugai M, Komatsuzawa H (2010) Distinct two-component systems in methicillin-resistant *Staphylococcus aureus* can change the susceptibility to antimicrobial agents. J Antimicrob Chemother 65(7):1536–1537. doi:10.1093/jac/dkq141

McCallum N, Meier PS, Heusser R, Berger-Bachi B (2011) Mutational analyses of open reading frames within the *vraSR* operon and their roles in the cell wall stress response of *Staphylococcus aureus*. Antimicrob Agents Chemother 55(4):1391–1402. doi:10.1128/AAC.01213-10

McEvoy CR, Tsuji B, Gao W, Seemann T, Porter JL, Doig K, Ngo D, Howden BP, Stinear TP (2013) Decreased vancomycin susceptibility in *Staphylococcus aureus* caused by IS256 tempering of WalKR expression. Antimicrob Agents Chemother 57(7):3240–3249. doi:10.1128/AAC.00279-13

Meehl M, Herbert S, Gotz F, Cheung A (2007) Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 51(8):2679–2689. doi:10.1128/AAC.00209-07

Meier S, Goerke C, Wolz C, Seidl K, Homerova D, Schulthess B, Kormanec J, Berger-Bachi B, Bischoff M (2007) σB and the σB-dependent *arlRS* and *yabJ-spoVG* loci affect capsule formation in *Staphylococcus aureus*. Infect Immun 75(9):4562–4571. doi:10.1128/IAI.00392-07

Memmi G, Nair DR, Cheung A (2012) Role of ArlRS in autolysis in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus strains*. J Bacteriol 194(4):759–767. doi:10.1128/JB.06261-11

Mensa B, Howell GL, Scott R, DeGrado WF (2014) Comparative mechanistic studies of brilacidin, daptomycin, and the antimicrobial peptide LL16. Antimicrob Agents Chemother 58 (9):5136–5145. doi:10.1128/AAC.02955-14

Mike LA, Dutter BF, Stauff DL, Moore JL, Vitko NP, Aranmolate O, Kehl-Fie TE, Sullivan S, Reid PR, DuBois JL, Richardson AR, Caprioli RM, Sulikowski GA, Skaar EP (2013) Activation of heme biosynthesis by a small molecule that is toxic to fermenting *Staphylococcus aureus*. Proc Natl Acad Sci USA 110(20):8206–8211. doi:10.1073/pnas.1303674110

Miyazaki S, Matsumoto Y, Sekimizu K, Kaito C (2012) Evaluation of *Staphylococcus aureus* virulence factors using a silkworm model. FEMS Microbiol Lett 326(2):116–124. doi:10.1111/j.1574-6968.2011.02439.x

Monteiro JM, Fernandes PB, Vaz F, Pereira AR, Tavares AC, Ferreira MT, Pereira PM, Veiga H, Kuru E, VanNieuwenhze MS, Brun YV, Filipe SR, Pinho MG (2015) Cell shape dynamics during the staphylococcal cell cycle. Nat Commun 6:8055. doi:10.1038/ncomms9055

Morfeldt E, Taylor D, von Gabain A, Arvidson S (1995) Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII. EMBO J 14(18):4569–4577

Moscoso JA, Schramke H, Zhang Y, Tosi T, Dehbi A, Jung K, Grundling A (2015) Binding of c-di-AMP to the *Staphylococcus aureus* sensor kinase KdpD occurs via the USP domain and down-regulates the expression of the Kdp potassium transporter. J Bacteriol. doi:10.1128/JB.00480-15

Mullner M, Hammel O, Mienert B, Schlag S, Bill E, Unden G (2008) A PAS domain with an oxygen labile $[4Fe–4S]^{2+}$ cluster in the oxygen sensor kinase NreB of *Staphylococcus carnosus*. Biochemistry 47(52):13921–13932. doi:10.1021/bi8014086

Muzamal U, Gomez D, Kapadia F, Golemi-Kotra D (2014) Diversity of two-component systems: insights into the signal transduction mechanism by the *Staphylococcus aureus* two-component system GraSR. F1000Res 3:252. doi:10.12688/f1000research.5512.2

Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, Scully IL, McNeil LK, Aste-Amezaga JM, Cooper D, Jansen KU, Anderson AS (2012) Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Hum Vaccines Immunotherapeutics 9(3):480–487

Neoh HM, Cui L, Yuzawa H, Takeuchi F, Matsuo M, Hiramatsu K (2008) Mutated response regulator *graR* is responsible for phenotypic conversion of *Staphylococcus aureus* from heterogeneous vancomycin-intermediate resistance to vancomycin-intermediate resistance. Antimicrob Agents Chemother 52(1):45–53. doi:10.1128/AAC.00534-07

Niemann V, Koch-Singenstreu M, Neu A, Nilkens S, Gotz F, Unden G, Stehle T (2014) The NreA protein functions as a nitrate receptor in the staphylococcal nitrate regulation system. J Mol Biol 426(7):1539–1553. doi:10.1016/j.jmb.2013.12.026

Nilkens S, Koch-Singenstreu M, Niemann V, Gotz F, Stehle T, Unden G (2014) Nitrate/oxygen co-sensing by an NreA/NreB sensor complex of *Staphylococcus carnosus*. Mol Microbiol 91 (2):381–393. doi:10.1111/mmi.12464

Novick RP, Geisinger E (2008) Quorum sensing in staphylococci. Annu Rev Genet 42:541–564. doi:10.1146/annurev.genet.42.110807.091640

Novick RP, Jiang D (2003) The staphylococcal *saeRS* system coordinates environmental signals with *agr* quorum sensing. Microbiology 149(Pt 10):2709–2717

Novick RP, Muir TW (1999) Virulence gene regulation by peptides in staphylococci and other Gram-positive bacteria. Curr Opin Microbiol 2(1):40–45

Novick RP, Ross HF, Projan SJ, Kornblum J, Kreiswirth B, Moghazeh S (1993) Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J 12 (10):3967–3975

Novick RP, Projan SJ, Kornblum J, Ross HF, Ji G, Kreiswirth B, Vandenesch F, Moghazeh S (1995) The *agr* P2 operon: an autocatalytic sensory transduction system in *Staphylococcus aureus*. Mol Gen Genet 248(4):446–458

Nygaard TK, Pallister KB, Ruzevich P, Griffith S, Vuong C, Voyich JM (2010) SaeR binds a consensus sequence within virulence gene promoters to advance USA300 pathogenesis. J Infect Dis 201(2):241–254. doi:10.1086/649570

Ohki R, Giyanto Tateno K, Masuyama W, Moriya S, Kobayashi K, Ogasawara N (2003) The BceRS two-component regulatory system induces expression of the bacitracin transporter, BceAB, in *Bacillus subtilis*. Mol Microbiol 49(4):1135–1144

Olson ME, Nygaard TK, Ackermann L, Watkins RL, Zurek OW, Pallister KB, Griffith S, Kiedrowski MR, Flack CE, Kavanaugh JS, Kreiswirth BN, Horswill AR, Voyich JM (2013) *Staphylococcus aureus* nuclease is an SaeRS-dependent virulence factor. Infect Immun 81 (4):1316–1324. doi:10.1128/IAI.01242-12

Oun S, Redder P, Didier JP, Francois P, Corvaglia AR, Buttazzoni E, Giraud C, Girard M, Schrenzel J, Linder P (2013) The CshA DEAD-box RNA helicase is important for quorum sensing control in *Staphylococcus aureus*. RNA Biol 10(1):157–165. doi:10.4161/rna.22899

Painter KL, Krishna A, Wigneshweraraj S, Edwards AM (2014) What role does the quorum-sensing accessory gene regulator system play during *Staphylococcus aureus* bacteremia? Trends Microbiol. doi:10.1016/j.tim.2014.09.002

Palazzolo-Ballance AM, Reniere ML, Braughton KR, Sturdevant DE, Otto M, Kreiswirth BN, Skaar EP, DeLeo FR (2008) Neutrophil microbicides induce a pathogen survival response in community-associated methicillin-resistant *Staphylococcus aureus*. J Immunol 180(1):500–509

Pantrangi M, Singh VK, Wolz C, Shukla SK (2010) Staphylococcal superantigen-like genes, *ssl5* and *ssl8*, are positively regulated by Sae and negatively by Agr in the Newman strain. FEMS Microbiol Lett 308(2):175–184. doi:10.1111/j.1574-6968.2010.02012.x

Pantrangi M, Singh VK, Shukla SK (2015) Regulation of Staphylococcal superantigen-like gene, *ssl8*, expression in *Staphylococcus aureus* strain, RN6390. Clin Med Res 13(1):7–11. doi:10.3121/cmr.2014.1226

Parish T, Smith DA, Kendall S, Casali N, Bancroft GJ, Stoker NG (2003) Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. Infect Immun 71(3):1134–1140

Park SY, Chong YP, Park HJ, Park KH, Moon SM, Jeong JY, Kim MN, Kim SH, Lee SO, Choi SH, Woo JH, Kim YS (2013) *agr* dysfunction and persistent methicillin-resistant *Staphylococcus aureus* bacteremia in patients with removed eradicable foci. Infection 41(1):111–119. doi:10.1007/s15010-012-0348-0

Patton TG, Yang SJ, Bayles KW (2006) The role of proton motive force in expression of the *Staphylococcus aureus cid* and *lrg* operons. Mol Microbiol 59(5):1395–1404. doi:10.1111/j.1365-2958.2006.05034.x

Paulander W, Nissen Varming A, Baek KT, Haaber J, Frees D, Ingmer H (2013) Antibiotic-mediated selection of quorum-sensing-negative *Staphylococcus aureus*. MBio 3(6):e00459–00412. doi:10.1128/mBio.00459-12

Peschel A, Otto M (2013) Phenol-soluble modulins and staphylococcal infection. Nat Rev Microbiol 11(10):667–673. doi:10.1038/nrmicro3110

Pietiainen M, Francois P, Hyyrylainen HL, Tangomo M, Sass V, Sahl HG, Schrenzel J, Kontinen VP (2009) Transcriptome analysis of the responses of *Staphylococcus aureus* to antimicrobial peptides and characterization of the roles of *vraDE* and *vraSR* in antimicrobial resistance. BMC Genom 10:429. doi:10.1186/1471-2164-10-429

Pozzi C, Waters EM, Rudkin JK, Schaeffer CR, Lohan AJ, Tong P, Loftus BJ, Pier GB, Fey PD, Massey RC, O'Gara JP (2012) Methicillin resistance alters the biofilm phenotype and attenuates virulence in *Staphylococcus aureus* device-associated infections. PLoS Pathog 8(4):e1002626. doi:10.1371/journal.ppat.1002626

Pozzi C, Lofano G, Mancini F, Soldaini E, Speziale P, De Gregorio E, Rappuoli R, Bertholet S, Grandi G, Bagnoli F (2015) Phagocyte subsets and lymphocyte clonal deletion behind ineffective immune response to *Staphylococcus aureus*. FEMS Microbiol Rev. doi:10.1093/femsre/fuv024

Pragman AA, Yarwood JM, Tripp TJ, Schlievert PM (2004) Characterization of virulence factor regulation by SrrAB, a two-component system in *Staphylococcus aureus*. J Bacteriol 186(8):2430–2438

Pragman AA, Herron-Olson L, Case LC, Vetter SM, Henke EE, Kapur V, Schlievert PM (2007a) Sequence analysis of the *Staphylococcus aureus srrAB* loci reveals that truncation of *srrA* affects growth and virulence factor expression. J Bacteriol 189(20):7515–7519. doi:10.1128/JB.00547-07

Pragman AA, Ji Y, Schlievert PM (2007b) Repression of *Staphylococcus aureus* SrrAB using inducible antisense *srrA* alters growth and virulence factor transcript levels. Biochemistry 46(1):314–321. doi:10.1021/bi0603266

Qin Z, Zhang J, Xu B, Chen L, Wu Y, Yang X, Shen X, Molin S, Danchin A, Jiang H, Qu D (2006) Structure-based discovery of inhibitors of the YycG histidine kinase: new chemical leads to combat *Staphylococcus epidermidis* infections. BMC Microbiol 6:96. doi:10.1186/1471-2180-6-96

Qin Z, Lee B, Yang L, Zhang J, Yang X, Qu D, Jiang H, Molin S (2007) Antimicrobial activities of YycG histidine kinase inhibitors against *Staphylococcus epidermidis* biofilms. FEMS Microbiol Lett 273(2):149–156. doi:10.1111/j.1574-6968.2007.00749.x

Qiu R, Pei W, Zhang L, Lin J, Ji G (2005) Identification of the putative staphylococcal AgrB catalytic residues involving the proteolytic cleavage of AgrD to generate autoinducing peptide. J Biol Chem 280(17):16695–16704. doi:10.1074/jbc.M411372200

Queck SY, Jameson-Lee M, Villaruz AE, Bach TH, Khan BA, Sturdevant DE, Ricklefs SM, Li M, Otto M (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. doi:10.1016/j.molcel.2008.08.005

Rampone H, Martinez GL, Giraudo AT, Calzolari A, Nagel R (1996) In vivo expression of exoprotein synthesis with a Sae mutant of *Staphylococcus aureus*. Can J Vet Res 60(3):237–240

Ravcheev DA, Best AA, Tintle N, Dejongh M, Osterman AL, Novichkov PS, Rodionov DA (2011) Inference of the transcriptional regulatory network in *Staphylococcus aureus* by integration of experimental and genomics-based evidence. J Bacteriol 193(13):3228–3240. doi:10.1128/JB.00350-11

Recsei P, Kreiswirth B, Oreilly M, Schlievert P, Gruss A, Novick RP (1986) Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr*. Mol Gen Genet 202(1):58–61. doi:10.1007/Bf00330517

Reed SB, Wesson CA, Liou LE, Trumble WR, Schlievert PM, Bohach GA, Bayles KW (2001) Molecular characterization of a novel *Staphylococcus aureus* serine protease operon. Infect Immun 69(3):1521–1527. doi:10.1128/IAI.69.3.1521-1527.2001

Regassa LB, Betley MJ (1992) Alkaline pH decreases expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. J Bacteriol 174(15):5095–5100

Regassa LB, Novick RP, Betley MJ (1992) Glucose and nonmaintained pH decrease expression of the accessory gene regulator (*agr*) in *Staphylococcus aureus*. Infect Immun 60(8):3381–3388

Reinhart F, Huber A, Thiele R, Unden G (2010) Response of the oxygen sensor NreB to air *in vivo*: Fe-S-containing NreB and apo-NreB in aerobically and anaerobically growing *Staphylococcus carnosus*. J Bacteriol 192(1):86–93. doi:10.1128/JB.01248-09

Reyes D, Andrey DO, Monod A, Kelley WL, Zhang G, Cheung AL (2011) Coordinated regulation by AgrA, SarA, and SarR to control agr expression in *Staphylococcus aureus*. J Bacteriol 193 (21):6020–6031. doi:10.1128/JB.05436-11

Richardson AR, Dunman PM, Fang FC (2006) The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. Mol Microbiol 61(4):927–939. doi:10.1111/j.1365-2958.2006.05290.x

Rietkotter E, Hoyer D, Mascher T (2008) Bacitracin sensing in *Bacillus subtilis*. Mol Microbiol 68 (3):768–785. doi:10.1111/j.1365-2958.2008.06194.x

Rogasch K, Ruhmling V, Pane-Farre J, Hoper D, Weinberg C, Fuchs S, Schmudde M, Broker BM, Wolz C, Hecker M, Engelmann S (2006) Influence of the two-component system SaeRS on global gene expression in two different *Staphylococcus aureus* strains. J Bacteriol 188 (22):7742–7758. doi:10.1128/JB.00555-06

Rooijakkers SH, Ruyken M, van Roon J, van Kessel KP, van Strijp JA, van Wamel WJ (2006) Early expression of SCIN and CHIPS drives instant immune evasion by *Staphylococcus aureus*. Cell Microbiol 8(8):1282–1293. doi:10.1111/j.1462-5822.2006.00709.x

Rudkin JK, Edwards AM, Bowden MG, Brown EL, Pozzi C, Waters EM, Chan WC, Williams P, O'Gara JP, Massey RC (2012) Methicillin resistance reduces the virulence of health-care-associated methicillin-resistant *Staphylococcus aureus* by interfering with the *agr* quorum sensing system. J Infect Dis 205(5):798–806. doi:10.1093/infdis/jir845

Saenz HL, Augsburger V, Vuong C, Jack RW, Gotz F, Otto M (2000) Inducible expression and cellular location of AgrB, a protein involved in the maturation of the staphylococcal quorum-sensing pheromone. Arch Microbiol 174(6):452–455

Salgado-Pabon W, Schlievert PM (2014) Models matter: the search for an effective *Staphylococcus aureus* vaccine. Nat Rev Microbiol 12(8):585–591. doi:10.1038/nrmicro3308

Schafer D, Lam TT, Geiger T, Mainiero M, Engelmann S, Hussain M, Bosserhoff A, Frosch M, Bischoff M, Wolz C, Reidl J, Sinha B (2009) A point mutation in the sensor histidine kinase SaeS of *Staphylococcus aureus* strain Newman alters the response to biocide exposure. J Bacteriol 191(23):7306–7314. doi:10.1128/JB.00630-09

Schauder S, Shokat K, Surette MG, Bassler BL (2001) The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 41(2):463–476

Schlag S, Fuchs S, Nerz C, Gaupp R, Engelmann S, Liebeke M, Lalk M, Hecker M, Gotz F (2008) Characterization of the oxygen-responsive NreABC regulon of *Staphylococcus aureus*. J Bacteriol 190(23):7847–7858. doi:10.1128/JB.00905-08

Schroder W, Bernhardt J, Marincola G, Klein-Hitpass L, Herbig A, Krupp G, Nieselt K, Wolz C (2014) Altering gene expression by aminocoumarins: the role of DNA supercoiling in *Staphylococcus aureus*. BMC Genom 15:291. doi:10.1186/1471-2164-15-291

Sharma-Kuinkel BK, Mann EE, Ahn JS, Kuechenmeister LJ, Dunman PM, Bayles KW (2009) The *Staphylococcus aureus* LytSR two-component regulatory system affects biofilm formation. J Bacteriol 191(15):4767–4775. doi:10.1128/JB.00348-09

Sheehan BJ, Foster TJ, Dorman CJ, Park S, Stewart GS (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

Shoji M, Cui L, Iizuka R, Komoto A, Neoh HM, Watanabe Y, Hishinuma T, Hiramatsu K (2011) *walK* and *clpP* mutations confer reduced vancomycin susceptibility in *Staphylococcus aureus*. Antimicrob Agents Chemother 55(8):3870–3881. doi:10.1128/AAC.01563-10

Singh R, Ray P (2014) Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. Future Microbiol 9(5):669–681. doi:10.2217/fmb.14.31

Smyth DS, Kafer JM, Wasserman GA, Velickovic L, Mathema B, Holzman RS, Knipe TA, Becker K, von Eiff C, Peters G, Chen L, Kreiswirth BN, Novick RP, Shopsin B (2012) Nasal carriage as a source of *agr*-defective *Staphylococcus aureus* bacteremia. J Infect Dis 206 (8):1168–1177. doi:10.1093/infdis/jis483

Srivastava SK, Rajasree K, Fasim A, Arakere G, Gopal B (2014) Influence of the AgrC-AgrA complex on the response time of *Staphylococcus aureus* quorum sensing. J Bacteriol 196 (15):2876–2888. doi:10.1128/JB.01530-14

Stauff DL, Torres VJ, Skaar EP (2007) Signaling and DNA-binding activities of the *Staphylococcus aureus* HssR-HssS two-component system required for heme sensing. J Biol Chem 282(36):26111–26121. doi:10.1074/jbc.M703797200

Stauff DL, Bagaley D, Torres VJ, Joyce R, Anderson KL, Kuechenmeister L, Dunman PM, Skaar EP (2008) *Staphylococcus aureus* HrtA is an ATPase required for protection against heme toxicity and prevention of a transcriptional heme stress response. J Bacteriol 190 (10):3588–3596. doi:10.1128/JB.01921-07

Su J, Iehara M, Yasukawa J, Matsumoto Y, Hamamoto H, Sekimizu K (2015) A novel mutation in the *vraS* gene of *Staphylococcus aureus* contributes to reduce susceptibility against daptomycin. J Antibiot (Tokyo). doi:10.1038/ja.2015.42

Sun J, Zheng L, Landwehr C, Yang J, Ji Y (2005) Identification of a novel essential two-component signal transduction system, YhcSR *Staphylococcus aureus*. J Bacteriol 187 (22):7876–7880. doi:10.1128/JB.187.22.7876-7880.2005

Sun F, Li C, Jeong D, Sohn C, He C, Bae T (2010) In the *Staphylococcus aureus* two-component system *sae*, the response regulator SaeR binds to a direct repeat sequence and DNA binding requires phosphorylation by the sensor kinase SaeS. J Bacteriol 192(8):2111–2127. doi:10.1128/JB.01524-09

Sun F, Ji Q, Jones MB, Deng X, Liang H, Frank B, Telser J, Peterson SN, Bae T, He C (2012) AirSR, a [2Fe–2S] cluster-containing two-component system, mediates global oxygen sensing and redox signaling in *Staphylococcus aureus*. J Am Chem Soc 134(1):305–314. doi:10.1021/ja2071835

Szurmant H, Nelson K, Kim EJ, Perego M, Hoch JA (2005) YycH regulates the activity of the essential YycFG two-component system in *Bacillus subtilis*. J Bacteriol 187(15):5419–5426. doi:10.1128/JB.187.15.5419-5426.2005

Szurmant H, Zhao H, Mohan MA, Hoch JA, Varughese KI (2006) The crystal structure of YycH involved in the regulation of the essential YycFG two-component system in *Bacillus subtilis* reveals a novel tertiary structure. Protein Sci 15(4):929–934. doi:10.1110/ps.052064406

Szurmant H, Mohan MA, Imus PM, Hoch JA (2007) YycH and YycI interact to regulate the essential YycFG two-component system in *Bacillus subtilis*. J Bacteriol 189(8):3280–3289. doi:10.1128/JB.01936-06

Thakker M, Park JS, Carey V, Lee JC (1998) *Staphylococcus aureus* serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect Immun 66(11):5183–5189

Thoendel M, Kavanaugh JS, Flack CE, Horswill AR (2011) Peptide signaling in the staphylococci. Chem Rev 111(1):117–151. doi:10.1021/cr100370n

Thomsen LE, Gottlieb CT, Gottschalk S, Wodskou TT, Kristensen HH, Gram L, Ingmer H (2010) The heme sensing response regulator HssR in *Staphylococcus aureus* but not the homologous RR23 in *Listeria monocytogenes* modulates susceptibility to the antimicrobial peptide plectasin. BMC Microbiol 10:307. doi:10.1186/1471-2180-10-307

Throup JP, Zappacosta F, Lunsford RD, Annan RS, Carr SA, Lonsdale JT, Bryant AP, McDevitt D, Rosenberg M, Burnham MK (2001) The *srhSR* gene pair from *Staphylococcus aureus*: genomic and proteomic approaches to the identification and characterization of gene function. Biochemistry 40(34):10392–10401

Toledo-Arana A, Merino N, Vergara-Irigaray M, Debarbouille M, Penades JR, Lasa I (2005) *Staphylococcus aureus* develops an alternative, ica-independent biofilm in the absence of the *arlRS* two-component system. J Bacteriol 187(15):5318–5329. doi:10.1128/JB.187.15.5318-5329.2005

Torres VJ, Stauff DL, Pishchany G, Bezbradica JS, Gordy LE, Iturregui J, Anderson KL, Dunman PM, Joyce S, Skaar EP (2007) A *Staphylococcus aureus* regulatory system that responds to host heme and modulates virulence. Cell Host Microbe 1(2):109–119. doi:10.1016/j.chom.2007.03.001

Truong-Bolduc QC, Dunman PM, Eidem T, Hooper DC (2011) Transcriptional profiling analysis of the global regulator NorG, a GntR-like protein of *Staphylococcus aureus*. J Bacteriol 193(22):6207–6214. doi:10.1128/JB.05847-11

Tu Quoc PH, Genevaux P, Pajunen M, Savilahti H, Georgopoulos C, Schrenzel J, Kelley WL (2007) Isolation and characterization of biofilm formation-defective mutants of *Staphylococcus aureus*. Infect Immun 75(3):1079–1088. doi:10.1128/IAI.01143-06

Tuchscherr L, Loffler B, Buzzola FR, Sordelli DO (2010) *Staphylococcus aureus* adaptation to the host and persistence: role of loss of capsular polysaccharide expression. Future Microbiol 5(12):1823–1832. doi:10.2217/fmb.10.147

Ulrich M, Bastian M, Cramton SE, Ziegler K, Pragman AA, Bragonzi A, Memmi G, Wolz C, Schlievert PM, Cheung A, Doring G (2007) The staphylococcal respiratory response regulator SrrAB induces ica gene transcription and polysaccharide intercellular adhesin expression, protecting *Staphylococcus aureus* from neutrophil killing under anaerobic growth conditions. Mol Microbiol 65(5):1276–1287. doi:10.1111/j.1365-2958.2007.05863.x

van Wamel W, Xiong YQ, Bayer AS, Yeaman MR, Nast CC, Cheung AL (2002) Regulation of *Staphylococcus aureus* type 5 capsular polysaccharides by *agr* and *sarA in vitro* and in an experimental endocarditis model. Microb Pathog 33(2):73–79

Vandenesch F, Kornblum J, Novick RP (1991) A temporal signal, independent of *agr*, is required for *hla* but not *spa* transcription in *Staphylococcus aureus*. J Bacteriol 173(20):6313–6320

Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR (2005) Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. Nat Rev Microbiol 3(5):383–396. doi:10.1038/nrmicro1146

Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR (2005) Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. J Immunol 175(6):3907–3919

Voyich JM, Vuong C, DeWald M, Nygaard TK, Kocianova S, Griffith S, Jones J, Iverson C, Sturdevant DE, Braughton KR, Whitney AR, Otto M, Deleo FR (2009) The SaeR/S gene regulatory system is essential for innate immune evasion by *Staphylococcus aureus*. J Infect Dis 199(11):1698–1706. doi:10.1086/598967

Wakeman CA, Stauff DL, Zhang Y, Skaar EP (2014) Differential activation of *Staphylococcus aureus* heme detoxification machinery by heme analogues. J Bacteriol 196(7):1335–1342. doi:10.1128/JB.01067-13

Walker JN, Crosby HA, Spaulding AR, Salgado-Pabon W, Malone CL, Rosenthal CB, Schlievert PM, Boyd JM, Horswill AR (2013) The *Staphylococcus aureus* ArlRS two-component system is a novel regulator of agglutination and pathogenesis. PLoS Pathog 9(12):e1003819. doi:10.1371/journal.ppat.1003819

Watanabe T, Hashimoto Y, Yamamoto K, Hirao K, Ishihama A, Hino M, Utsumi R (2003) Isolation and characterization of inhibitors of the essential histidine kinase, YycG in *Bacillus subtilis* and *Staphylococcus aureus*. J Antibiot (Tokyo) 56(12):1045–1052

White MJ, Boyd JM, Horswill AR, Nauseef WM (2014) Phosphatidylinositol-specific phospholipase C contributes to survival of *Staphylococcus aureus* USA300 in human blood and neutrophils. Infect Immun 82(4):1559–1571. doi:10.1128/IAI.01168-13

Winzer K, Hardie KR, Burgess N, Doherty N, Kirke D, Holden MT, Linforth R, Cornell KA, Taylor AJ, Hill PJ, Williams P (2002) LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. Microbiology 148(Pt 4):909–922

Wolz C, Pohlmann-Dietze P, Steinhuber A, Chien YT, Manna A, van Wamel W, Cheung A (2000) *Agr*-independent regulation of fibronectin-binding protein(s) by the regulatory locus *sar* in *Staphylococcus aureus*. Mol Microbiol 36(1):230–243

Wright JS 3rd, Traber KE, Corrigan R, Benson SA, Musser JM, Novick RP (2005) The *agr* radiation: an early event in the evolution of staphylococci. J Bacteriol 187(16):5585–5594. doi:10.1128/JB.187.16.5585-5594.2005

Xiong YQ, Willard J, Yeaman MR, Cheung AL, Bayer AS (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 doi:JID36622 [pii]

Xue T, You Y, Hong D, Sun H, Sun B (2011) The *Staphylococcus aureus* KdpDE two-component system couples extracellular K+ sensing and Agr signaling to infection programming. Infect Immun 79(6):2154–2167. doi:10.1128/IAI.01180-10

Yan M, Yu C, Yang J, Ji Y (2011) The essential two-component system YhcSR is involved in regulation of the nitrate respiratory pathway of *Staphylococcus aureus*. J Bacteriol 193 (8):1799–1805. doi:10.1128/JB.01511-10

Yan M, Hall JW, Yang J, Ji Y (2012) The essential *yhcSR* two-component signal transduction system directly regulates the lac and *opuCABCD* operons of *Staphylococcus aureus*. PLoS ONE 7(11):e50608. doi:10.1371/journal.pone.0050608

Yang SJ, Xiong YQ, Yeaman MR, Bayles KW, Abdelhady W, Bayer AS (2013) Role of the LytSR two-component regulatory system in adaptation to cationic antimicrobial peptides in *Staphylococcus aureus*. Antimicrob Agents Chemother 57(8):3875–3882. doi:10.1128/AAC.00412-13

Yarwood JM, Schlievert PM (2003) Quorum sensing in *Staphylococcus* infections. J Clin Invest 112(11):1620–1625. doi:10.1172/JCI20442

Yarwood JM, McCormick JK, Schlievert PM (2001) Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus*. J Bacteriol 183(4):1113–1123. doi:10.1128/JB.183.4.1113-1123.2001

Yin S, Daum RS, Boyle-Vavra S (2006) VraSR two-component regulatory system and its role in induction of *pbp2* and *vraSR* expression by cell wall antimicrobials in *Staphylococcus aureus*. Antimicrob Agents Chemother 50(1):336–343. doi:10.1128/AAC.50.1.336-343.2006

Yoshida Y, Matsuo M, Oogai Y, Kato F, Nakamura N, Sugai M, Komatsuzawa H (2011) Bacitracin sensing and resistance in *Staphylococcus aureus*. FEMS Microbiol Lett 320(1):33–39. doi:10.1111/j.1574-6968.2011.02291.x

Zhang S, Stewart GC (2000) Characterization of the promoter elements for the staphylococcal enterotoxin D gene. J Bacteriol 182(8):2321–2325

Zhang L, Gray L, Novick RP, Ji G (2002) Transmembrane topology of AgrB, the protein involved in the post-translational modification of AgrD in *Staphylococcus aureus*. J Biol Chem 277 (38):34736–34742. doi:10.1074/jbc.M205367200

Zhao L, Xue T, Shang F, Sun H, Sun B (2010) *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. Infect Immun 78(8):3506–3515. doi:10.1128/IAI.00131-10

Zhao F, Cheng BL, Boyle-Vavra S, Alegre ML, Daum RS, Chong AS, Montgomery CP (2015) Proteomic identification of *saeRS*-dependent targets critical for protective humoral immunity against *Staphylococcus aureus* skin infection. Infect Immun. doi:10.1128/IAI.00667-15

Staphylococcus aureus-Associated Skin and Soft Tissue Infections: Anatomical Localization, Epidemiology, Therapy and Potential Prophylaxis

Reuben Olaniyi, Clarissa Pozzi, Luca Grimaldi and Fabio Bagnoli

Abstract Skin and soft tissue infections (SSTIs) are among the most common infections worldwide. They range in severity from minor, self-limiting, superficial infections to life-threatening diseases requiring all the resources of modern medicine. Community (CA) and healthcare (HA) acquired SSTIs are most commonly caused by *Staphylococcus aureus*. They have variable presentations ranging from impetigo and folliculitis to surgical site infections (SSIs). Superficial SSTIs may lead to even more invasive infections such as bacteraemia and osteomyelitis. Here we describe the anatomical localization of the different SSTI associated with *S. aureus*, the virulence factors known to play a role in these infections, and their current epidemiology. Current prevention and treatment strategies are also discussed. Global epidemiological data show increasing incidence and severity of SSTIs in association with methicillin-resistant *S. aureus* strains (MRSA). CA-SSTIs are usually less morbid compared to other invasive infections caused by *S. aureus*, but they have become the most prevalent, requiring a great number of medical interventions, extensive antibiotic use, and therefore a high cost burden. Recurrence of SSTIs is common after initial successful treatment, and decolonization strategies have not been effective in reducing recurrence. Furthermore, decolonization approaches may be contributing to the selection and maintenance of multi-drug resistant strains. Clinical studies from the early 1900s and novel autovaccination approaches suggest an alternative strategy with potential effectiveness: using vaccines to control *S. aureus* cutaneous infections.

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Abbreviations

SSTI	Skin and soft tissue infection
CA	Community acquired
HA	Hospital acquired
SSIs	Surgical site infections
MRSA	Methicillin-resistant *Staphylococcus aureus*
CA-SSTIs	Community acquired skin and soft tissue infections
SA-SSTIs	*Staphylococcus aureus* skin and soft tissue infections
PRR	Pattern recognition receptor
PAMPs	Pathogen-associated molecular patterns
MSSA	Methicillin sensitive *Staphylococcus aureus*
SSSS	Staphylococcal scalded skin syndrome
SCC*mec*	Staphylococcal chromosome cassette *mec*
NNIS	National nosocomial infections surveillance
HA-MRSA	Hospital-associated methicillin-resistant *Staphylococcus aureus*
CA-MRSA	Community-associated methicillin resistant *Staphylococcus aureus*
SA-SSIs	*Staphylococcus aureus* surgical site infections
ICU	Intensive care unit
SEA	Staphylococcal enterotoxins A
SEB	Staphylococcal enterotoxins B
TSST-1	Toxic shock syndrome toxin-1
Th2	T-helper cell 2
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
FnBPs	Fibronectin-binding proteins
ClfA and ClfB	Clumping factor A and B
IsdA	Iron surface determinant A
WTA	Wall teichoic acid
ETA and ETB	Exfoliative toxins A and B
PVL	Panton–Valentine leukocidin
Hla	Alpha hemolyisin
ACME	Arginine catabolic mobile element
SIRS	Systemic inflammatory response syndrome
ED	Emergency department
CoNS	Coagulase negative *S. aureus*

Contents

1 Introduction

Staphylococcus aureus is both a commensal and a major pathogen in humans. On the one hand, *S. aureus* ubiquitously colonizes the moist skin of the anterior nares of approximately 30 % of healthy, asymptomatic adults (Kluytmans et al. 1997; Eriksen et al. 1995). Surveys of non-nasal skin sites such as the axillae and the perianal region suggest an even higher percentage of asymptomatic persistent and transient *S. aureus* carriers (Wertheim et al. 2005). On the other hand, *S. aureus* is a leading cause of bacteraemia, infective endocarditis, osteomyelitis, pneumonia, indwelling medical device related infections, as well as skin and soft tissue infections (SSTIs). Colonization of the skin and mucosa with *S. aureus* may precede and increase the risk of invasive infections. One of the most susceptible populations to *S. aureus* infection are HIV-infected patients, who have a high rate of *S. aureus* carriage in the anterior nares, increasing the incidence of superficial and deep dermal pathology and consequently invasive infections (Berger 1993). The emergence of multidrug-resistant strains has increased the mortality and morbidity rates associated with these infections in the community and hospital environment.

S. aureus SSTIs (SA-SSTIs) affect patients of all ages and manifest in a diverse array of clinical presentations, varying from superficial and harmless, to severe and life-threatening. Uncomplicated SSTIs account for a large number of patients seeking medical care annually. The number of ambulatory care visits has been reported to range between 11.6 and 14.2 million in the United States (McCaig et al. 2006; Hersh et al. 2008). Although low in severity compared to other forms of *S. aureus* infections, uncomplicated SSTIs account for the most prevalent form of staphylococcal infections and *S. aureus* represents the most frequent cause of

SSTIs, in both adult and paediatric populations (Ray et al. 2013a). SA-SSTIs are also associated with the development of serious complications, as bacteria can gain access to deeper tissue and disseminate to the bloodstream causing invasive disease such as endocarditis, osteomyelitis, deep tissue abscesses, sepsis and pneumonia (Tattevin et al. 2012). *S. aureus* is also the leading cause of surgical site infections (SSIs) (Deverick and Anderson 2009). Recurrent SSTIs are common, and the emergence of multidrug-resistant *S. aureus* strains limits available antimicrobial therapies (Creech et al. 2015).

This review provides an overview of the various SA-SSTIs, worldwide epidemiology of community acquired (CA) and hospital acquired (HA) infections, virulence factors and pathogenesis, as well as current antimicrobial therapy and prophylaxis.

2 Human Skin Anatomy

The skin is the largest organ of the body corresponding to 15 % of the total body weight in adults. Human skin has three main compartments: the epidermis, the dermis and the subcutis (Fig. 1). The epidermis is the outermost layer of the skin consisting of an avascular stratified epithelium. Its thickness is about 0.2 mm on average and this thickness varies depending on the part of the body and the amount of water that it contains.

The epidermis is composed of five layers. The innermost *stratum basale* contains actively mitotic stem cells that divide and move towards the outer surface to become part of the more superficial layers. The basal layer is responsible for constantly renewing the cells of the epidermis. One cycle of turnover of the epidermis takes approximately 28 days. Basal keratinocytes differentiate and move to the *stratum spinosum* to begin a maturation process, but also divide to replenish the basal layer. T cells (mainly $CD8^+$ T cells), melanin-producing cells called melanocytes and Langerhans cells can be found in the *stratum basale*. The *stratum spinosum* is the thickest layer of the epidermis with 10–20 layers of cells (50–150 μm) that lie on top of the basal layer (Anderson and Parrish 1982). Cells that move into the *stratum spinosum* become polygonal in shape and synthesize keratins (keratin 1 and 10) that are distinct from the basal layer keratins (keratin 5 and 14). The term *stratum spinosum* refers to the shape of the keratinocytes in histological sections whose spiny appearance is due to shrinking of the microfilaments between desmosomes. In addition, the *stratum spinosum* contains melanocytes and Langerhans cells, which are the main skin-resident immune cell. The third layer, *stratum granulosum* is comprised of a population of keratinocytes whose keratin content is higher and moisture content lower in comparison with the preceding two layers. The *stratum lucidum*, the fourth layer, is visible by light microscopy only in areas of thick skin, which are found on the palms of the hands and the soles of the feet. The cells are flattened and tightly packed. The fifth layer is the *stratum corneum*. Cells in this layer, known as corneocytes or horny cells, are dead

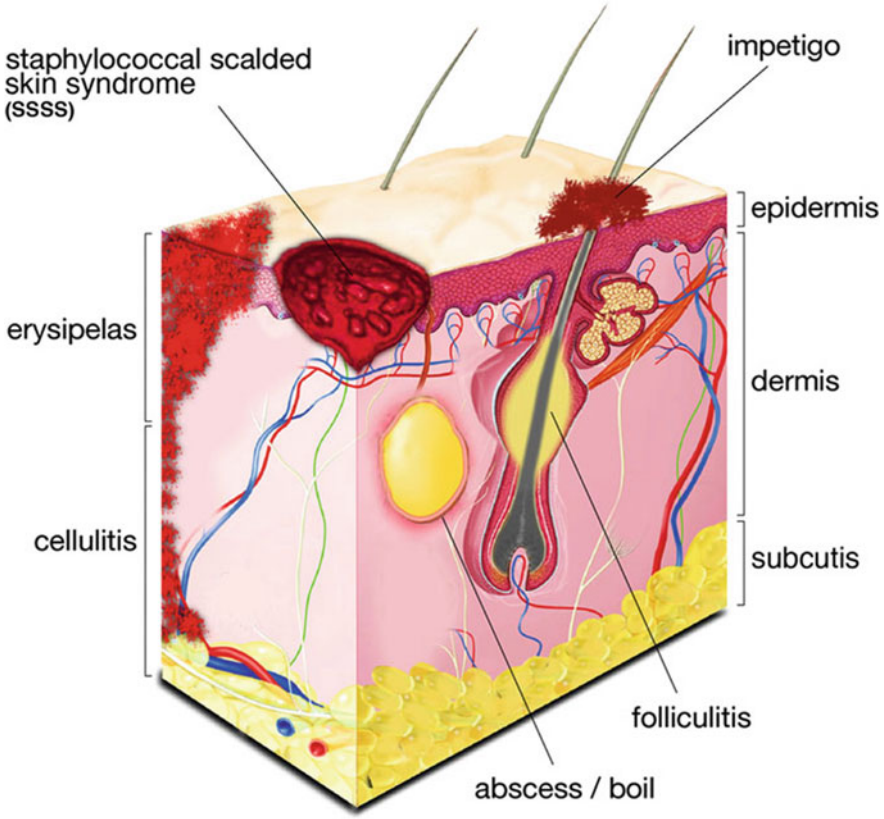

Fig. 1 Anatomical localization of skin infections. The image illustrates main superficial skin infections. The epidermis can be affected by impetigo, staphylococcal scalded skin syndrome (SSSS) and erysipelas. The dermis and subcutis are affected by erysipelas and cellulitis. Manifestations affecting hair follicles include folliculitis, furuncles and carbuncles. Structural components of the skin are indicated on the *right*

keratinocytes that lack organelles. They provide the barrier against many toxic agents and prevents dehydration (Proksch et al. 2008; Krueger and Stingl 1989).

One of the skin's most important functions is to form a protective barrier against environmental insults. The epidermis comprises physical, chemical/biochemical and cellular (both innate and adaptive) defences. The physical barrier is represented primarily by the *stratum corneum* but the other layers of the epidermis containing nucleated cells also contribute to the barrier through cell–cell junctions (tight and adherens junctions) and cytoskeletal proteins. Keratinocytes and Langerhans cells in the epidermis, as well as dermal mast cells, dendritic cells and macrophages, release antimicrobial peptides, cytokines and chemotactic proteins as a danger signals (Kupper and Fuhlbrigge 2004; Murphy et al. 2000). The *stratum corneum* contains antimicrobial peptides such as β-defensin 2, β-defensin 3, cathelicidins and

RNase 7 which have bacteriostatic and bactericidal activity against skin pathogens (Schauber and Gallo 2009; Otto 2010). Keratinocytes of the granular, *spinosum*, and basal layers express pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) of invading microorganisms, initiating a prompt cutaneous immune response (Fournier and Philpott 2005; Kawai and Akira 2010). Adaptive immunity of the skin consists of both humoral and cellular components orchestrated by intradermal B and T cells respectively (Kupper and Fuhlbrigge 2004; Nestle et al. 2009).

3 Overview of SSTIs

Classification of SSTIs is dependent on the location and depth of the infection. In particular impetigo is superficially localized in the *stratum corneum* of the epidermis, ecthyma on the superficial epidermis, while erysipelas, cellulitis and necrotizing fasciitis are confined to a deeper level of the dermis. Carbuncles and furuncles involve the hair follicles (Fig. 1 and Table 1).

3.1 Superficial Skin Infections—Impetigo and Ecthyma

Impetigo, the most common bacterial infection in children is a localized purulent superficial skin infection. *S. aureus* has been reported to be a cause of both bullous and non-bullous impetigo. Bullous impetigo begins as a vesicle, but then forms a flaccid bulla. The bulla soon ruptures and forms a thin light brown crust (Gemmell 1995; Ladhani et al. 1999; Melish and Glasgow 1970).

Non-bullous impetigo (impetigo contagiosa) begins as a single red papule that becomes a vesicle. After the vesicle ruptures the contents dry out and form honey-coloured crusts. Non-bullous impetigo typically arises on the face or extremities following minor cutaneous trauma.

Staphylococcal scalded skin syndrome (SSSS) predominantly affects neonates, infants, and children (Farrell 1999). SSSS is characterized by acute onset of erythema on the face and extremities, followed by the formation of clear and superficial bullous lesions which break to reveal bright red moist skin. SSSS ranges in severity from discrete regions of local blistering to a widespread scalding of a significant area of the skin. SSSS is a systemic manifestation of a toxin producing strain infection, which in a more localized form causes bullous impetigo. These distinctive, fluid-filled lesions result from the action of secreted staphylococcal exfoliative toxins (ETA), which cleave host proteins in the epidermal *stratum granulosum* and lead to the sloughing of this blistered layer of tissue (Nishifuji et al. 2008).

Ecthyma is a deeper form of impetigo in which ulcerations form underneath crusted plaques penetrating the superficial dermis. The lower extremities are the most common site of involvement, and scarring is common.

Table 1 Types of *S. aureus* infection in skin and soft tissue (SSTI)

Skin disease	Skin layer	Disease manifestation	Age range	Predisposing risk factors
Impetigo (bullous and non-bullous)	Epidermis (*Stratum corneum*)	Flaccid purulent blisters up to 2 cm in diameter The roof of the blister ruptures easily with shiny and wet basis. **Bullous** impetigo occurs most commonly in regions such as the diaper area, axillae and neck, palms and soles. The **non-bullous** impetigo is characterized by the rupture of the vesicles that dries as an adhering and yellowish (honey-coloured) crust. Lesions occur in exposed areas, especially in the limbs and face	Most common in newborn and young children, also in adults	Atopic dermatitis (Adachi et al. 1998; Hartman-Adams et al. 2014), scabies (Lejbkowicz et al. 2005; Romani et al. 2015), HIV (Donovan et al. 1992), diabetes mellitus, hemodialysis, chemotherapy, radiation therapy, corticosteroid treatment, leukemia, chronic granulomatous disease (Pereira 2014)
Staphylococcal scalded skin syndrome (SSSS)	Epidermis	Acute exfoliation of the skin typically following an erythematous cellulitis. Severity varies from a few blisters localized to the site of infection to a severe exfoliation affecting almost the entire body	Infants and children	Poor renal function, impetigo (Nichols and Florman 2001)
Erysipelas	Epidermis–dermis	Form of cellulitis with marked superficial inflammation, typically affecting the lower limbs and the face	Infants, young children and elderly	Diabetes (Raya-Cruz et al. 2014), immune-suppressed, obesity (Scheinfeld 2004), eczema, tinea pedis (Bonnetblanc and Bedane 2003), venous insufficiency (Jorup-Ronstrom and Britton 1987)

(continued)

Table 1 (continued)

Skin disease	Skin layer	Disease manifestation	Age range	Predisposing risk factors
Folliculitis	Follicule	Pyoderma that arises within a hair follicle. These lesions occur most frequently in the moist areas of the body and in areas subject to friction and perspiration	Adults	HIV (Holmes et al. 2002; Berger et al. 1990), diabetes mellitus, corticosteroid treatment, obesity, blood dyscrasias, defects in neutrophil function, transplant-related immunosuppression, acquired immunodeficiency syndrome (Nichols and Florman 2001)
Abscess	Follicule (furuncles and carbuncles)	Deeper infections of hair follicle can lead to furuncles or carbuncles. A furuncle affects hair-bearing area that discharges purulent, necrotic debris. Carbuncles are multiple furuncles that combine to form large, deep, organized abscesses	Adults	Diabetes (Shah et al. 1987; Meurehg-Haik and Garcia-Velasco 1974), obesity, steroid therapy, atopic dermatitis, kidney failure
	Epidermis–Dermis	Abscess caused by surgical site infections, abrasions, anal ulcerations, punctures and minor breaks	Any age	Military training, physical exercise sport practice, surgical procedures
Ecthyma	Dermis	Ecthyma is a deeper form of impetigo in which ulcerations form beneath crusted plaques. Lesions affect the lower extremities	Children and elderly	Diabetes, neutropenia (Pechter et al. 2012), immunosuppressive medication, malignancy (Avolio et al. 2012), HIV (Ungprasert et al. 2013), transplant-related immunosuppression (Nakai et al. 2008), impetigo (Edlich et al. 2005)

(continued)

Table 1 (continued)

Skin disease	Skin layer	Disease manifestation	Age range	Predisposing risk factors
Cellulitis	Dermis and subcutaneous	Caused by spreading bacterial inflammation of the skin, with redness, pain, lymphangitis, fever and raised white blood cell count	Adults	Diabetes (Raya-Cruz et al. 2014), lymphedema (Dupuy et al. 1999; Morris 2008), obesity (Scheinfeld 2004),eczema, impetigo (Nichols and Florman 2001), immunosuppression (Nichols and Florman 2001), tinea pedis (Semel and Goldin 1996)

3.2 *Follicular Infections—Folliculitis, Furunculosis, Carbunculosis*

S. aureus folliculitis is characterized by pustules within hair follicles. Carriage of *S. aureus* in the nares may lead to sycosis vulgaris, also known as sycosis barbae, which is characterized by an inflammatory chronic infection of the chin and beard area. Deeper infections of hair follicles can lead to furuncles or carbuncles. A furuncle, or boil, is a small abscess of a deep hair follicle that forms a nodule filled with purulent and necrotic debris. Carbuncles are larger lesions formed by coalescence of multiple furuncles that combine to form large, deep, organized abscesses.

Abscess formation begins as a severe localized inflammatory process triggered by the host response to the invading bacteria. It appears as swollen, red tender, fluctuant mass surrounded by cellulitis and generally involves trunk and extremities. Polymorphonuclear leukocytes (PMNs) play a key role in the formation and resolution of the abscess with necrotic and live neutrophils forming the collection of pus inside the abscess. The tissue–specific immune mechanisms influence the development of the abscess formation whereas the overall structure of the abscess is consistent regardless the anatomical location (Kobayashi et al. 2015).

S. aureus is one of the most common cause of perineal, prostatic and breast abscess (Talan and Singer 2014; Lachant et al. 2013). *S. aureus* is also the predominant pathogen in postpartum breast abscesses, accounting for 32–100 % of all culture-confirmed cases (Stafford et al. 2008; Moazzez et al. 2007; Dener and Inan 2003)

3.3 *Intradermal Infections—Erysipelas, Cellulitis, Necrotizing Fasciitis*

Erysipelas is an infection of the superficial dermis that also affects the superficial lymphatics. Since it involves the more superficial dermis the lesions are well demarcated and very erythematous. Erysipelas can affect the lower extremities (70 % of cases) and the face (20 %). However, erysipelas is typically caused by *Streptococcus pyogenes*.

Cellulitis involves the subcutaneous dermis and fat and is frequently caused by either *S. pyogenes* or *S. aureus* (Chiller et al. 2001). The affected area becomes tender, warm, erythematous, and swollen. Because the infection involves the looser subcutaneous tissue and not only the upper dermis, the involved areas may be poorly delimited. The organism responsible for cellulitis is difficult to identify, since diffuse infections without abscess formation are difficult to culture. Local abscesses, necrotizing fasciitis, and septicemia may complicate cellulitis (Chiller et al. 2001).

Necrotizing fasciitis is a potentially life-threatening infection of the subcutaneous tissue that rapidly spreads along tissue fascial planes (Tharakaram and Keczkes 1988). Erythema and edema progress to violaceous, dusky plaques within 48 h which quickly become necrotic. The majority of monomicrobial necrotizing fasciitis are caused by *Streptococcus pyogenes*, but CA-MRSA is emerging as another predominant causative agent in recent years (Miller et al. 2005).

4 Epidemiology of *Staphylococcus aureus* SSTIs

4.1 *Community-Acquired SSTIs*

Community-acquired SA-SSTIs are generally frequent, but incidence is quite variable from country to country. Annual incidence rates of SA-STTIs based on US military health system data was estimated to be between 122.7 and 168.9 per 100,000 person-years (Landrum et al. 2012). In the US community incidence of SA-SSTIs has been increasing due to the high prevalence of CA-MRSA, with certain populations affected disproportionately (i.e. children, the elderly, and African Americans) (Ray et al. 2013a; Hersh et al. 2008; Marra et al. 2012; Suaya et al. 2014). Between 1997 and 2005 was observed an increase of visits to physician offices and emergency departments with SA-SSTIs (from 32.1 to 48.1 visits per 1000 population) in the United States (Hersh et al. 2008). The incidence rate of boils or abscesses in the UK between years 1995–2010 has been observed to be 450 per 100,000 person-years (Shallcross et al. 2014). Similar incidence rate of SSTIs has been observed in Oceania, with 108 per 100,000 person-years in New Zealand (Williamson et al. 2014).

Recurrent SA-SSTIs are common and problematic, with rates ranging between 9 and 70 % in the US (Creech et al. 2015; Miller et al. 2007; Fritz et al. 2012; Montgomery et al. 2015; Miller et al. 2015). As many as 70 % of patients with CA-SSTIs experience recurrent SSTIs over 1 year, even after successful initial treatment (Kaplan et al. 2014; Duong et al. 2010; Chen et al. 2009; Williams et al. 2011). Interestingly, Chen et al. observed that recurrent infections were usually more common after infection by MRSA than with MSSA (Chen et al. 2009; Williams et al. 2011). The rate of repeat consultation for a boil or abscess in the UK increased from 66 per 100,000 person-years in 1995 to peak at 97 per 100,000 person-years in 2006, which remained stable thereafter (Shallcross et al. 2014).

Though several pathogens such as *Pseudomonas aeruginosa*, *Enterococcus* spp., *Escherichia coli* and *Streptococcus pyogenes* (beta-hemolytic streptococci) are known to cause SSTIs (Dryden 2009; Moet et al. 2007), *S. aureus* is the most frequently isolated pathogen from SSTIs across many countries (Moet et al. 2007; Mawalla et al. 2011; Ojulong et al. 2009; Kesah et al. 2003; Anguzu and Olila 2007; Seni et al. 2013). This pathogen is associated with 20–45 % of all SSTIs in the African continent (Mawalla et al. 2011; Ojulong et al. 2009; Kesah et al. 2003; Anguzu and Olila 2007; Seni et al. 2013), 1–40 % in Europe (>50 % in Romania and Portugal) (Moet et al. 2007; Jonathan and Otter 2010; Bassetti et al. 2014; Lorette et al. 2009; Niniou et al. 2008), 34 % in Latin America, 45 % in North America (Moet et al. 2007), and 1–17 % in Asia (Song et al. 2011; Zhao et al. 2012). Worldwide, CA-MRSA infections are caused by more than 20 distinct genetic lineages. Five of these CA-*S. aureus* strains are globally predominant, including ST1-IV (WA-1, USA400), ST8-IV (USA300), ST30-IV (South West Pacific clone), ST59-V (Taiwan clone), and ST80-IV (European clone) (Monecke et al. 2011). ST8-IV (USA300) and ST30-IV may be considered pandemic, as they have been isolated repeatedly from every continent (Monecke et al. 2011; DeLeo et al. 2010). CA-MRSA can be responsible for a significant percentage of *S. aureus* SSTIs (Moet et al. 2007; Moran et al. 2006). In the US, CA-MRSA associated SSTIs peaked at 62 % in 2006 before decreasing annually to 52 % in 2010 (Landrum et al. 2012). USA300 represents the most prevalent CA-MRSA strain, and is isolated in up to 90 % of staphylococcal CA-SSTIs in the United States (Daum 2007; DeLeo et al. 2010; Talan et al. 2011; King et al. 2006; Jones et al. 2007). Moreover, recent evidence using mathematical models predict that community-acquired MRSA strains will eventually displace traditional health care-acquired MRSA strains in hospitals, with significant clinical and public health implications (D'Agata et al. 2009).

4.2 Surgical Site Infections (SSIs)

In addition to CA-SSTIs, *S. aureus* is the leading cause of surgical site infection (SA-SSIs) (Table 2). SSIs are one of the most prevalent causes of morbidity and mortality. They are the second most common cause of nosocomial infections in the

Table 2 Relative abundance of bacterial species associated with SSIs

Relative abundance of bacterial species in SSIs (%)													
S. aureus	Coagulase negative staphylococci	*Enterococcus* spp.	*Streptococcus* spp.	*Pseudomonas* spp.	*Escherichia coli*	*Klebsiella* spp.	*Proteus* spp.	*Proteus mirabilis*	*Citrobacter* spp.	*Enterobacter* spp.	*Acinetobacter* spp.	Country	References
32.2 27.3	14.8 9.7	2.7 5.5	1.9 –	12.8 18.2	8.9 18.2	4.7 9.1	2.7 –	1.6 –	2.3 –	1.2 1.8	5.8 1.8	India	(Gayathree Naik 2011; Amutha and Viswanathan 2014)
15.6[a] 20 15.5 33 34	11 – 6.4 11 9	11.6 12 14.5 8 12	– – 7.3 3 6	6.7 8 6.4 4 4	13.5 8 12.7 6 12	11.4[b] 3 13.6 4 6	– – – – – –	7.1 3 4.5 – –	– – – – –	– 7 4.5 – 4	– 1 1.8 – –	US	(Edwards 1976; National Nosocomial Infections Surveillance (NNIS) 1996) (Magill et al. 2014) (Anderson et al. 2007) (Baker et al. 2016)
18.2 28.6 45.1	4 – –	3.2 – –	– – –	11.1 – 9.9	13 25 –	1.6 17.9 7.0	– –	1.6 – 11.3	0.8 – –	– – 2.8	– – –	Africa	(Akinkunmi et al. 2014) (Mawalla et al. 2011) (Anguzu and Olila 2007)

(continued)

Table 2 (continued)

Relative abundance of bacterial species in SSIs (%)													
S. aureus	Coagulase negative staphylococci	*Enterococcus* spp.	*Streptococcus* spp.	*Pseudomonas* spp.	*Escherichia coli*	*Klebsiella* spp.	*Proteus* spp.	*Proteus mirabilis*	*Citrobacter* spp.	*Enterobacter* spp.	*Acinetobacter* spp.	Country	References
24.3 17.4	13.8 12	8.3 14.3	4.0 3.3	7.0 5.1	– 18.0	– 3.5	– 3.3	– –	– 1.2	– 4.2	0.4 0.6	Europe	(Health Protection Agency 2011) (European Centre for Disease Prevention and Control 2013)

[a]Coagulase positive staphylococci; [b]Klebsiella–Enterobacter spp

United States after urinary tract infections (UTIs) (Gayathree Naik 2011; Edwards 1976; National Nosocomial Infections Surveillance (NNIS) 1996; Akinkunmi et al. 2014; Amutha and Viswanathan 2014). Patients suffering from SSI are twice as likely to die, 60 % more likely to spend time in an intensive care unit (ICU), and more than five times more likely to be re-admitted (Kirkland et al. 1999). Post-surgical site infections account for 20 % of all SSTIs in hospitalized patients (Wilson 2003) and they have been shown to represent one of the most common foci of more severe infections such as bacteraemia (Tong et al. 2015). Indeed, during or after surgical operations bacteria may gain access to the different layers of the skin and eventually enter into the bloodstream. Data from national Nosocomial Infections Surveillance (NNIS) System and other sources indicate that *S. aureus,* and in particular MRSA, is the most isolated pathogen associated with SSIs and often associated with higher mortality rate (Anderson et al. 2007; Weigelt et al. 2010; Centers for Disease, C. and Prevention 1996; Magill et al. 2014).

Of note, in the US, 90 % of HA-MRSA infections are SSIs (World Health Organization 2013). Based on data published by Anderson et al. (2010), from over 96,000 orthopaedic, neurosurgical, cardiothoracic, and plastic surgical procedures performed in adults, the overall rate of invasive *S. aureus* infections was 0.47 infections per 100 procedures. Cardiothoracic surgery has the highest incidence of invasive SSIs (0.79 per 100 procedures). Orthopaedic, neurosurgical, and plastic surgery procedures have incidence rates of 0.37, 0.62, and 0.32 infections per 100 procedures respectively. In other studies the incidence in orthopaedic patients have been found to be around 0.8, 1.0 % in cardiothoracic, and 1.8 % in neurosurgery (Noskin et al. 2007). In order to better understand the relevance of SA-SSIs we should also consider the size of the affected populations. In the US approximately 2,200,000 patients undergo cardiovascular surgery, 2,500,000 orthopaedic surgery, and 290,000 neurosurgery annually (Noskin et al. 2007). Given the increasing age of the worldwide population these numbers are predicted to increase further in the future and SA-SSI infections are expected to increase in parallel (Noskin et al. 2007; Kaye et al. 2005; United Nations 2013).

4.3 Affected Populations and Medical Cost of Hospitalizations Associated with SA-SSTIs

Children, the elderly, and African Americans are disproportionately affected by CA-MRSA SSTIs in the United States (Ray et al. 2013a; Hersh et al. 2008; Ray et al. 2013b; Suaya et al. 2009). Apart from age and race, other high-risk groups include military personnel, prisoners and athletes. Several risk factors have been identified for SSTIs in these groups. These include living in crowded facilities, in close contact with other people harbouring CA-MRSA strains, extremes of age, and having a break in skin integrity due to medical interventions or trauma. Other risk factors include low socioeconomic status, smoking, previous SSTIs, poor hygiene,

and sharing of personal items (Knox et al. 2015; Diederen and Kluytmans 2006; Ellington et al. 2009). Furthermore, there are a number of underlying diseases that increase the risk for SSTIs, such as atopic dermatitis, HIV, diabetes, immune deficiencies, lymphedema, obesity, poor renal function, neutropenia (Table 1). SSTIs incidence has also been shown to follow a strong annual seasonal variability, with peak incidence occurring in early September (Wang et al. 2013; Sahoo et al. 2014). Other studies have reported similar results (Elegbe 1983; Frei et al. 2010; Kaier et al. 2010; Leekha et al. 2012; Mermel et al. 2011; Perencevich et al. 2008; Szczesiul et al. 2007). Another environmental factor associated with SSTIs are contaminated household fomites, particularly in cases of recurrent SSTIs (Ray et al. 2013a; Miller et al. 2015).

Hospitalizations due to SA-SSTIs in the US have significantly increased recently, and they represent about half of hospitalizations due to *S. aureus* infections. Total annual cost of SA-SSTI hospitalizations has been reported to be $4.5 billion of the $14.5 billion cost due to all staphylococcal infections. The elderly are the demographic group with the highest number of hospitalizations due to SA-SSTIs. However, the highest incidence increase has been lately observed in children 0–17 years of age (Suaya et al. 2009).

4.4 Paediatric SA-SSTIs

Paediatric SSTIs deserve a special consideration given the recent increase of the incidence observed in this population (Creech et al. 2015; Ray et al. 2013b). Skin colonization is a strong risk factor for developing SA-SSTIs in both children and adults, and most infections occurring outside the hospital are caused by the patient's colonizing strain. It has been noted that *S. aureus* skin colonization rates of neonates and children are considerably higher than in adults, with carriage rates uniformly decreasing with age. By day 5 post-birth, neonatal carriage rates may be as high as 90 % with the umbilicus and perianal skin colonized prior to the nares (Hurst 1960). Within the first 6 months of life, some studies find that colonization rates drop to between 21 and 28 % (Lebon et al. 2008), perhaps reflecting important shifts in the skin and mucosal microbiota, as well as immunological maturation.

While manifestations of SA-SSTIs described for adults also occur in paediatric patients, *S. aureus* impetigo is the most common bacterial skin infection in children (Table 1).

Recurrence of infection is a major feature of SA-SSTIs, both in adult and, more prominently, in paediatric patient populations. Some studies estimate that up to 70 % of paediatric patients with SSTIs caused by CA-MRSA will experience a recurrence of infection within one year, regardless of successful initial treatment (Creech et al. 2015; Bocchini et al. 2013).

5 Virulence Factors and Pathogenesis of *S. aureus*-Associated Skin Infections

In order to increase human nasal mucosa and skin colonization, *S. aureus* expresses various factors that facilitate skin colonization and infection (Fig. 2). *S. aureus* colonization is favoured by elevated levels of Th2 cytokines. In this respect SEA and SEB (staphylococcal enterotoxins A and B) and TSST-1(toxic shock syndrome toxin-1) can switch the cutaneous immune response towards the Th2 cytokines, associated with increased colonization of *S. aureus* in atopic dermatitis patients (Laouini et al. 2003). High levels of Th2 cytokines also enhance the expression of two classes of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) involved in *S. aureus* colonization, fibronectin-binding proteins (FnBPs) and fibrinogen-binding proteins (ClfA and ClfB) (Burian et al. 2010; Clarke et al. 2006). Another major MSCRAMM surface protein IsdA (Iron-surface determinant A), which is expressed under iron-depleted conditions, mediates binding to cornified envelope proteins of the human desquamated epithelial cells (Clarke et al. 2009) and governs antimicrobial fatty acids (AFAs) resistance by

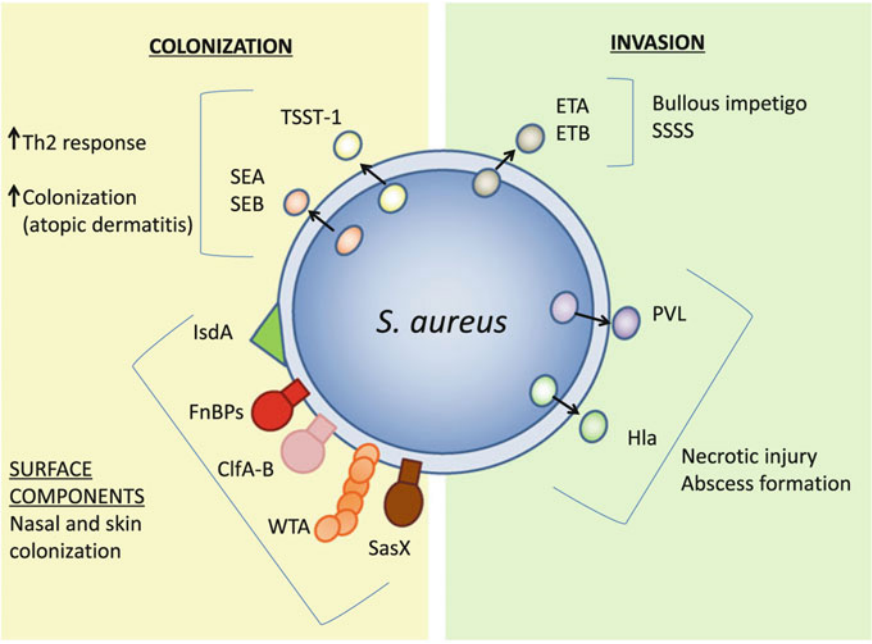

Fig. 2 *S. aureus* virulence factors associated with SSTIs. Th2 cytokines switching and increased colonization of *S. aureus* in atopic dermatitis is mediated by SEA, SEB and TSST-1. Surface proteins FnBPs, ClfA and ClfB (ClfA-B), IsdA and SasX as well as the cell wall glycopolimer WTA are involved in nasal and skin colonization. Toxins mediating invasion of the skin include ETs (bullous impetigo and SSSS), PVL and Hla (necrotic lesions and subcutaneous abscess)

increasing bacterial surface hydrophilicity (Clarke et al. 2007). In addition to proteins, cell wall glycopolymers such as wall teichoic acids protects *S. aureus* from skin AFAs (Kohler et al. 2009). Furthermore, wall techoic acids have been identified as an essential factor for nasal colonization in cotton rats showing specific interactions with airway epithelial cells (Weidenmaier et al. 2004). *S. aureus* expresses factors that enable it to directly counter host cationic antimicrobial peptides encountered in the epidermis. The extracellular metalloproteinase named aureolysin impedes cathelicidin antimicrobial activity (Sieprawska-Lupa et al. 2004). Exfoliative toxins (ETA and ETB) are serine proteases indicated to be the major toxins responsible for bullous impetigo and SSSS (Amagai et al. 2000). They cause exfoliation of the epidermis by the cleavage of the cadherin family member protein desmoglein 1, thereby disrupting the desmosome and abolishing the cell–cell integrity in the upper epidermis.

Panton–Valentine leucocidin (PVL) appears to be a marker for severity and recurrence in skin infection (Moran et al. 2006; Gordon and Lowy 2008; Zetola et al. 2005). PVL appears to be a possible virulence factor associated with necrotic lesions of the skin and subcutaneous abscess (e.g. furuncles). The PVL gene was found in 86 % of strains PVL-related infection commonly manifests as a localized necrotizing skin lesion resembling a Loxosceles spider bite (Couppie et al. 1994).

Alpha-Toxin (Hla) is required for dermonecrotic injury during infection and contributes to abscess size in animal models (Kennedy et al. 2010). Binding of Hla to the host receptor ADAM10 leads to E-cadherin cleavage and consequent breakdown of epithelial barrier (Berube and Bubeck Wardenburg 2013). In addition, Hla toxicity is regulated by several components of the adherens junctions (Popov et al. 2015). Increased Hla expression has been observed in CA-MRSA strain USA300, as well as historically in clinical isolates associated with epidemics of skin infections with increased severity of SSTIs (DeLeo et al. 2011).

A recent finding (Thurlow et al. 2013) provided evidence that links the functional activities of ACME (arginine catabolic mobile element) genes with the virulence of USA300 during SSTIs. The arginine deiminase system encoded by ACME would confer an enhanced acid tolerance of USA300 in the presence of exogenous lactic acid, the major organic acid present on human skin. Recently the newly identified staphylococcal surface-anchored protein SasX has been demonstrated to significantly enhance nasal colonization and abscess formation in animal models (Li et al. 2012). *sas*X is a mobile genetic element-encoded gene, which is typically harboured by strains belonging to the sequence type (ST) 239 (predominant in Asian countries), but it is quickly spreading to invasive clones belonging to other ST strains (Li et al. 2012).

6 Therapy for SA-SSTIs

Antibiotics are currently recommended for CA-SSTI patients at the extremes of age and those with severe or extensive disease, associated comorbidities or immunosuppression, rapidly progressing cellulitis, abscess in an anatomic location that precludes adequate drainage, systemic illness, associated septic phlebitis, or failure to improve (Liu et al. 2011). The administration of antibiotic as a supplement to incision and drainage is often based upon presence or absence of systemic inflammatory response syndrome (SIRS), such as fever (Diekema et al. 2001).

However, the increasing prevalence of multi-drug resistant strains has limited treatment options. Notwithstanding, there are still few effective drugs in clinical use for which little resistance has been observed. In the US vancomycin, linezolid, clindamycin, daptomycin, ceftaroline, minocycline and also trimethoprim–sulfamethoxazole are the most commonly used antibiotics for infections caused by MRSA. Nafcillin, and cefazolin, or their oral counterparts, dicloxacillin and cephalexin, are the antibiotics of choice for STTIs caused by MSSA (Stevens et al. 2014). Vancomycin is the drug of choice for initial empiric treatment of infections requiring parenteral treatment where MRSA is suspected (Stevens et al. 2014). However, vancomycin resistance, or more often intermediate resistant strains of *S. aureus,* have been reported in various regions of the world. Linezolid has been shown to be as effective as vancomycin for the treatment of MRSA SSTIs, but its use is limited by cost and toxicity. Other antibiotics have also been shown to be effective against SA-SSTIs. Daptomycin for instance was shown to be non-inferior to either vancomycin or β-lactams in the treatment of staphylococcal SSTIs (Arbeit et al. 2004). Rare, resistant strains have also been reported for linezolid and daptomycin (Gu et al. 2013).

7 Prevention of SA-SSTIs

Since SSTIs are often caused by strains carried by the patient (Al-Zubeidi et al. 2014), decolonization with mupirocin and chlorhexidine has been attempted as a strategy to reduce carriage and prevent infections. Mupirocin treatment was shown to eradicate nasal colonization in treated subjects over several months in a study conducted by Fritz et al. (Fritz et al. 2012). Nasal decolonization with mupirocin has been suggested to reduce the incidence of infection (Hacek et al. 2008), but efficacy varies by study and patients tend to become recolonized over time (Creech et al. 2015). As with many other antibiotics, cases of resistance have also been reported for mupirocin (Patel et al. 2009; Miller et al. 1996; Upton et al. 2003). Interestingly, the prescription of antibiotics for SSTIs treatment (Schmitz et al. 2010; Duong et al. 2010), as well as the choice of antibiotic (e.g. clindamycin) (Williams et al. 2011), may reduce incidence of recurrent infection. This suggests that recurrence may, at least in part, be due to lack of sufficient bacterial clearance following treatment. Good personal and

environmental hygiene may also contribute to reduce recurrent infections, and many hospitals have adopted policies of chlorhexidine baths prior to surgery to decrease the incidence of SSIs (Liu et al. 2011).

An alternative prophylaxis strategy against SA-SSTIs is vaccination. Interestingly, the majority of preliminary efforts in vaccine development date back to the pre-antibiotic era; with a paucity of research during the antibiotic era. Now, however, there is a resurgence of interest in the strategy of vaccination, which could potentially be effective against both antibiotic sensitive and resistant strains, and, unlike antibiotics, would not increase the selective pressure for antibiotic resistance.

Historic attempts to prevent SSTIs by vaccination targeted Hla as an antigen. Preliminary studies conducted in the 1930s showed promising results against recurrent furunculosis using a staphylococcal culture filtrate enriched in Hla. Twenty-four patients (age varying from 16 to 52 years) with recurrence episodes of furunculosis were studied. The toxin preparations were diluted with a saline solution and administered hypodermically at intervals of about a week. At least ten injections were given to each patient. A favourable response to the treatment was observed after the fourth to the sixth injection in the majority of the patients. Vaccination was associated with a rapid decrease in size and/or resolution of the furuncles. New furuncles were smaller than previous lesions and resolved more rapidly. The interval between the recurrence of new lesions was lengthened, and some patients remained disease free. Nineteen out twenty-four patients ceased to have furuncles, and several of these were followed from three months to two years remained infection free (Weise 1930).

Additional studies were conducted with formaldehyde-inactivated Hla with high success rate (93 % efficacy in 3000 SSTIs patients) (Parish and Cannon 1960). Results obtained with active vaccination were in agreement with the ones using anti-Hla serum therapy. An article published in 1934 reported that all 24 patients with various types of staphylococcal SSTIs recovered rapidly following serum therapy (Dolman 1934).

Despite these promising observations, other studies have contrasting results which raised debate regarding the therapeutic value of Hla vaccination (Harrison 1963). A study published in 1963 enrolled patients suffering from chronic furunculosis (patients over 14 years old suffering from boils for at least 2 months) (Harrison 1963). The clinical course of those vaccinated with Hla vs placebo was followed for one year. Of the 61 patients who received Hla, 19 (30.5 %) were completely cured. Of the placebo group 5 (12.5 %) of the 40 patients enrolled were found to be free of infection at the study end. However, statistical analysis of the data (which included evaluation of symptoms and relapses) did not confirm a significant disease improvement. Nonetheless, after immunization with Hla, only 40 out of 50 patients (80 %) showed a rise in titre, 6 (12 %) showed no change, and in 4 (8 %) the antibody level fell. Interestingly, all patients having a severe reaction to Hla immunization were completely cured for the follow-up period of one year.

In another study conducted in 1963, Hla vaccination was tested against skin infections in one chronic mental hospital. In this study, a follow up after vaccination of about 7 months was conducted. Of the 12 patients presenting boils before

vaccination with Hla, 5 continued to have the infection at the study end. In the placebo group, of the 11 patients presenting boils before enrolment to the trial, 7 continued to have the infection at the study end. The authors concluded that this study had too many variables and was impossible to estimate a beneficial effect of the vaccine.

Discrepancy of the results obtained in the trials described above could be due to many variables. For example, it was shown that there was a large variability in the antigenic potency of toxoid preparations found in the open market (Farrell 1941). Furthermore, the staphylococcal culture filtrate enriched in Hla used in the early studies likely contained traces of other bacterial antigens that might have contributed to protection. Therefore, vaccine efficacy could have been largely affected by its purity and by the detoxification methods used in the different studies.

Finally, the studies were conducted using different clinical endpoints, study design, patients, and in some case too few subjects were enrolled.

Autologous vaccination, which employs vaccines prepared from the autologous infecting bacterial strain, has been used since the beginning of the twentieth century to treat chronic staphylococcal infections (Halasa et al. 1978; Holtfreter et al. 2011; Wright 1902). Autologous vaccination is still in use in some Eastern European countries, including Poland and the Czech Republic for patients with chronic *S. aureus* infections that are refractory to standard therapy (Halasa et al. 1978; Novotný et al. 1990). A recent pilot study provided some preliminary indications that this form of vaccination may reduce the frequency and severity of furunculosis or result in complete remission (Holtfreter et al. 2011). Unfortunately this study was not designed to test the efficacy of autovaccination, but rather to characterize the kinetics of the antibody responses in a small cohort and did not include placebo controls. Four patients suffering from chronic or recurrent furunculosis were vaccinated with autovaccines individually prepared from the causative *S. aureus* strain, which was isolated from furuncles. It is interesting to note that previous antibiotic treatment and surgical intervention had been unsuccessful in these patients although none of the patients showed signs of immune suppression (Holtfreter et al. 2011). Two out of four patients enrolled for this study were free of symptoms after autovaccination treatment. While the other two still developed single, self-healing furuncles, which did not require medical treatment (Holtfreter et al. 2011). Jozef et al. (Halasa et al. 1978) evaluated immunological parameters in the course of autovaccine treatment in patients with chronic ostitis and carbuncles, and they observed an intensification of phagocytic activity after autovaccination. Results from other studies however reported downregulation of Th1 cell function and reduction in delayed type hypersensitivity reactions by autovaccination (Ring et al. 1976; Nolte et al. 2001; Rusch et al. 2001). Although autovaccination may represent a valid approach as a therapeutic strategy against antibiotic refractory SSTIs, considering staphylococcal strain variability, and the unknown mechanism of action and unpredictable safety profile of autovaccination, its value as a prophylactic approach remains elusive.

Finally, to date investigation of vaccination against alpha-toxin antigen and autovaccination are far from conclusive. Indeed, it is difficult to interpret the results

of the early clinical trials and the studies on autovaccination are based on too few subjects. Nevertheless, given the lack of known correlates of protection against *S. aureus* in humans, and the significant medical need for a vaccine against this pathogen, we find these data of high interest and importance.

8 Discussion

SSTIs are very common in all age groups and treatment represents a significant cost burden. *S. aureus* is the leading pathogen causing SSTIs worldwide, and multidrug-resistant strains are increasingly associated with these infections. Extensive use of antibiotics for treating SSTIs can promote further increase of resistant strains. Furthermore, neither successful antibiotic therapy of primary infections nor decolonization procedures are sufficiently effective in reducing recurrent infections. Given that severity and incidence of SSTIs significantly increased in the last twenty years, innovative medical interventions are required. *S. aureus* vaccines are not yet available on the market and recent clinical trials targeting different staphylococcal antigens have failed to show efficacy (Bagnoli et al. 2012; Pozzi et al. 2015). However, those failures could be due to the fact that the vaccines targeted one single antigen, did not contain adjuvants, and that trials were performed in patients with severe comorbidities, such as end-stage renal disease and those undergoing cardiothoracic surgery (Fattom et al. 2015; Fowler et al. 2013). On the other hand, observational studies conducted in the first decades of the 1900s utilizing *S. aureus* vaccines containing formaldehyde-denatured α-toxin showed promising results against cutaneous infections. And, although for several reasons we cannot derive any firm conclusions from those studies, they suggest that vaccination could induce protection against *S. aureus* SSTIs in humans. Therefore, it is possible that new generation vaccines which target multiple antigens and contain adjuvants able to stimulate cell-mediated immunity may be efficacious against *S. aureus* SSTIs. Such vaccines may not only prevent skin infections, but also reduce antibiotic use and potentially contain the staphylococcal epidemic, including new hospital outbreaks which might be caused by staphylococcal strains currently circulating mainly in the community. Indeed, it has been predicted that CA-MRSA will replace current HA strains. Primary targets for a vaccine against SSTIs may include populations at increased risk of such infections such as surgical patients, military trainees, athletes, nursing home patients, and subjects at risk for recurrent infections. In addition, a vaccine against SSTIs may also be considered for children and the elderly who are disproportionately affected by CA staphylococcal SSTIs. Finally, given that SSTIs can also be a source of other disease outcomes such as bacteraemia, a vaccine efficacious against *S. aureus* SSTI may also prevent more invasive infections.

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References

Adachi J et al (1998) Increasing incidence of streptococcal impetigo in atopic dermatitis. J Dermatol Sci 17(1):45–53

Akinkunmi EO, Adesunkanmi AR, Lamikanra A (2014) Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. Afr Health Sci 14(4):802–809

Al-Zubeidi D et al (2014) Molecular epidemiology of recurrent cutaneous methicillin-resistant infections in children. J Pediatric Infect Dis Soc 3(3):261–264

Amagai M et al (2000) Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nat Med 6(11):1275–1277

Amutha B, Viswanathan T (2014) A retrospective study on the pattern of pathogens isolated from surgical site wound infection in tertiary care hospital in Coimbatore, India. Int Res J Med Sci 2 (10):1–6

Anderson RR, Parrish JA (1982) Lasers in dermatology provide a model for exploring new applications in surgical oncology. Int Adv Surg Oncol 5:341–358

Anderson DJ et al (2007) Severe surgical site infection in community hospitals: epidemiology, key procedures, and the changing prevalence of methicillin-resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol 28(9):1047–1053

Anderson DJ et al (2010) Variation in the type and frequency of postoperative invasive *Staphylococcus aureus* infections according to type of surgical procedure. Infect Control Hosp Epidemiol 31(7):701–709

Anguzu JR, Olila D (2007) Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr Health Sci 7(3):148–154

Arbeit RD et al (2004) The safety and efficacy of daptomycin for the treatment of complicated skin and skin-structure infections. Clin Infect Dis 38(12):1673–1681

Avolio M, Bonea M, Camporese A (2012) Molecular diagnosis of *Staphylococcus aureus* prosthetic aortic graft infection: a case report. Infez Med 20(4):276–278

Bagnoli F, Bertholet S, Grandi G (2012) Inferring reasons for the failure of *Staphylococcus aureus* vaccines in clinical trials. Front Cell Infect Microbiol 2:16

Baker AW, Dicks KV, Durkin MJ, Weber DJ, Lewis SS, Moehring RW, Chen LF, Sexton DJ, Anderson DJ (2016) Epidemiology of surgical site infection in a community hospital network. Infect Control Hosp Epidemiol 37(5):519–526

Bassetti M et al (2014) European perspective and update on the management of complicated skin and soft tissue infections due to methicillin-resistant *Staphylococcus aureus* after more than 10 years of experience with linezolid. Clin Microbiol Infect 20(Suppl 4):3–18

Berger TG (1993) Treatment of bacterial, fungal, and parasitic infections in the HIV-infected host. Semin Dermatol 12(4):296–300

Berger TG, Obuch ML, Goldschmidt RH (1990) Dermatologic manifestations of HIV infection. Am Fam Physician 41(6):1729–1742

Berube BJ, Bubeck Wardenburg J (2013) *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. Toxins (Basel) 5(6):1140–1166

Bocchini CE et al (2013) Recurrent community-associated *Staphylococcus aureus* infections in children presenting to Texas Children's Hospital in Houston, Texas. Pediatr Infect Dis J 32 (11):1189–1193

Bonnetblanc JM, Bedane C (2003) Erysipelas: recognition and management. Am J Clin Dermatol 4(3):157–163

Burian M et al (2010) Temporal expression of adhesion factors and activity of global regulators during establishment of *Staphylococcus aureus* nasal colonization. J Infect Dis 201(9):1414–1421

Centers for Disease, Control and Prevention (1996) National nosocomial infections surveillance (NNIS) report, data summary from October 1986–April 1996, issued May 1996. A report from the national nosocomial infections surveillance (NNIS) system. Am J Infect Control 24:380–388

Chen AE et al (2009) Discordance between *Staphylococcus aureus* nasal colonization and skin infections in children. Pediatr Infect Dis J 28(3):244–246

Chiller K, Selkin BA, Murakawa GJ (2001) Skin microflora and bacterial infections of the skin. J Investig Dermatol Symp Proc 6(3):170–174

Clarke SR et al (2006) Identification of in vivo-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. J Infect Dis 193(8):1098–1108

Clarke SR et al (2007) The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. Cell Host Microbe 1(3):199–212

Clarke SR et al (2009) Iron-regulated surface determinant protein A mediates adhesion of *Staphylococcus aureus* to human corneocyte envelope proteins. Infect Immun 77(6):2408–2416

Couppie P, Cribier B, Prevost G (1994) Leukocidin from *Staphylococcus aureus* and cutaneous infections: an epidemiologic study. Arch Dermatol 130(9):1208–1209

Creech CBA-Z, Zubeidi DN, Fritz SA (2015) Prevention of recurrent staphylococcal skin infections. Infect Dis Clin North Am 29(3):429–464

D'Agata EMC et al (2009) Modeling the invasion of community-acquired methicillin-resistant *Staphylococcus aureus* into hospitals. Clin Infect Dis: Off Publ Infect Dis Soc Am 48(3):274–284

Daum RS (2007) Clinical practice. Skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*. N Engl J Med 357(4):380–390

DeLeo FR et al (2010) Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375(9725):1557–1568

DeLeo FR et al (2011) Molecular differentiation of historic phage-type 80/81 and contemporary epidemic *Staphylococcus aureus*. Proc Natl Acad Sci USA 108(44):18091–18096

Dener C, Inan A (2003) Breast abscesses in lactating women. World J Surg 27(2):130–133

Deverick J, Anderson KSK (2009) Staphylococcal surgical site infections. Infect Dis Clin N Am 23(1):53–72

Diederen BMW, Kluytmans JAJW (2006) The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. J Infect 52(3):157–168

Diekema DJ et al (2001) Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997–1999. Clin Infect Dis 32(Suppl 2):S114–s132

Dolman CE (1934) Staphylococcus antitoxic serum in the treatment of acute staphylococcal infections and toxax, milas*. Can Med Assoc J:601

Donovan B et al (1992) Bullous impetigo in homosexual men–a risk marker for HIV-1 infection? Genitourin Med 68(3):159–161

Dryden MS (2009) Skin and soft tissue infection: microbiology and epidemiology. Int J Antimicrob Agents 34(Suppl 1):S2–S7

Duong M et al (2010) Randomized, controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. Ann Emerg Med 55(5):401–407

Dupuy A et al (1999) Risk factors for erysipelas of the leg (cellulitis): case-control study. BMJ 318 (7198):1591–1594

Edlich RF et al (2005) Bacterial diseases of the skin. J Long Term Eff Med Implants 15(5):499–510

Edwards LD (1976) The epidemiology of 2056 remote site infections and 1966 surgical wound infections occurring in 1865 patients: a four year study of 40,923 operations at Rush-Presbyterian-St. Luke's Hospital, Chicago. Ann Surg 184(6):758–766

Elegbe IA (1983) Influence of seasonal and weather variation on the incidence of coagulase positive Staphylococci isolates among Nigerians with boil infections. J R Soc Health 103 (3):118–119

Ellington MJ et al (2009) Clinical and molecular epidemiology of ciprofloxacin-susceptible MRSA encoding PVL in England and Wales. Eur J Clin Microbiol Infect Dis 28(9):1113–1121

Eriksen NH et al (1995) Carriage of Staphylococcus aureus among 104 healthy persons during a 19-month period. Epidemiol Infect 115(1):51–60

European Centre for Disease Prevention and Control (2013) Surveillance of surgical site infections in Europe 2010–2011

Farrell N (1941) The potency of staphylococcal toxoid. J Immunol 41(1):119–126

Farrell AM (1999) Staphylococcal scalded-skin syndrome. The Lancet 354(9182):880–881

Fattom A et al (2015) Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: phase III randomized study. Hum Vaccin Immunother 11 (3):632–641

Fournier B, Philpott DJ (2005) Recognition of Staphylococcus aureus by the innate immune system. Clin Microbiol Rev 18(3):521–540

Fowler VG et al (2013) Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. JAMA 309(13):1368–1378

Frei CR et al (2010) Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections as a common cause of hospitalization in United States children. J Pediatr Surg 45(10):1967–1974

Fritz SA et al (2012) Household versus individual approaches to eradication of community-associated *staphylococcus aureus* in children: a randomized trial. Clin Infect Dis 54 (6):743–751

Gayathree Naik SRD (2011) A study on surgical site infections caused by *Staphylococcus aureus* with a special search for methicillin-resistant isolates. J Clin Diagn Res 5(3)

Gemmell CG (1995) Staphylococcal scalded skin syndrome. J Med Microbiol 43(5):318–327

Gordon RJ, Lowy FD (2008) Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 46(Suppl 5):S350–S359

Gu B et al (2013) The emerging problem of linezolid-resistant Staphylococcus. J Antimicrob Chemother 68(1):4–11

Hacek D et al (2008) *Staphylococcus aureus* nasal decolonization in joint replacement surgery reduces infection. Clin Orthop Relat Res 466(6):1349–1355

Halasa J et al (1978a) Evaluation of certain immunological parameters in the course of autovaccine treatment in patients with chronic ostitis and carbunculosis. Arch Immunol Ther Exp (Warsz) 26(1–6):589–593

Halasa J, Giedrys-Galant S, Podkowinska I, Joanna Braun J, Strzelecka G, Dabrowski W (1978) Evaluation of certain immunologic parametersin the course of autovaccine treatment inpatients with chronic ostitis and carbuncles. Arch Immunologjae et therapiae Exp 26:589

Harrison K (1963) Clinical trial of coagulase and alpha-hemolysin toxoids in chronic forunculosis. Br Med J

Hartman-Adams H, Banvard C, Juckett G (2014) Impetigo: diagnosis and treatment. Am Fam Physician 90(4):229–235

Health Protection Agency (2011/2012) Surveillance of surgical site infections in NHS hospitals in England London. Health Protection Agency

Hersh AL, Chambers HF, Maselli JH, Gonzales R (2008) National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. Arch Intern Med 168(14) 1585–1591

Hersh AL et al (2008b) National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. Arch Intern Med 168(14):1585–1591

Holmes RB, Martins C, Horn T (2002) The histopathology of folliculitis in HIV-infected patients. J Cutan Pathol 29(2):93–95

Holtfreter S et al (2011) Antibody responses in furunculosis patients vaccinated with autologous formalin-killed *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis 30(6):707–717

Hurst V (1960) Transmission of hospital staphylococci among newborn infants. II. Colonization of the skin and mucous membranes of the infants. Pediatrics 25:204–214

Jonathan A, Otter GLF (2010) Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. Lancet Infect Dis 10:227–239

Jones RN et al (2007) Molecular characterization of *Staphylococcus aureus* isolates from a 2005 clinical trial of uncomplicated skin and skin structure infections. Antimicrob Agents Chemother 51(9):3381–3384

Jorup-Ronstrom C, Britton S (1987) Recurrent erysipelas: predisposing factors and costs of prophylaxis. Infection 15(2):105–106

Kaier K et al (2010) Seasonal and ascending trends in the incidence of carriage of extended-spectrum ß-lactamase-producing *Escherichia coli* and Klebsiella species in 2 German hospitals. Infect Control Hosp Epidemiol 31(11):1154–1159

Kaplan SL et al (2014) Randomized trial of "Bleach Baths" plus routine hygienic measures vs routine hygienic measures alone for prevention of recurrent infections. Clin Infect Dis 58 (5):679–682

Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol 11(5):373–384

Kaye KS et al (2005) The effect of increasing age on the risk of surgical site infection. J Infect Dis 191(7):1056–1062

Kennedy AD et al (2010) Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 202(7):1050–1058

Kesah C et al (2003) Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin Microbiol Infect 9(2):153–156

King MD et al (2006) Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med 144(5):309–317

Kirkland KB et al (1999) The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. Infect Control Hosp Epidemiol 20(11):725–730

Kluytmans J, van Belkum A, Verbrugh H (1997) Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10(3):505–520

Knox J, Uhlemann AC, Lowy FD (2015) *Staphylococcus aureus* infections: transmission within households and the community. Trends Microbiol

Kobayashi SD, Malachowa N, DeLeo FR (2015) Pathogenesis of *Staphylococcus aureus* abscesses. Am J Pathol 185(6):1518–1527

Kohler T, Weidenmaier C, Peschel A (2009) Wall teichoic acid protects *Staphylococcus aureus* against antimicrobial fatty acids from human skin. J Bacteriol 191(13):4482–4484

Krueger GG, Stingl G (1989) Immunology/inflammation of the skin–a 50-year perspective. J Invest Dermatol 92(4 Suppl):32S–51S

Kupper TS, Fuhlbrigge RC (2004) Immune surveillance in the skin: mechanisms and clinical consequences. Nat Rev Immunol 4(3):211–222

Lachant DJ, Apostolakos M, Pietropaoli A (2013) Methicillin resistant *Staphylococcus aureus* prostatic abscess with bacteremia. Case Rep Infect Dis 2013:613961

Ladhani S et al (1999) Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 12(2):224–242
Landrum ML et al (2012) Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005–2010. JAMA 308(1):50–59
Laouini D et al (2003) Epicutaneous sensitization with superantigen induces allergic skin inflammation. J Allergy Clin Immunol 112(5):981–987
Lebon A et al (2008) Dynamics and determinants of *staphylococcus aureus* carriage in infancy: the generation R study. J Clin Microbiol 46(10):3517–3521
Leekha S, Diekema DJ, Perencevich EN (2012) Seasonality of staphylococcal infections. Clin Microbiol Infect 18(10):927–933
Lejbkowicz F et al (2005) Impetigo in soldiers after hand-to-hand combat training. Mil Med 170 (11):972–974
Li M et al (2012) MRSA epidemic linked to a quickly spreading colonization and virulence determinant. Nat Med 18(5):816–819
Liu C et al (2011) Clinical practice guidelines by the infectious diseases society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin Infect Dis 52(3):e18–e55
Lorette G et al (2009) Superficial community-acquired skin infections: prevalence of bacteria and antibiotic susceptibility in France. J Eur Acad Dermatol Venereol 23(12):1423–1426
Magill SS et al (2014) Multistate point-prevalence survey of health care-associated infections. N Engl J Med 370(13):1198–1208
Marra F et al (2012) Population-based study of the increased incidence of skin and soft tissue infections and associated antimicrobial use. Antimicrob Agents Chemother 56(12):6243–6249
Mawalla B et al (2011) Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. BMC Surg 11:21
McCaig LF et al (2006) Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis 12(11):1715–1723
Melish ME, Glasgow LA (1970) The staphylococcal scalded-skin syndrome. N Engl J Med 282 (20):1114–1119
Mermel LA, Machan JT, Parenteau S (2011) Seasonality of MRSA infections. PLoS ONE 6(3): e17925
Meurehg-Haik C, Garcia-Velasco J (1974) Carbuncle: discussion and a report of two cases. Int J Dermatol 13(2):63–68
Miller MA et al (1996) Development of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* after widespread use of nasal mupirocin ointment. Infect Control Hosp Epidemiol 17(12):811–813
Miller LG et al (2005) Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles. N Engl J Med 352(14):1445–1453
Miller LG et al (2007) A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. Clin Infect Dis 44(4):483–492
Miller LG et al (2015) *Staphylococcus aureus* skin infection recurrences among household members: an examination of host, behavioral, and pathogen-level predictors. Clin Infect Dis 60 (5):753–763
Moazzez A et al (2007) Breast abscess bacteriologic features in the era of community-acquired methicillin-resistant *Staphylococcus aureus* epidemics. Arch Surg 142(9):881–884
Moet GJ et al (2007) Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY antimicrobial surveillance program (1998–2004). Diagn Microbiol Infect Dis 57(1):7–13
Monecke S et al (2011) A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS ONE 6(4):e17936
Montgomery CP, David MZ, Daum RS (2015) Host factors that contribute to recurrent staphylococcal skin infection. Curr Opin Infect Dis 28(3):253–258

Moran GJ et al (2006) Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 355(7):666–674
Morris AD (2008) Cellulitis and erysipelas. BMJ Clin Evid
Murphy JE, Robert C, Kupper TS (2000) Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J Invest Dermatol 114(3):602–608
Nakai N, Takenaka H, Kishimoto S (2008) Ecthyma gangrenosum without pseudomonas septicemia in a kidney transplant recipient. J Dermatol 35(9):585–589
National Nosocomial Infections Surveillance (NNIS) (1996) Report, data summary from october 1986-april 1996. AJIC Am J Infect control 24:380–388
Nestle FO et al (2009) Skin immune sentinels in health and disease. Nat Rev Immunol 9(10):679–691
Nichols RL, Florman S (2001) Clinical presentations of soft-tissue infections and surgical site infections. Clin Infect Dis 33(Suppl 2):S84–S93
Niniou I et al (2008) Clinical and molecular epidemiology of community-acquired, methicillin-resistant *Staphylococcus aureus* infections in children in central Greece. Eur J Clin Microbiol Infect Dis 27(9):831–837
Nishifuji K, Sugai M, Amagai M (2008) Staphylococcal exfoliative toxins: "molecular scissors" of bacteria that attack the cutaneous defense barrier in mammals. J Dermatol Sci 49(1):21–31
Nolte O et al (2001) Autovaccination of dairy cows to treat post partum metritis caused by Actinomyces pyogenes. Vaccine 19(23–24):3146–3153
Noskin GA et al (2007) National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6-year period (1998–2003). Clin Infect Dis 45(9):1132–1140
Novotný Z et al (1990) Treatment with autovaccines and the immunologic profile of patients. Cesk Otolaryngologie 39(1):40–47
Ojulong J et al (2009) Relative prevalence of methicilline resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. Tanzan J Health Res 11(3):149–153
Otto M (2010) Staphylococcus colonization of the skin and antimicrobial peptides. Expert Rev Dermatol 5(2):183–195
Parish HJ, Cannon DA (1960) Staphylococcal infection: antitoxic immunity. Br Med J
Patel JB, Gorwitz RJ, Jernigan JA (2009) Mupirocin resistance. Clin Infect Dis 49(6):935–941
Pechter PM et al (2012) Ecthyma gangrenosum secondary to *Staphylococcus aureus* in an infant with transient neutropenia. Pediatr Dermatol 29(3):320–323
Pereira LB (2014) Impetigo—review. An Bras Dermatol 89(2):293–299
Perencevich EN et al (2008) Summer peaks in the incidences of gram-negative bacterial infection among hospitalized patients. Infect Control Hosp Epidemiol 29(12):1124–1131
Popov LM et al (2015) The adherens junctions control susceptibility to *Staphylococcus aureus* alpha-toxin. Proc Natl Acad Sci USA 112(46):14337–14342
Pozzi C et al (2015) Phagocyte subsets and lymphocyte clonal deletion behind ineffective immune response to *Staphylococcus aureus*. FEMS Microbiol Rev 39(5):750–763
Proksch E, Brandner JM, Jensen JM (2008) The skin: an indispensable barrier. Exp Dermatol 17 (12):1063–1072
Ray GT, Suaya JA, Baxter R (2013a) Incidence, microbiology, and patient characteristics of skin and soft-tissue infections in a U.S. population: a retrospective population-based study. BMC Infect Dis 13:252
Ray GT, Suaya JA, Baxter R (2013b) Microbiology of skin and soft tissue infections in the age of community-acquired methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 76(1):24–30
Raya-Cruz M et al (2014) Skin and soft-tissue infections in hospitalized patients: epidemiology, microbiological, clinical and prognostic factors. Enferm Infecc Microbiol Clin 32(3):152–159
Ring J et al (1976) [Chronic posttraumatic osteomyelitis. Attempt at oral autovaccine therapy]. Fortschr Med 94(5):264–265, 268

Romani L et al (2015) Scabies and impetigo prevalence and risk factors in Fiji: a national survey. PLoS Negl Trop Dis 9(3):e0003452

Rusch V et al (2001) Results of an open, non-placebo controlled pilot study investigating the immunomodulatory potential of autovaccine. Arzneimittelforschung 51(8):690–697

Sahoo KC et al (2014) Climatic factors and community - associated methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infections—a time-series analysis study. Int J Environ Res Public Health 11(9):8996–9007

Schauber J, Gallo RL (2009) Antimicrobial peptides and the skin immune defense system. J Allergy Clin Immunol 124(3 Suppl 2):R13–R18

Scheinfeld NS (2004) Obesity and dermatology. Clin Dermatol 22(4):303–309

Schmitz GR et al (2010) Randomized controlled trial of trimethoprim-sulfamethoxazole for uncomplicated skin abscesses in patients at risk for community-associated methicillin-resistant *Staphylococcus aureus* infection. Ann Emerg Med 56(3):283–287

Semel JD, Goldin H (1996) Association of athlete's foot with cellulitis of the lower extremities: diagnostic value of bacterial cultures of ipsilateral interdigital space samples. Clin Infect Dis 23 (5):1162–1164

Seni J et al (2013) Molecular Characterization of *Staphylococcus aureus* from patients with surgical site infections at Mulago Hospital in Kampala, Uganda. PLoS ONE 8(6):e66153

Shah AM, Supe AN, Samsi AB (1987) Carbuncle–a conservative approach. J Postgrad Med 33 (2):55–57

Shallcross LJ et al (2014) Evidence for increasing severity of community-onset boils and abscesses in UK General Practice. Epidemiol Infect:1–4

Sieprawska-Lupa M et al (2004) Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. Antimicrob Agents Chemother 48(12):4673–4679

Song JH et al (2011) Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 66(5):1061–1069

Stafford I et al (2008) Community-acquired methicillin-resistant *Staphylococcus aureus* among patients with puerperal mastitis requiring hospitalization. Obstet Gynecol 112(3):533–537

Stevens DL et al (2014) Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the infectious diseases society of America. Clin Infect Dis 59 (2):147–159

Suaya JA, Cassidy A, Meera RM, O'Hara P, Amrine-Madsen H, Burstin S, Miller LG (2009) Incidence and cost of hospitalizations associated with Staphylococcus aureus skin and soft tissue infections in the United States from 2001 through 2009. BMC Infect Dis 14(296)

Suaya JA et al (2014) Incidence and cost of hospitalizations associated with Staphylococcus aureus skin and soft tissue infections in the United States from 2001 through 2009. BMC Infect Dis 14:296

Szczesiul JM et al (2007) No decrease in clindamycin susceptibility despite increased use of clindamycin for pediatric community-associated methicillin-resistant *Staphylococcus aureus* skin infections. Pediatr Infect Dis J 26(9):852–854

Talan DA, Singer AJ (2014) Management of skin abscesses. N Engl J Med 370(23):2245–2246

Talan DA et al (2011) Comparison of *Staphylococcus aureus* from skin and soft-tissue infections in US emergency department patients, 2004 and 2008. Clin Infect Dis 53(2):144–149

Tattevin P et al (2012) Concurrent epidemics of skin and soft tissue infection and bloodstream infection due to community-associated methicillin-resistant Staphylococcus aureus. Clin Infect Dis 55(6):781–788

Tharakaram S, Keczkes K (1988) Necrotizing fasciitis. A report of five patients. Int J Dermatol 27 (8):585–588

Thurlow LR et al (2013) Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant *Staphylococcus aureus*. Cell Host Microbe 13(1):100–107

Tong SY et al (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661

Ungprasert P et al (2013) A rare case of ecthyma gangrenosum associated with methicillin-resistant *Staphylococcus aureus* infection. J Infect Chemother 19(4):761–763
United Nations (2013) Department of economic and social affairs, population division. World population ageing. **ST/ESA/SER.A/348**
Upton A, Lang S, Heffernan H (2003) Mupirocin and *Staphylococcus aureus*: a recent paradigm of emerging antibiotic resistance. J Antimicrob Chemother 51(3):613–617
Wang X et al (2013) A population based study of seasonality of skin and soft tissue infections: implications for the spread of CA-MRSA. PLoS ONE 8(4):e60872
Weidenmaier C et al (2004) Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med 10(3):243–245
Weigelt JA et al (2010) Surgical site infections: causative pathogens and associated outcomes. Am J Infect Control 38(2):112–120
Weise EC (1930) Staphylococcus toxin in the treatment of furunculosis: preliminary report. J Am Med Assoc 95(5):324–326
Wertheim HFL et al (2005) The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 5(12):751–762
Williams DJ et al (2011) Comparative effectiveness of antibiotic treatment strategies for pediatric skin and soft-tissue infections. Pediatrics 128(3):e479–e487
Williamson DA et al (2014) *Staphylococcus aureus* infections in New Zealand, 2000–2011. Emerg Infect Dis 20(7):1156–1161
Wilson MA (2003) Skin and soft-tissue infections: impact of resistant gram-positive bacteria. Am J Surg 186(5A):35S–41S; discussion 42S–43S, 61S–64S
World Health Organization (2013) MRSA surveys network [powerpoint slides]. Retrieved from http://www.who.int/patientsafety/patients_for_patient/PFPS-webinar1_2013.pdf
Wright AE (1902) Notes on the treatment of furunculosis, sycosis, and acne by the inoculation of a staphylococcus vaccine. The Lancet 159(4100):874–884
Zetola N et al (2005) Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis 5(5):275–286
Zhao C et al (2012) Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. PLoS ONE 7 (6):e38577

Staphylococcus aureus-Associated Musculoskeletal Infections

Evgeny A. Idelevich, Carolin Kreis, Bettina Löffler and Georg Peters

Abstract Musculoskeletal infections caused by *Staphylococcus aureus* are among the most difficult-to-treat infections. *S. aureus* osteomyelitis is associated with a tremendous disease burden through potential for long-term relapses and functional deficits. Although considerable advances have been achieved in diagnosis and treatment of osteomyelitis, the management remains challenging and impact on quality of life is still enormous. *S. aureus* acute arthritis is relatively seldom in general population, but the incidence is considerably higher in patients with predisposing conditions, particularly those with rheumatoid arthritis. Rapidly destructive course with high mortality and disability rates makes urgent diagnosis and treatment of acute arthritis essential. *S. aureus* pyomyositis is a common disease in tropical countries, but it is very seldom in temperate regions. Nevertheless, the cases have been increasingly reported also in non-tropical countries, and the physicians should be able to timely recognize this uncommon condition and initiate appropriate treatment. The optimal management of *S. aureus*-associated musculoskeletal infections requires a strong interdisciplinary collaboration between all involved specialists.

Contents

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1 Introduction

Musculoskeletal infections caused by *Staphylococcus aureus* are among the most difficult-to-treat infections (Davis 2005; Ciampolini and Harding 2000). Osteomyelitis is an inflammation of bone, usually caused by bacterial infections (Lew and Waldvogel 2004). *S. aureus* osteomyelitis is associated with 6 % mortality and tremendous disease burden through potential for long-term relapses (32 %) and neurological deficits (32 %) (Mylona et al. 2009). Economic impact of osteomyelitis is enormous, and it also substantially affects quality of life (Lew and Waldvogel 2004). *S. aureus* infection of native joints is seldom in general population, but its rapidly destructive course with high mortality and disability rates makes urgent diagnosis and treatment essential (Shirtliff and Mader 2002). Pyomyositis, a common disease in tropical countries (Chauhan et al. 2004; Chiedozi 1979), is very seldom observed in temperate areas. However, also in non-tropical countries, the cases have been increasingly reported (Christin and Sarosi 1992), and the physicians should be able to recognize this condition in order to initiate appropriate treatment.

2 *Staphylococcus aureus* Osteomyelitis

2.1 Epidemiology

The incidence of osteomyelitis in children amounts to 13 per 100,000 children per year (Riise et al. 2008), but it is more common in the developing countries (Dartnell et al. 2012). Patients under the age of three are more frequently affected than older children (Riise et al. 2008). Boys are affected about twice as often as girls (Dartnell et al. 2012; Malcius et al. 2005). The incidence of hematogenous osteomyelitis in children has reduced over last decades. A study from Scotland has shown a decline of 44 % in incidence from 1990 to 1997 (Blyth et al. 2001), which mirrors the decline of over 50 % reported between 1970 and 1990 for population of the same area (Craigen et al. 1992). This reduction is supposedly due to progress in treatment of acute bacterial infections, improved standards of living and hygiene (Blyth et al. 2001), as well as broad introduction of *Haemophilus influenzae* type B vaccination (Howard et al. 1999). However, the reduced incidence was not confirmed by the

studies from some other countries (Malcius et al. 2005) and was not observed in adults. The incidence of vertebral osteomyelitis, the most common form of hematogenous osteomyelitis in adults, is 2.4 per 100,000 inhabitants. Noteworthy, the incidence is increasing with age reaching 6.5 per 100,000 in patients >70 years of age (Grammatico et al. 2008). The disease has a male predominance with a male: female ratio of 1.5 (Grammatico et al. 2008). Vertebral osteomyelitis appears to be on increase as a result of higher life expectancy, the rise in the intravenous drug abusers, immunocompromised patients, and those undergoing hemodialysis and spinal instrumentation (Gasbarrini et al. 2005). Posttraumatic osteomyelitis is also seen more frequently now (Aytaç et al. 2014), probably due to increased number of surgery, particularly that involving foreign body material.

Hematogenous osteomyelitis is mostly monomicrobial (85 % of cases) (Mylona et al. 2009), and *S. aureus* is the most common causative organism of osteomyelitis in adults (Zimmerli 2010) and children (Dartnell et al. 2012). However, in children under the age of four, *Kingella kingae* is being increasingly reported as the major cause of osteomyelitis (Ceroni et al. 2010; Dartnell et al. 2012). *S. aureus* accounts for 32–67 % of isolates in patients with vertebral osteomyelitis (Mylona et al. 2009; Gupta et al. 2014). Also for posttraumatic osteomyelitis, *S. aureus* is the most frequently isolated organism, ranging from 28 to 67 % (Aytaç et al. 2014; Gross et al. 2002; Merritt 1988). The rate of methicillin-resistant *S. aureus* (MRSA) is dependent on the geographical area. In postoperative osteomyelitis, coagulase-negative staphylococci (CoNS) are common pathogens, particularly in the setting of foreign body-associated infections (Gross et al. 2002; Trampuz and Zimmerli 2006b).

2.2 Pathogenesis

2.2.1 General Pathogenesis and Classification

Acute osteomyelitis is characterized by suppurative inflammation causing destruction of bone trabeculae and bone matrix. Blood vessels are compressed by the inflammatory process, and the resulting interruption of blood supply contributes to bone necrosis. Ischemic portions of bone can be separated to form pieces of dead bone (sequestra). At this stage, necrotic areas are hardly reached by antibiotics indicating the need for surgical treatment of (chronic) osteomyelitis. At the bone necrosis borders, new bone formation occurs (Lew and Waldvogel 2004). While there is no clear division between acute and chronic osteomyelitis, some authors distinguish acute (course of disease up to several weeks) and subacute or chronic (lasting several months or years) forms (Zimmerli 2010). Specific feature of chronic osteomyelitis is the development of bone necrosis, classically presenting as sequestrum (Lew and Waldvogel 2004). Other common features of chronic osteomyelitis include involucrum (a sheath of new bone surrounding a sequestrum),

local osteoporosis, and sinus tract in case of extension through cortical bone. Furthermore, chronic osteomyelitis is typically characterized by some extent of inflammation and clinical relapses with persistent detection of pathogens (Lew and Waldvogel 2004). Recently, a novel mouse model has been suggested which allows precise investigation of complex pathogenesis in *S. aureus* chronic osteomyelitis (Horst et al. 2012). Horst et al. detected bone destruction and remodeling processes by magnetic resonance and X-ray imaging after 3 weeks of infection. Histological examination also revealed areas of bone destruction due to the osteoclasts' resorbing activity at the beginning of chronic stage. Individual activated osteoclasts produce the typical resorption lacunae, resulting in bone segments with ruffled border. The activity of osteoclasts further leads to the separation of dead bone, generating a sequestrum. These processes are accompanied by the reactive bone formation with the typical irregular structure of woven bone with increased thickness. Biomechanical testing demonstrates decreased flexibility and increased torsional rigidity in chronically infected bones, thus increasing the risk of fractures (Horst et al. 2012). Chronic osteomyelitis became uncommon with modern principles of treatment of acute hematogenous osteomyelitis.

Osteomyelitis develops (i) hematogenously, (ii) by spread from adjacent structures (per continuitatem) or due to direct inoculation through open fracture or surgical intervention, or (iii) secondary to vascular insufficiency (e.g., diabetic foot) (Lew and Waldvogel 2004). Cierny and Mader suggested a staging classification of osteomyelitis based on the affected portion of the bone and physiology of the host (Mader et al. 1997).

In hematogenous osteomyelitis, the microorganisms are disseminated into the bone from a primary focus of infection, e.g., heart valves, skin, or urinary tract infection (McHenry et al. 2002). However, in some cases, no apparent infection focus can be elicited. A considerable proportion of *S. aureus* bacteremia episodes have been shown to be of endogenous origin since they originate from the bacteria in the nasal mucosa (von Eiff et al. 2001). About one-third of acute hematogenous osteomyelitis is correlated with acute trauma, as it is known that a hematoma located at the metaphysis after trauma promotes the colonization of bacteria (Jaramillo 2011). While the metaphyses of long bones are mainly affected in children (Peltola and Pääkkönen 2014; Waldvogel et al. 1970a), vertebral osteomyelitis is the most common localization form of hematogenous osteomyelitis in adults (Waldvogel et al. 1970a). In vertebral osteomyelitis, two neighboring vertebra are frequently affected by the infection due to the blood supply from one bifurcating arterial vessel (Lew and Waldvogel 2004). Involvement of their intervertebral disk explains the term spondylodiscitis which is used synonymously with vertebral osteomyelitis.

The reason for the development of a contiguous focus (per continuitatem) osteomyelitis is the dissemination from neighboring structures as joint spaces and soft tissue or due to trauma or surgery with direct colonization of the pathogen (Lew and Waldvogel 2004). The posttraumatic osteomyelitis is a bone infection of exogenous origin. It develops in consequence of a bone fracture or bone surgery (Aytaç et al. 2014; Gross et al. 2002). The incidence of posttraumatic osteomyelitis

is on the increase due to the number of sport, traffic, and conflict incidents as well as rising numbers of surgical interventions with bone involvement (e.g., heart surgery). The infection rate after an open fracture depends on the extent of the injury and occurs in up to 26 % (Merritt 1988; Patzakis and Wilkins 1989). Also in postoperative osteomyelitis, staphylococci (in particular *S. aureus*) are most common causative organisms (Aytaç et al. 2014; Gross et al. 2002; Trampuz and Zimmerli 2006b). Coagulase-negative staphylococci are frequently responsible for osteomyelitis associated with orthopedic implants and pin track infections (Gross et al. 2002). Mixed flora is common (Trampuz and Zimmerli 2006b), particularly after open fractures and in chronic cases with formation of fistulae. A special form of osteomyelitis represents the prosthetic joint infection after joint replacement, or implant-associated forms of osteomyelitis after fracture treatment that can occur in early or late stages (Davis 2005). The presence of an implant considerably increases the risk of infection (implant-associated osteomyelitis) (Merritt 1988), because biofilm formation is facilitated on the surface of foreign body (Trampuz and Zimmerli 2006b). Furthermore, even after years of remission, survived bacteria can be released from biofilm and cause relapse of infection. Postoperative sternum osteomyelitis is one of the most common and hard-to-treat forms of the postoperative osteomyelitis, as described below.

The infections of prosthetic joints are not in the scope of this review due to specific pathogenesis, manifestations, and management, and it is referred to the recent literature on this topic (Osmon et al. 2013; Tande and Patel 2014).

Osteomyelitis due to vascular insufficiency has a tendency to affect the feet and usually occurs in combination with chronic ulcers (Caputo et al. 1994). This form, referred to as diabetic foot infection (Wagner 1981), is due to microvascular, macrovascular, and/or neuropathic changes, which are commonly observed in but not restricted to patients with diabetes mellitus (Lew and Waldvogel 2004). This form, which is characterized by specific pathogenesis, microbial spectrum, and treatment modalities (Waldvogel et al. 1970b), has recently been reviewed elsewhere (Uckay et al. 2014, 2015) and is beyond the scope of this work. In the context of differential diagnosis, the Charcot arthropathy has to be taken into account. It is the most grievous complication of diabetes mellitus and most commonly can be found in the joints of the lower extremity at the foot and ankle. It is characterized by a progressive destruction of the musculoskeletal system and is accompanied by pathologic fractures, joint dislocation, deformity, ulceration, infection, and the need for amputation in advanced stages. Because of its clinical picture, the Charcot arthropathy is often misdiagnosed as osteomyelitis, deep vein thrombosis, or rheumatoid arthritis (Idusuyi 2015) (Fig. 1).

2.2.2 Molecular and Cellular Pathogenesis

S. aureus osteomyelitis represents a tissue infection that involves bacterial invasion and bacterial persistence in bone tissue (Wright and Nair 2010). This process includes several steps that are most likely similar in different forms of osteomyelitis,

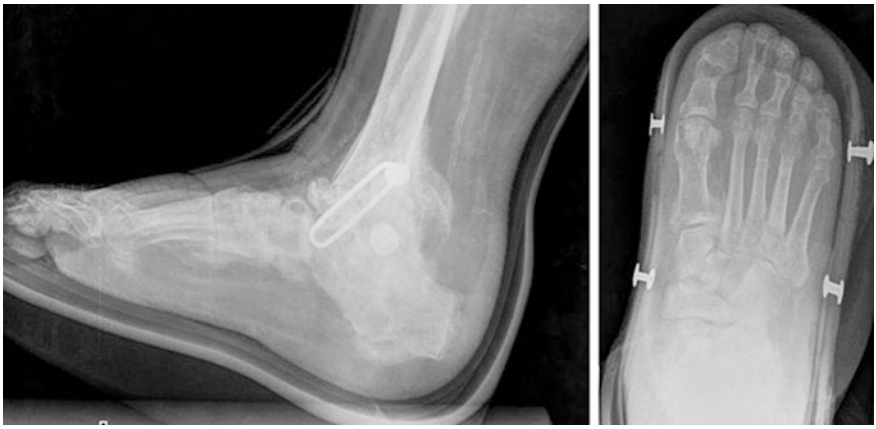

Fig. 1 Native radiological picture of a Charcot arthropathy in the foot and ankle in an advanced stage shows progressive destruction of the bone and joints with joint dislocation and deformation

as in hematogenous or local osteomyelitis (Lew and Waldvogel 2004; Wright and Nair 2010). In a **first step,** the bacteria need to **adhere to bone tissue**. For this, the *S. aureus* expresses a multitude of surface proteins with adhesive functions, called adhesins. *S. aureus* is endowed with a multitude of adhesins with redundant and overlapping functions (Clarke and Foster 2006). The adhesins collagen-binding protein (Cna) and bone sialoprotein (BSP) have been associated with hematogenous osteomyelitis in murine models and clinical samples of bone infections (Campoccia et al. 2009; Elasri et al. 2002; Tung et al. 2000). Yet, a single adhesin was not found to be clearly responsible for the development of osteomyelitis, suggesting that a combined action of several adhesins contributes to the formation of osteomyelitis (Wright and Nair 2010). In this context, accumulative growth leading to the establishment of a mixed biofilm consisting of multiple interadhering cell clusters of *S. aureus* as well as host products (extracellular matrix) is also very important. The staphylococcal factors involved in this complex process of biofilm production and its role in surface colonization and pathogenesis are extensively reviewed elsewhere (Becker et al. 2014; Otto 2009). In a **second step,** the bacteria **invade bone tissue**, including osteoblasts (Ellington et al. 1999; Tuchscherr et al. 2015). The vast majority of clinical *S. aureus* isolates expresses the adhesins fibronectin-binding proteins FnBPs that provide tight adhesion to integrin on the surface of host cells, such as osteoblasts (Sinha et al. 1999). Adhesion to host cells is followed by the uptake of bacteria that are located within phagosomes in the cytoplasm (Tuchscherr et al. 2015). It is extremely difficult to investigate whether the intracellular location of *S. aureus* plays a major role during osteomyelitis development or whether the bacteria are mainly adherent to extracellular matrix (Garzoni and Kelley 2009). However, a high invasiveness of host cells is a general characteristic of pathogenic *S. aureus* isolates (Kalinka et al. 2014; Valour et al. 2015), indicating an important contribution of host cell invasion in infection

development. After host cell invasion, different ways of infection are possible. If the bacteria release high levels of secreted virulence factors within the intracellular location, the host cells become activated and/or undergo cell death (Haslinger-Löffler et al. 2005; Kalinka et al. 2014; Rasigade et al. 2013). For osteoblasts, the phenol-soluble adhesins have been described as strong pro-inflammatory factors that can induce bone inflammation and cell death induction (Rasigade et al. 2013). In this way, the bacteria destroy host tissue, which enable them to invade deep tissue structures. In a **third step,** the bacteria need to **adapt for long-term persistence**, which can cause chronic and difficult-to-treat infections (Lew and Waldvogel 2004). For this, the bacteria downregulate their metabolism and the expression of secreted virulence factors to silently persist within the intracellular location for long-time periods (Tuchscherr et al. 2010, 2011). Hence, within their intracellular shelter, the bacteria are largely protected against treatments and against the host immune system. Nevertheless, when they leave the intracellular location, they can rapidly revert back to the fully aggressive phenotype, which can induce a new form of an acute infection (Tuchscherr et al. 2011). This mechanism is most likely also relevant within biofilm, as not only the intracellular location triggers SCVs but also SCV phenotypes produce enhanced levels of biofilm (Mitchell et al. 2013). Taken together, the dynamic SCV formation is strongly associated with chronic infection processes that cause massive bone destructions and deformations.

To rapidly adapt to the infection process in the acute as well as in the chronic stage, the bacteria change the expression of virulence factors by dynamic regulatory systems (Tuchscherr et al. 2011, 2015). *S. aureus* disposes of complex regulatory networks that closely sense the environment during the infection (Novick 2000). In the acute stage of infection, the bacteria show an activated *accessory gene regulator* (*agr)* system, which is a quorum-sensing system that upregulates the expression of many secreted virulence factors (Novick 2003). In the chronic stage of the infection, the bacteria downregulate their *agr* system and activate the sigma factor B (SigB) (Tuchscherr et al. 2015; Tuchscherr and Löffler 2016). SigB is a transcription factor that promotes bacterial survival in stress conditions, such as heat and nutrition deprivation (Bischoff et al. 2004). During the infection process, SigB most likely protects the bacteria against the hostile intracellular conditions. SigB induces a downregulation of secreted virulence factors and an upregulation of adhesins. In this way, the bacteria change from a highly aggressive to a persisting phenotype that largely avoids detection by the immune systems and silently persist for long-time periods (Tuchscherr et al. 2015).

Due to the changes in regulatory mechanisms, the bacteria do not only adapt their virulence factor expression, but also downregulate their metabolism. Consequently, the bacteria grow very slowly and change their phenotypes to small-colony variants (SCVs) (Proctor et al. 2006), which are adapted phenotypes for long-term persistence (Tuchscherr et al. 2010). SCVs are defined as colonies that are less than one-tenth of the wild-type phenotype colony size (Proctor et al. 2006). Experimentally, SCVs can be generated by defects in the electron transport system, e.g., *hemB* mutants (von Eiff et al. 1997b). Additionally, SCVs can appear

in clinical samples and have been associated with chronic infection processes, such as clinical cases of chronic osteomyelitis (Proctor et al. 1995). Mostly, a defined gene mutation could not be detected in clinical SCVs, and often, clinical SCVs are not stable, but can revert to the normal wild-type phenotype upon subcultivation. Analysis of the gene expression profiles in different SCVs revealed that clinical SCVs are very heterogenous bacterial subpopulations that most likely arise via different mechanisms (Kriegeskorte et al. 2011). Recent research demonstrated that SCVs form very dynamically during infection and are an integral part of any invasive tissue infection process. Early after infection bacteria gradually use their regulatory systems to adapt to the intracellular milieu/host tissue. For this, they downregulate the *agr* system and upregulate SigB that enable the bacteria to form dynamic SCV phenotypes. In this way, the bacteria can escape from the host immune response and from antimicrobial treatments (Tuchscherr et al. 2016), but nevertheless have the capacity to rapidly revert back to the fully aggressive wild-type phenotype when they leave their shelter (Tuchscherr et al. 2011). At a later stage of infection, when the host organism was not successful to clear the infection focus, chronically infecting bacteria can additionally make use of mutations to form permanent SCVs, such as defined defects in the electron transporter system. The stable SCVs with defined mutations are less flexible, but apparently better ensure for long-term persistence within the host organism (Proctor et al. 2006) (Fig. 2).

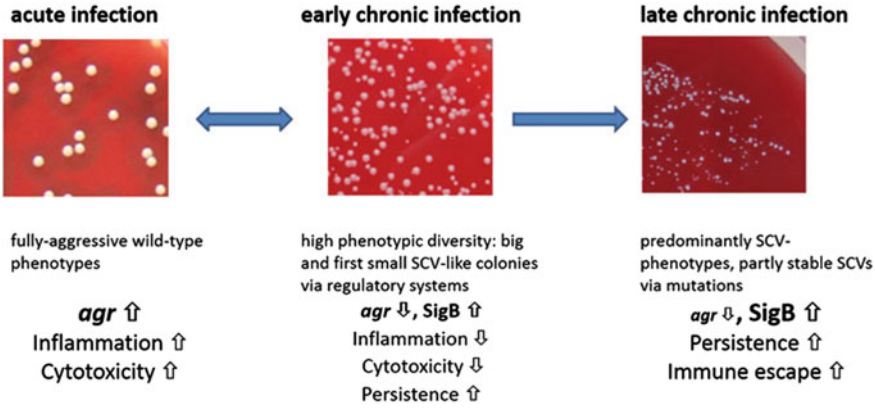

Fig. 2 Schemata of the bacterial adaptation mechanisms from acute to chronic bone infection. In the acute infection, the bacteria predominantly show the aggressive wild-type phenotype that induces high levels of inflammation and cytotoxicity. If the infection proceeds (early chronic infection), the development of first small SCV-like colonies appears. These early SCVs are highly dynamic and are based on regulatory processes. During long-term persistence, more and more SCV phenotypes develop. Additionally, bacterial mutations occur that can result in stable SCVs

The formation of metabolically downregulated SCV-like phenotypes has important implications on the efficacy of antibiotic treatments and the host immune system. It is well known from cell culture and animal models that antibiotics have variable capacity to penetrate into host cells and biofilm. Some compounds, such as rifampicin and ciprofloxacin, efficiently accumulate within host cells, whereas other antibiotic classes are less effective to penetrate to the intracellular milieu, such as β-lactams (Van Bambeke et al. 2006). Further on, many antibiotics loose effect, when bacteria downregulate their metabolism and enter in the stage of SCV formation. In many experimental conditions, rifampicin was detected as compound that efficiently penetrates in host cells and still is active against metabolically inactive SCV phenotypes (Tuchscherr et al. 2016).

Similar to the efficacy of antibiotic treatment, the immune system loses activity to clear persisting bacteria. Already cell culture models demonstrate that SCV phenotypes induce less inflammatory responses than wild-type phenotypes (Tuchscherr et al. 2010, 2011). Not only the innate immune responses largely fail to respond to persisting bacteria, but also the adaptive immune system is altered during chronic infection. Very recent experimental data suggest that especially in *S. aureus,* osteomyelitis parts of the cellular adaptive immune system seem to be more important. In the acute phase, T cells become heavily stimulated indicating their possible role in specific responses. However, in further progress of the disease, i.e., if the osteomyelitis becomes more subacute or even chronic, the T cells switch to an anergic state, most possibly by the influence of innate lymphoid cells (Ziegler et al. 2011; Tebartz et al. 2015). The possible clinical relevance of these interesting data is still beyond the possibility of a reliable judgement.

2.3 Clinical Manifestations and Diagnosis

The clinical picture of an acute hematogenous osteomyelitis impresses with local pain, warmth, tenderness, and fever up to a clinical picture of severe sepsis. However, vertebral and pelvic osteomyelitis may show symptoms that are less differentiated, e.g., pain that cannot be specifically assigned and general infection symptoms that negatively influence general condition. In contrast to the acute phase, the chronic phase of an osteomyelitis is associated with long periods without clinical symptoms followed by acute infection exacerbation with local signs of inflammation. Furthermore, chronic osteomyelitis impresses with non-healing ulcers approximate to the affected bone and a draining sinus possibly with the presence of pus (Davis 2005; Torda et al. 1995) (Figs. 3 and 4).

Careful examination, laboratory diagnostic tools, and imaging techniques are helpful for the detection of an osteomyelitis as well as the microbiological identification/characterization of the pathogen for an effective and successful treatment. In acute osteomyelitis, laboratory examination reveals increased peripheral white blood cell count, erythrocyte sedimentation rate, and serum C-reactive protein (CRP), whereas the CRP is a good marker for appropriate

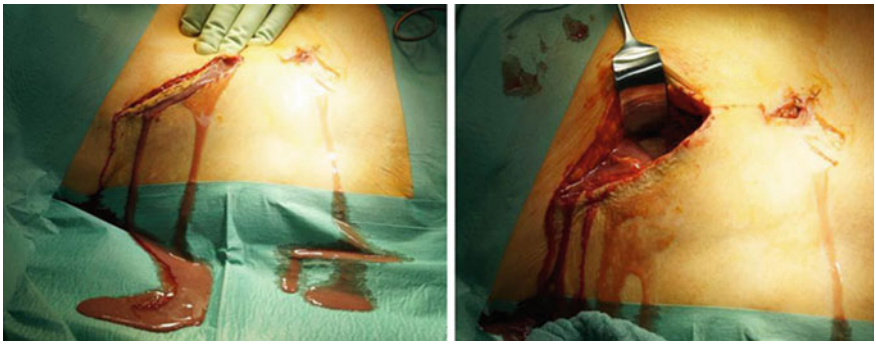

Fig. 3 Intraoperative picture of an acute implant-associated infection of the pelvic with local pain, redness, tenderness, swelling, and the presence of pus. The clinical picture shows an anterior approach to the pelvic

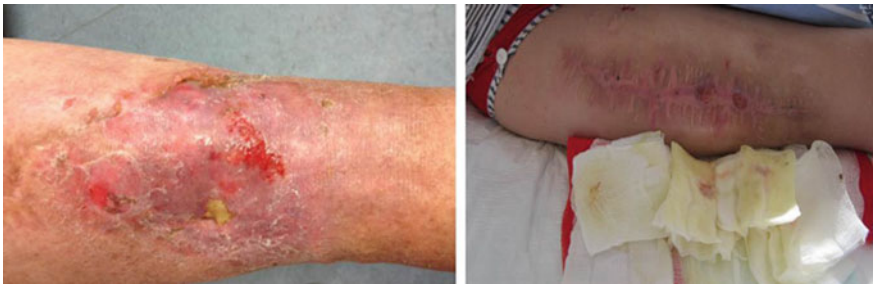

Fig. 4 Clinical picture of two examples of a chronic osteomyelitis (*lower leg on the left side* and *upper leg/hip on the right side*) with non-healing ulcera, a draining sinus and the presence of pus

therapy, treatment failure, or relapse. Overall, it has to be remembered that normal laboratory markers do not exclude an osteomyelitis (Davis 2005). Different imaging tools are available to diagnose osteomyelitis. Computed tomography (CT) scan, which demonstrates bone destruction, is more precise than X-ray imaging and magnetic resonance imaging (MRI) for the detection of soft tissue infection and its involvement. It takes a minimum of 3 weeks to detect bone changes by X-ray in cases of acute osteomyelitis. The acute form of osteomyelitis is associated with the 3 signs in X-ray imaging: soft tissue swelling, periosteal reaction, and bone destruction, which is represented as subperiosteal bone resorption and cortical erosions and in the course spread to the medullary cavity with lytic lesions (Barrington 1988; Davis 2005; Gold et al. 1991). In cases of chronic osteomyelitis, the X-ray imaging reveals bone sclerosis, periosteal new bone formation, sequestra, and sinuses up to circumscribed lytic lesions named Brodie abscess. The differentiation between infected bone and neuropathic destruction is impossible just by X-ray. Next to radiological examinations, microbiological and histological analysis is necessary to differentiate between these two entities. CT scans are more precise

compared to X-ray imaging to detect sequestra, cortical changes, involucrum, and intraosseous gas (Davis 2005). MRI imaging so far is the best imaging modality for detecting purulent collections, representation of soft tissue affection, early changes of the long bones (edema), and is especially useful in vertebral osteomyelitis and diabetic foot infections (Davis 2005). Bone scans with increased technetium-99 m-labeled diphosphonate uptake support the clinical diagnosis but have only poor specificity (Davis 2005). Further nuclear medicine imaging are technetium-99m (^{99m}Tc)-diphosphonate bone scintigraphy, gallium-67 (^{67}Ga), and in vitro labeled leukocyte imaging, which are helpful diagnostic tools for specific questions. The sensitivity of bone scintigraphy is very high in contrast to the specificity. ^{67}Ga is useful for the detection of tumor and trauma and in aseptic inflammation. It has to be taken into account that there is a time interval of 1–3 days between application and imaging. The disadvantage of the in vitro leukocyte labeling process is low availability. The most sensitive (95 %) diagnostic tool in bone and soft tissue infection is 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) positron-emission tomography (PET) with or without computed tomography (CT). Its specificity is 75–99 % (Love and Palestro 2016) (Fig. 5).

Microbiological diagnostics include detection, identification, and antimicrobial susceptibility testing of pathogen from biopsy specimens and blood. Multiple bone specimens should be taken (at least three, better four to five) to facilitate recognition of possible contamination. Detection of causative microorganism is essential for optimization of antimicrobial treatment. Diagnostic biopsy is important in cases where blood cultures remained negative (Berbari et al. 2015). Fistula or wound swabs are less useful for microbiological diagnosis because of difficulties to differentiate contaminating physiological flora from pathogen. Nevertheless, wound

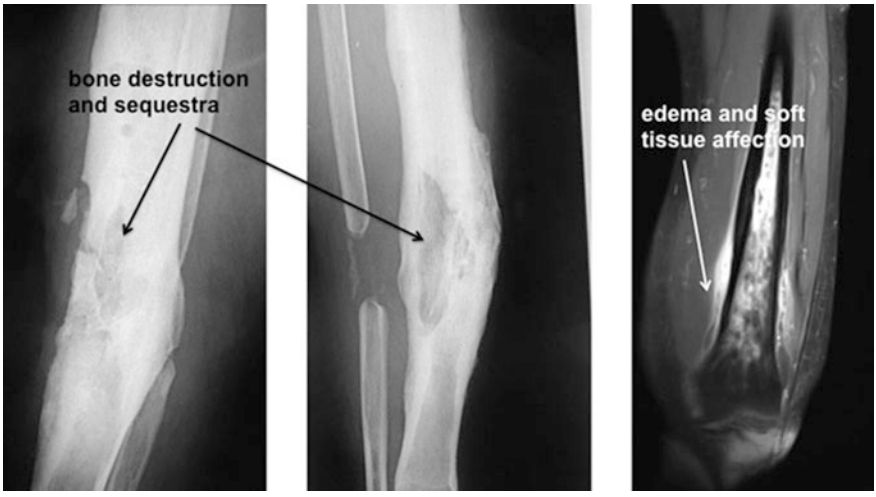

Fig. 5 X-ray and magnetic resonance imaging in case of a chronic osteomyelitis show bone destruction, sequestra, edema, and soft tissue affection

swabs can deliver helpful information if taken from purulent foci, and several swabs yield *S. aureus*. At least two sets of blood cultures should be taken, each consisting of an aerobic and an anaerobic bottle (Berbari et al. 2015). In acute hematogenous osteomyelitis, the blood cultures are positive in about 58 % of cases (Mylona et al. 2009). Specimens for microbiological analysis should be taken prior to the antibiotic therapy to increase the yield probability (Kim et al. 2012). If antibiotic therapy has already been established, the consideration should be made to withhold antibiotics for several days prior to microbiological sampling when feasible (Zimmerli 2010). Noteworthy, a recent single-center retrospective study did not confirm the association of prebiopsy antibiotics with culture results and concluded that antibiotic exposure should not be the sole reason for foregoing biopsies (Marschall et al. 2011). Molecular diagnostics can improve pathogen detection in cases when culture remained negative. Broad-range PCR (16S rRNA gene amplification) with subsequent sequencing increased diagnostic yield in patients with spinal infection (Fuursted et al. 2008). Biopsy is additionally submitted for pathological investigation (Berbari et al. 2015) to underline the diagnosis of osteomyelitis in cases of negative microbiological examination or to rule out another reason for bone destruction (Sehn and Gilula 2012).

2.4 Special Forms of Osteomyelitis

2.4.1 Vertebral Osteomyelitis

Vertebral osteomyelitis, also referred to as spondylodiscitis, is infection of the intervertebral disk and the adjacent vertebrae. Vertebral osteomyelitis is almost always of hematogenous origin with endocarditis, catheter infections, skin and soft tissue infections, urinary tract, dental infections, and intravenous drug abuse being the most common primary source of infection (Zimmerli 2010; McHenry et al. 2002). More seldom, inoculation can occur by direct inoculation after penetrating trauma, open fractures, postoperatively or from a spreading infection of the surrounding soft tissue (Berbari et al. 2015; Boody et al. 2015; Zimmerli 2010). As for other localizations of hematogenous osteomyelitis, *S. aureus* is most commonly isolated (up to 67 %) (Babouee Flury et al. 2014; Grammatico et al. 2008; Boody et al. 2015). About 50–60 % of the cases occur in the lumbar spine, 30–40 % in the thoracic spine, and about 10 % of infection manifestation occurs in the cervical spine (Zimmerli 2010). Vertebral osteomyelitis is accompanied by complications due to seeding in different compartments, which leads to paravertebral, epidural, or psoas abscesses (Zimmerli 2010). Neurological complications could be observed in about 35 % of the cases (Boody et al. 2015; Pigrau et al. 2005). Diabetes mellitus, i. v. drug abuse, systemic infections, obesity, malignancy, immunodeficiency, malnutrition, and smoking are known to be risk factors for the development of a vertebral osteomyelitis (Boody et al. 2015; Zimmerli 2010). Next to general clinical signs of infections such as fever, chills, weight loss, swelling, or redness, the

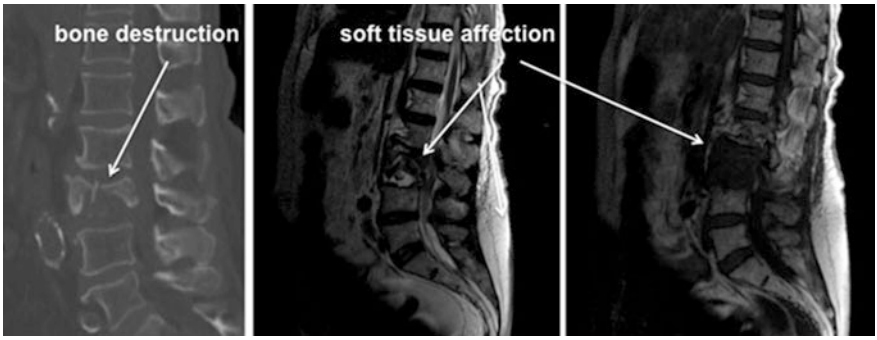

Fig. 6 CT and MRI of spondylodiscitis of the lumbar spine that shows the spread of infection

clinical picture of vertebral osteomyelitis is characterized by severe back pain on the site of infection, muscle spasms, painful or difficult urination, and neurological deficits in 10–20 % (radiculopathy, myelopathy) as a result of direct infection spreading on neural elements, compression due to an epidural abscess, or compression due to spine instability. Futhermore, the clinical picture can be dominated by the primary infection focus (Berbari et al. 2015; Zimmerli 2010). Careful neurological examination and laboratory diagnostic tools are helpful for the detection of a vertebral osteomyelitis as well as the microbiological identification of the pathogen for a successful treatment. Samples are collected either intraoperatively or through CT-guided biopsy sampling of the vertebra. Imaging tools are necessary to diagnose this kind of infection and to determine the location of infection, whereas an X-ray imaging is the first choice tool followed by CT-scan, which demonstrates bone destruction, and MRI for soft tissue infection involvement (Berbari et al. 2015; Boody et al. 2015; Zimmerli 2010) (Fig. 6).

2.4.2 Postoperative Sternum Osteomyelitis

A special form of postoperative osteomyelitis is sternum osteomyelitis. In cardiothoracic surgery, the median sternotomy is the most important operational approach to achieve an overall access to the heart and to the vessels as a possible approach for extracorporeal circular flow (Antunes et al. 1997; Julian et al. 1957). Disturbance of wound healing followed by sternal and mediastinal infection occurs in 0.4–8 % after heart thoracic surgery and is followed by high rates of mortality (14–50 %). Postoperative sternum osteomyelitis after median sternotomy occurs in 0.8–8 % (Grossi et al. 1985; Klesius et al. 2004; Loop et al. 1990). The spread of bone infection depends on various influencing factors, e.g., virulence of the pathogen, implanted surgical devices, soft tissue damage, bone vascularization, and bone damage. Furthermore, diabetes mellitus, obesity, chronic obstructive pulmonary disease, treatment with corticosteroids, intraoperative use of both Aa. mammaria interna, extended operation time, and prolonged artificial respiration as well as the

development of a postoperative pneumonia are known to be risk factors for the development of a sternum osteomyelitis (Loop et al. 1990). While *S. aureus* has been reported as the predominant pathogen in deep sternal wound infections (Borger et al. 1998; Gummert et al. 2002), some authors found coagulase-negative staphylococci to be most commonly isolated in sternum osteomyelitis (Tegnell et al. 2000). Next to various classifications, El Oakley and Wright classified postoperative mediastinitis in 1996 into the following: A) wound healing disorder after median sternotomy without clinical signs of infection and without microbiological detection of pathogens and B) clinical and microbiological signs of infection with infected soft tissue around the sternum and sternum osteomyelitis with or without mediastinal sepsis and with or without sternum instability. B is further subdivided into a) superficial wound infection of the cutaneous and subcutaneous tissue and b) infection with a sternum osteomyelitis that can be associated with a retrosternal infection (El Oakley and Wright 1996). The clinical symptoms of sternum osteomyelitis vary dependent on the phase of infection. In the beginning, the infection can be accompanied by almost no clinical symptoms followed by local signs of infection such as local pain, redness, hyperthermia, and swelling of the wound in advanced stages. Furthermore, the clinical picture can be accompanied by local infection signs such as osteocutaneous fistula, the presence of pus, wound secretion, and sternum instability up to sternum dehiscence. In addition, systemic infection signs such as fever, dyspnea, tachycardia, and chemical laboratory changes with the possibility of sepsis development may be present. Analogous to the clinical symptoms, it can be difficult to diagnose sternum osteomyelitis in its early stages. Sternum osteomyelitis can be diagnosed with the help of its clinical symptoms, laboratory and microbiological results, X-ray examination, CT, or MRI (Jurkiewicz et al. 1980; Ringelman et al. 1994).

2.5 Management

S. aureus-associated musculoskeletal infections in their chronic stages require a combination of antibiotic and surgical treatment, whereas antibiotic treatment alone may be sufficient to cure osteomyelitis in its early and acute stages (Davis 2005; Lew and Waldvogel 2004).

Antimicrobial treatment

Empiric antimicrobial regimens are used in cases where the causative organism is not known at the initiation of antimicrobial treatment. Those regimens might include a combination of vancomycin and a third- or fourth-generation cephalosporin. Alternatively, in case of intolerance, a combination of daptomycin and a quinolone might be used (Berbari et al. 2015). If *S. aureus* is anticipated as a possible cause of infection, the empiric use of penicillinase-stable penicillin (e.g., flucloxacillin or oxacillin) instead of vancomycin or daptomycin might be considered in areas with low prevalence of MRSA.

Table 1 Antimicrobial agents for the treatment of acute *S. aureus* osteomyelitis in adults[a, b]

Microorganism	Primary choice	Alternatives
Methicillin-susceptible *S. aureus*	Flucloxacillin (or oxacillin or nafcillin) 2 g intravenously every 4–6 h or Cefazolin[c] 1–2 g intravenously every 8 h	Vancomycin 15–20 mg/kg intravenously every 12 h or daptomycin 6–8 mg/kg intravenously every 24 h or levofloxacin 750 mg orally every 24 h and rifampin orally 300 mg every 12 h
Methicillin-resistant *S. aureus*	Vancomycin 15–20 mg/kg intravenously every 12 h (adjustment according to TDM[d])	Daptomycin 6–8 mg/kg intravenously every 24 h or levofloxacin 750 mg orally every 24 h and rifampin orally 300 mg every 12 h

[a]modified from Berbari et al. (2015) and Zimmerli (2010)
[b]choice of antimicrobial agents and dosages might be a subject of national recommendations and should be adjusted according to individual patient's characteristics as well as in vitro susceptibility. The list is not intended to include all possible treatments
[c]or other cephalosporins with activity against staphylococci in the respective intravenous dosages, e.g., cefuroxime
[d]TDM, therapeutic drug monitoring

As soon as *S. aureus* is identified from clinical specimens, the treatment is tailored to the targeted therapy. Appropriate antimicrobial agents are shown in Table 1. This includes flucloxacillin or other penicillinase-stable penicillin (up to 12 g/day). In case of penicillin allergy, a first-generation cephalosporin (e.g., cefazolin 2 g t.i.d.) can be chosen. However, in case of anaphylaxis due to β-lactams in the history, vancomycin must be preferred. Apart of β-lactam intolerance, vancomycin should not be used for treatment of infections caused by methicillin-susceptible *S. aureus* (MSSA). In a large retrospective study, the relative risk for recurrence of osteomyelitis due to *S. aureus* after vancomycin therapy was more than twice that for β-lactams (Tice et al. 2003). Initial dose for a normal weight person with normal renal function is 1 g b.i.d., which can be adapted according to the results of therapeutic drug monitoring. Recommended trough serum vancomycin concentration for *S. aureus* osteomyelitis is 15–20 mg/L (Rybak et al. 2009). However, the established pharmacokinetic–pharmacodynamic target is not achievable with conventional dosing, if vancomycin minimum inhibitory concentration (MIC) of *S. aureus* isolate is ≥ 2 mg/L (Rybak et al. 2009). In such case, alternative therapies should be considered (e.g., daptomycin). While daptomycin is not approved for the treatment of bone and joint infections, its efficacy in osteomyelitis has been demonstrated (Malizos et al. 2016; Roux et al. 2016) and recent guidelines suggest this antibiotic as alternative therapy (Berbari et al. 2015). The guidelines recommend higher dosages of 6–8 mg/kg/day (Berbari et al. 2015), which is supported by data from clinical reports (Roux et al. 2016; Seaton et al. 2015). Novel cephalosporins ceftaroline and ceftobiprole exhibit activity against MRSA, but resistant strains have been described (Schaumburg et al. 2016;

Strommenger et al. 2015). Therefore, MIC determination should be performed prior to use of these cephalosporins.

Antimicrobial therapy should be administered intravenously for at least 14 days. If considerable improvement has been achieved, the treatment can be switched to oral antibiotics (Babouee Flury et al. 2014). For oral therapy, antibiotics with high bioavailability are used. Chinolones, rifampin, linezolid, and clindamycin have excellent bioavailability and can be administered orally without concern. Oral β-lactam antibiotics (e.g., cefuroxime axetil) are characterized by suboptimal bioavailability and should be avoided for the treatment of osteomyelitis (Zimmerli 2010).

The combination of an oral quinolone and rifampin resulted in success rates similar to that of the standard intravenous therapy in several studies (Schrenzel et al. 2004; Viale et al. 2009). Linezolid has an excellent bioavailability, but due to its bacteriostatic mode of action, it should only be used if other antimicrobials cannot be given (Berbari et al. 2015). Clinical data for another oxazolidinone antibiotic, tedizolid, in bone and joint infections are lacking. Clindamycin is also not recommended as first-line monotherapy of acute hematogenous osteomyelitis, because of lack of bactericidal activity (Zimmerli 2010; Berbari et al. 2015).

Monotherapy is usually sufficient if *S. aureus* has been identified as causative agent. However, in some situations, administering a second drug can be advantageous. Particularly in case of the presence of a foreign body, e.g., osteosynthesis material at the infection focus, rifampin should additionally be administered (Trampuz and Zimmerli 2006a). Rifampin is an old antibiotic with a very good in vitro activity against *S. aureus* biofilm (Trampuz and Zimmerli 2006a). Rifampin has very good bioavailability and can be given orally subsequent to intravenous treatment. Rifampin must not be given as a single drug, because of rapid resistance development. Some experts even recommend to add rifampin after several days of effective therapy with the "main" antibiotic to prevent the emergence of rifampin resistance (Habib et al. 2015; Sendi and Zimmerli 2012). Rifampin is a potent inducer of hepatic drug metabolism, which increases the metabolism of many coadministered drugs, thereby decreasing their serum concentrations (Venkatesan 1992). Therefore, careful review of possible interactions with patient's concomitant medication is essential. Clindamycin may be considered as a "combination partner," e.g., in case of severe infection. It inhibits production of *S. aureus* toxins (Otto et al. 2013), additionally to the exhibition of its antibacterial activity. Combination of clindamycin with rifampin has also been suggested (Czekaj et al. 2011), but recent pharmacokinetic studies demonstrated dramatic reduction of clindamycin serum concentration if combined with rifampin (Bernard et al. 2015a; Join-Lambert et al. 2014). Therefore, caution is recommended regarding this combination until more data become available.

While only low-quality evidence is available on optimal duration of antimicrobial therapy in osteomyelitis, there is a consensus to treat for a total duration of 6 weeks (Berbari et al. 2015). A recent randomized study demonstrated non-inferiority of 6-week antimicrobial treatment versus 12-week treatment for vertebral osteomyelitis (Bernard et al. 2015b). Also, the results of an observational

study on patients with vertebral osteomyelitis suggest that antibiotic therapy could safely be shortened to 6 weeks without increasing the risk of relapse (Roblot et al. 2007). Prolongation of antibiotic therapy for up to 3 month and longer can be necessary in some cases (Berbari et al. 2015), and the respective decision is made based on individual response to treatment including information from laboratory (Carragee et al. 1997) and imaging investigations (Carragee 1997; Kowalski et al. 2006). In addition, prolonged antimicrobial treatment can be necessary in case of particular microorganisms isolated, e.g., fungi (Cornely et al. 2012).

Several studies on children with osteomyelitis have shown that early switch to oral therapy is safe also in this population (Jagodzinski et al. 2009; Peltola et al. 2010). According to a systematic review (Howard-Jones and Isaacs 2010), most children with chronic osteomyelitis received 4–6 weeks of parenteral antibiotics followed by oral antibiotics for a total duration of 3–6 months in 14 observational case series. The success rate ranged from 80 to 100 %. Small observational studies found comparable cure rates with only 5–14 days of parenteral and 3–6 weeks of oral antibiotics (Howard-Jones and Isaacs 2010). However, large randomized controlled trials comparing short-course parenteral and oral antibiotics with longer antibiotic duration in chronic osteomyelitis in children are lacking.

The targeted antimicrobial therapy of posttraumatic or postoperative osteomyelitis upon identification of *S. aureus* as a causative organism is similar to that of hematogenous osteomyelitis and is performed according to general principles described above. The presence of an implant or other foreign material is a crucial factor for the choice of treatment of posttraumatic (postoperative) osteomyelitis, because penetration of the most antibiotics into biofilm is minimal (Trampuz and Zimmerli 2006a). If the removal of foreign material is not possible, a biofilm-active antibiotic rifampin is added (Trampuz and Zimmerli 2006a), as described above. While limited data suggest a role of rifampin combinations for the treatment of orthopedic implant-related staphylococcal infections (Zimmerli et al. 1998), larger randomized controlled trials are needed to definitely confirm this beneficial effect.

Surgical treatment

In addition to antibiotic treatment, the surgical therapy plays an important role in advanced phases of osteomyelitis. It includes debridement of necrotic bone and soft tissue as well as meticulous soft tissue management (Davis 2005). Surgical debridement is known to be a very effective treatment component in case of musculoskeletal infections leading to an improved wound healing (Henke et al. 2005).

In vertebral osteomyelitis with refractory infection, neurologic deficits, or progressive deformity or instability, the surgical debridement in combination with neurological decompression and spinal stabilization has to be performed. Depending on the spread of infection, the anterior debridement and the implantation of a graft with or without posterior instrumentation become necessary, which is considered as the gold standard in the operative treatment of vertebral osteomyelitis. Sometimes, the posterior debridement and decompression alone is sufficient and indicated (Berbari et al. 2015; Boody et al. 2015; Zimmerli 2010).

Surgical treatment also has a decisive status in advanced phases of the postoperative sternum osteomyelitis. Surgery includes an extended debridement, removal of infected bone, removal of foreign material, and, if necessary, the stabilization of the thorax to achieve a spontaneous and stable breathing situation combined with a stable soft tissue status. Furthermore, it is important to take samples for microbiological analysis. The thoracic wound can be either treated open with a vacuum sealing bandage or, if closed, with a suction rinse system (Agarwal et al. 2005; Doss et al. 2002; Hersh et al. 2001; Milano et al. 1999). Restabilization of the sternum can be achieved by wiring according to Robicsek (Losanoff et al. 2002; Robicsek et al. 1977). Defects after debridement can be covered by muscle flap surgery, general flap surgery, or omentum plastic surgery (Falagas and Rosmarakis 2006; Nahai et al. 1989).

The four main surgical aspects in chronic musculoskeletal infections are as follows: (1) debridement, (2) soft tissue reconstruction, (3) management of bony dead space, and (4) stabilization of mechanically compromised bone (Parsons and Strauss 2004). In chronic osteomyelitis, it is most important to perform a very radical debridement which includes resection of all infected or necrotic bone till healthy, and bleeding bone is visible. Radical debridement can either be performed at once or in two or even more stage revision surgeries (Davis 2005). In cases of implant-associated infections, the surgical treatment has to be extended with the removal of the implant with fracture/bone stabilization after implant removal and the two-step replacement of the implant in advance additionally to the four main aspects of surgical treatment in musculoskeletal infections mentioned above (Ciampolini and Harding 2000; Lew and Waldvogel 2004) (Fig. 7).

Besides systemic antibiotic treatment, the surgical treatment offers the option to apply antibiotic substances locally in different ways. With the help of this technique, high antibiotic concentrations can be achieved locally while avoiding negative side effects of high concentrated systemic antibiotic treatment. Antibiotic-loaded bone cement is a method to achieve those concentrations as it serves as local drug delivery system. Its use is established for more than 40 years with good long-term results. The use of a local drug delivery system has been reported to lower the rate of infection (Breusch and Kuhn 2003; Buchholz et al. 1981; Raschke and Schmidmaier 2004). In most cases, polymethylmethacrylate

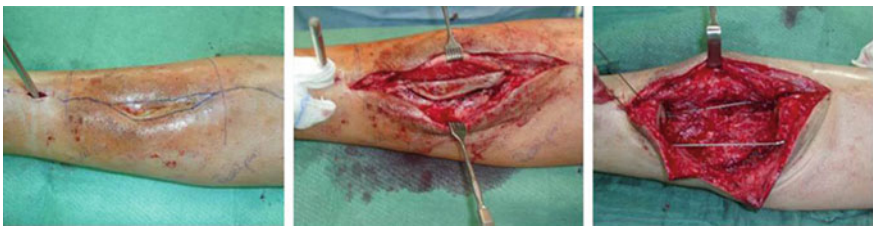

Fig. 7 Documentation of the surgical procedure in case of a chronic osteomyelitis of the tibia: preparation of bone stabilization with the help on an external fixation, marking of the infected soft tissue that has to be removed/debrided, resection of the chronically infected bone till healthy bleeding bone is visible

(PMMA) bone cement is loaded with gentamicin, which is characterized by an initial peak of antibiotic release for hours till days followed by a continuous release of low concentrations up to weeks after implantation. The antibiotic application can be changed to vancomycin, depending on local epidemiology of *S. aureus* or susceptibility testing results in a particular case. Furthermore, in 80 % of cases, the use of gentamicin-loaded PMMA-bone cement inhibits *S. aureus*-induced biofilm synthesis, which is known as one possible reason for therapy-refractory and chronic implant-associated infections (Anguita-Alonso et al. 2005; Breusch and Kuhn 2003; Neut et al. 2009; Reichert et al. 2007; Stengel et al. 2001). In context with *S. aureus*-associated musculoskeletal infections, antibiotic-loaded PMMA-bone cement is available and can be used in different products. Antibiotic-loaded PMMA-bone cement is produced as beads with different sizes and as a powder that can be processed individually to spacers as well as to industrial anatomically preformed shapes, which can be applied temporarily (Fig. 8).

In the treatment course, PMMA-bone cement beads or spacers have to be removed for a two-step bone replacement/reconstruction or two-step reimplantation of an implant/prosthesis. Timely removal is particularly important in the case of gentamicin-loaded bone cement, because subinhibitory gentamicin concentrations might select for staphylococcal small-colony variants (SCVs) (von Eiff et al. 1997a). Resorbable antibiotic-loaded materials such as sponges or powder are an alternative method for local application of antibiotic substances (McKee et al. 2002, 2010). Depending on the soft tissue status, wound closure and soft tissue reconstruction can be achieved by primary wound closure, skin grafts, or different kind of flaps. After infection healing by the methods mentioned above and verification of the absence of bacteria, the complex reconstruction of osseous defects has to be planned, which is practicable with several existing techniques (Davis 2005; Stengel et al. 2001). Osseous reconstruction can be aimed with autologous bone graft, vascularized free fibula, iliac crest bone grafts, extraction of autologous bone by the reamer–irrigator–aspirator system, or distraction osteogenesis (Pfeifer et al. 2011). Futhermore, the Masquelet technique has to be considered that leads to a foreign body-induced membrane, which stimulates the consolidation of the implanted bone

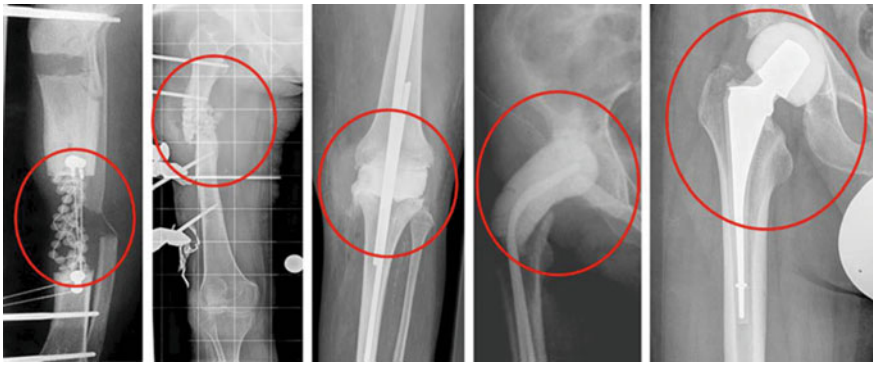

Fig. 8 Five examples of the use of antibiotic-loaded bone cement as beads or as spacers

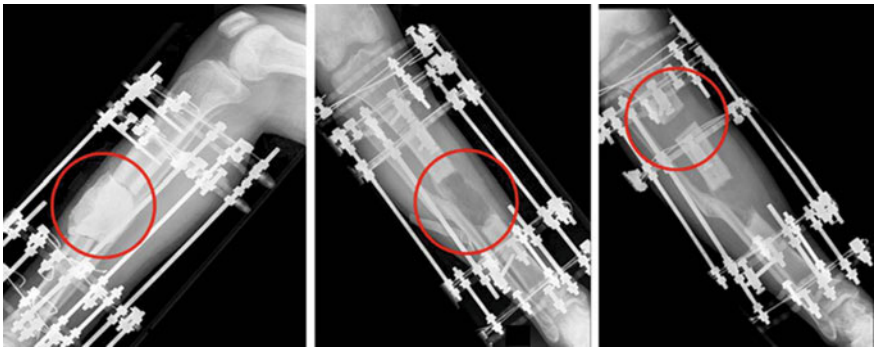

Fig. 9 Series of osseous defect reconstruction of the tibia. Radical surgical debridement and resection of infected bone, temporary local antibiotic application with the help of a PMMA-bone cement spacer, and fitting of an Ilizarov external fixateur. Removal of the cement spacer and bone osteotomy. Stepwise distraction of the bone

graft in cases of the need for osseous reconstruction (Masquelet and Begue 2010). For distraction osteogenesis, the Ilizarov technique is indicated. With the help of a ring-shaped external fixateur and stepwise distraction of the bone for a time period of weeks till months after bone osteotomy, osseous defects can be filled (Davis 2005) (Fig. 9). The management of bone healing can be determined individually. In some cases, the early change to an intramedullary stabilization advice, e.g., intramedullary nail, is indicated, which also exists as a gentamicin coated model. The use of coated implants as another option of a drug delivery system has the opportunity to prevent biofilm synthesis and the development of implant-associated infections (Schmidmaier et al. 2006). First studies and clinical experience with the use of coated implants reveal promising results to reduce and prevent implant-associated infections by this method (Fuchs et al. 2008).

Severe *S. aureus*-associated musculoskeletal infections can lead to joint arthrodesis, physical disability, or at worst limb amputation (Lew and Waldvogel 2004). In cases of osteomyelitis with vascular deficits, it is fundamental to ensure the blood supply—if necessary with the help of angioplasty or bypass operations (Davis 2005).

The optimal management of osteomyelitis requires a strong collaboration between all involved specialists (Berbari et al. 2015). This includes surgeons, radiologists, neurosurgeons, infectious disease specialists, and clinical microbiologists.

3 *Staphylococcus aureus* Infections of Native Joints

Acute arthritis, also called septic arthritis, is a seldom condition in general population with an incidence of about six cases per 100,000 inhabitants per year (Kaandorp et al. 1997a). These numbers are considerably higher in patients with

predisposing conditions. Incidence of bacterial arthritis in patents with rheumatoid arthritis ranges between 28 and 38 per 100,000 inhabitant per year (Kaandorp et al. 1997a). Other risk factors are intravenous drug abuse (Peterson et al. 2014), alcoholism, and diabetes as well as immunosuppression due to underlying disease or treatment (Mathews et al. 2010; Salar et al. 2014).

Acute arthritis of native joints is predominantly due to the hematogenous infection, while the direct infection of joint, e.g., through surgery or trauma, is rare (Kaandorp et al. 1997a). Skin infection is an important risk factor for hematogenously originated septic arthritis, particularly in patients with rheumatoid arthritis (Kaandorp et al. 1995). In the most cases, single joint is affected (80–90 % of patients), knees (approximately 50 %) being most commonly involved (Kaandorp et al. 1997a; Gupta et al. 2001; Weston et al. 1999). In children, the hip is most frequently affected (Shirtliff and Mader 2002).

Bacteria are able to enter the joint and may induce an acute inflammatory synovitis within hours. In the course, proliferative lining-cell hyperplasia occurs with an inflow of acute and chronic inflammatory cells. Released aggressive cytokines and proteases induce cartilage destruction and later permanent subchondral bone loss. In the case of infection spreading surrounding soft tissue, ligaments and tendons can also be affected and destroyed up to the formation of sinus tracts (Goldenberg 1998; Shirtliff and Mader 2002).

S. aureus is by far the most common causing organism found in about 50 % of septic arthritis cases in adults (Kaandorp et al. 1997a; Weston et al. 1999). This rate did not considerably change over decades (Dubost et al. 2002).

The patients with acute arthritis classically present with pain, swelling, warmth, redness, and loss of function in the affected joint (Margaretten et al. 2007). These local symptoms are often accompanied by fever (Margaretten et al. 2007; Gupta et al. 2001; Weston et al. 1999). Septic arthritis is characterized by a rapidly destructive course and is therefore considered an emergency (Davis 2005; Goldenberg 1998). In most severe cases, the joint function is permanently lost with resulting disability in 25–50 % of patients (Goldenberg 1998; Mathews et al. 2010; Shirtliff and Mader 2002). Patients who receive immunosuppressive treatments may exhibit minimal clinical manifestations (Salar et al. 2014).

Urgent diagnosis and management of sepsis arthritis are essential because of high mortality amounting to 10–15 % (Kaandorp et al. 1997b; Weston et al. 1999) and reaching 56 % in *S. aureus* polyarticular arthritis (Dubost et al. 1993). Urgent management should also minimize the risk of rapid joint destruction and long-time sequela (Kaandorp et al. 1997b).

Laboratory investigations often reveal increased inflammatory parameters (C-reactive protein, erythrocyte sedimentation rate, leukocytes) (Shirtliff and Mader 2002; Hariharan and Kabrhel 2011). Joint puncture provides purulent synovial fluid with low viscosity. Leukocyte count is >20,000/μl in 81 % of patients, with a neutrophil fraction of >75 % (Shmerling et al. 1990). Others have suggested that a leukocyte count value of >50,000/μl is discriminative for septic arthritis (Margaretten et al. 2007; Goldenberg and Reed 1985).

Direct Gram stain performed from synovial fluid helps to rapidly assume the group of microorganisms involved and to accordingly adapt antimicrobial treatment at the early stage. However, the sensitivity of Gram stain is about 50 % (Weston et al. 1999) compared to 67–100 % sensitivity of culture (Margaretten et al. 2007, Goldenberg and Reed 1985). There is some data that inoculation of synovial fluid into a blood culture bottle with liquid medium followed by the incubation in an automated blood culture system may increase the detection rate of microorganisms (Hughes et al. 2001). However, another study demonstrated that conventional culture on solid medium was not inferior to the processing in the blood culture system (Kortekangas et al. 1995). Incubation of liquid sample in a blood culture system necessitates a time-consuming subculture from the positive bottle upon growth detection, because isolated colonies are needed for identification and susceptibility testing. Another disadvantage of using blood culture bottles is that direct Gram stain is not possible after mixing with the prefilled broth. Therefore, a native synovial fluid should always be submitted for microbiological analysis, even if a blood culture bottle is additionally used (Mathews et al. 2010). Nucleic acid amplification methods, including multiplex PCR, have been suggested for rapid and sensitive detection and identification of bacteria and fungi from synovial fluid (Kim et al. 2010). Nevertheless, classical culture remains indispensable for microbiological diagnostics, because it provides isolated organisms for antimicrobial susceptibility testing. Additionally, at least two sets of blood cultures are to be drawn. Synovial fluid and blood specimens for cultural investigation should be obtained before the initiation of antimicrobial therapy.

While plain roentgenography examination has only limited value for early diagnosis of acute arthritis (Coakley et al. 2006), CT and particularly MRI (Coakley et al. 2006) enable sensitive imaging of septic arthritis on early stage (Shirtliff and Mader 2002). Ultrasonography can promptly provide information on the presence of joint effusion (Zieger et al. 1987).

The management of acute arthritis includes joint drainage and antimicrobial treatment (Coakley et al. 2006). Removal and draining of purulent fluid from the infected joint can be accomplished by needle aspiration, arthroscopy, or arthrotomy, with the method choice being made according to the clinical situation (Mathews et al. 2010; Shirtliff and Mader 2002). To our knowledge, there are no randomized clinical trials comparing antimicrobial agents or evaluating optimal duration of antimicrobial treatment for acute arthritis of native joints. Therefore, initial treatment is based on optimal coverage of possible pathogens, and targeted treatment of *S. aureus* arthritis includes antibiotics with specific and narrow activity against this pathogen taking into consideration their penetration into the joints. Initial treatment prior to the detection of a causative pathogen should consist of a broad-spectrum i. v. antimicrobial, which at the same time is active against *S. aureus*. This can be accomplished by intravenous application of a second-generation cephalosporin (e.g., cefuroxime) or amoxicillin/clavulanic acid (Clerc et al. 2011). In the areas with high incidence of MRSA, or in patients with risk factors for MRSA, vancomycin should be considered (Coakley et al. 2006; Mathews et al. 2010). As soon as *S. aureus* has been identified and results of susceptibility testing are available,

treatment should be changed to a specific therapy. Antistaphylococcal agents given are the same as for osteomyelitis (Table 1). *S. aureus* arthritis generally requires 4–6 weeks of treatment. Antibiotics should be given intravenously for at least 2 weeks, which can be followed by oral antibiotics with high bioavailability (Coakley et al. 2006; Mathews et al. 2010). Intra-articular application of antibiotic is not recommended (Shirtliff and Mader 2002).

4 *Staphylococcus aureus* Pyomyositis

Pyomyositis is an acute infection of skeletal muscles characterized by the primary intramuscular accumulation of pus. The most common organism causing pyomyositis is *S. aureus*, which is isolated in 90 % of the cases in tropical areas (Chauhan et al. 2004; Chiedozi 1979) and in 70 % of the cases in the temperate regions (Christin and Sarosi 1992). Pyomyositis is common in tropical countries (Chauhan et al. 2004; Chiedozi 1979) which explains the term "tropical pyomyositis." However, after the first description (Levin et al. 1971), cases in temperate regions have been increasingly reported (Christin and Sarosi 1992). Pyomyositis in non-tropical countries has frequently been associated with predisposing conditions. Immunocompromised patients, particularly those with human immunodeficiency virus (HIV) (Rodgers et al. 1993) or malignancy, have been reported to develop pyomyositis (Crum 2004) as well as intravenous drug users (Hsueh et al. 1996). Local mechanical trauma has been suggested as a factor facilitating development of pyomyositis and was reported in 25–63 % and 39 % of patients in tropical and non-tropical zones, respectively (Chiedozi 1979; Christin and Sarosi 1992). This is emphasized by the fact that the skeletal muscle is highly resistant to bacterial infection as muscle trauma was needed in in vivo experiments to establish pyomyositis (Miyake 1904). Thus, the pathogenesis of pyomyositis likely involves transient bacteremia in the setting of predisposing conditions and muscular injury (Crum 2004).

Pyomyositis usually affects single muscle, although in an evaluation of 676 patients 16.6 % were identified with multiple-site involvement (Bickels et al. 2002). The most commonly affected body sites are quadriceps, gluteal, and iliopsoas muscles (Chiedozi 1979). Psoas abscess is a particular type of muscle infection, which is characterized by specific localization and usually results from spread of infection from surrounding structures, e.g., as a complication of vertebral osteomyelitis (Shields et al. 2012). However, an abscess with psoas localization can also occur primarily by the hematogenous way (Shields et al. 2012; Ricci et al. 1986). In such cases, the pathogenesis is as in pyomyositis, and *S. aureus* is a most commonly isolated pathogen (Shields et al. 2012; Ricci et al. 1986; Desandre et al. 1995). Primary psoas abscess has been reported to occur more frequently in patients with trauma, immunocompromised patients, or children (Shields et al. 2012).

The clinical picture is divided into three stages (Bickels et al. 2002; Chauhan et al. 2004; Christin and Sarosi 1992). The first, or *invasive stage*, is characterized by a

subacute onset with local swelling, induration, pain, and variable erythema, as well as low-grade fever and general malaise. This picture progresses after one to three weeks to the *suppurative stage*. Fever is common, and most patients are seen at this stage. The abscess forms and needle aspiration reveal pus. If the infection is not appropriately treated, the disease enters the third or *late stage*. In this stage, generalization of infection results in systemic manifestations and, eventually, sepsis.

Laboratory investigations reveal leukocytosis with shift to the left and elevation of inflammation parameters (Bickels et al. 2002; Chauhan et al. 2004). At the suspicion of pyomyositis, imaging confirms diagnosis and supports the indication for surgical intervention. While MRI represents the best method for early diagnosis (Bickels et al. 2002), CT and ultrasonography are also important diagnostic options (Chauhan et al. 2004; Gordon et al. 1995). In the most cases, urgent surgery (incision and drainage) is necessary and represents the essential cornerstone of patient's management (Chauhan et al. 2004). Apart of the inherent treatment effect, it provides specimens for microbiological diagnostics. The latter is essential, because identification and susceptibility testing of causative organism is a prerequisite for the potential modification to an appropriate and targeted antimicrobial treatment. At least two sets of blood cultures are obligatory to confirm or exclude bacteremia. While 31 % of pyomyositis patients in North America were bacteremic (Christin and Sarosi 1992), only 5 % of patients had positive blood culture in tropical areas (Chiedozi 1979). This discrepancy might be attributed to the differences in blood culturing technique.

Upon clinical diagnosis, the intravenous antimicrobial treatment is urgently initiated with an antistaphylococcal agent, e.g., flucloxacillin, because *S. aureus* is a commonly found causative organism in pyomyositis. A decision to use initially an MRSA-active antimicrobial (e.g., vancomycin) is dependent on local epidemiology of methicillin resistance in *S. aureus*. In immunocompromised patients, the empiric therapy should additionally include broad-spectrum intravenous antibiotics (Chauhan et al. 2004), e.g., piperacillin/tazobactam or meropenem. As soon as pathogen is identified from clinical specimens and the results of antimicrobial susceptibility testing are available, modification to a definitive targeted treatment should follow. Penicillinase-stable penicillins (flucloxacillin, oxacillin, etc.) are primary agents to be used for pyomyositis caused by MSSA, although intravenous first- or second-generation cephalosporins can also be chosen (e.g., in case of penicillin allergy). Vancomycin is the first option if MRSA is detected. With the surgical drainage of abscesses and appropriate antimicrobial therapy, the prognosis of patients with pyomyositis is very good.

5 Conclusions

Although considerable advances have been achieved in diagnosis and treatment of *S. aureus*-associated musculoskeletal infections, the management remains challenging and the relapse rate of osteomyelitis is still high. The ultimate goal of

treatment is the complete eradication of infection and preservation of function. The collaboration of all specialists involved supports interdisciplinary approach and successful management of these difficult-to-treat infections.

References

Agarwal JP, Ogilvie M, Wu LC, Lohman RF, Gottlieb LJ, Franczyk M, Song DH (2005) Vacuum-assisted closure for sternal wounds: a first-line therapeutic management approach. Plast Reconstr Surg 116:1035–1040

Anguita-Alonso P, Hanssen AD, Osmon DR, Trampuz A, Steckelberg JM, Patel R (2005) High rate of aminoglycoside resistance among staphylococci causing prosthetic joint infection. Clin Orthop Relat Res 439:43–47

Antunes PE, Bernardo JE, Eugenio L, de Oliveira JF, Antunes MJ (1997) Mediastinitis after aorto-coronary bypass surgery. Eur J Cardiothorac Surg 12:443–449

Aytaç S, Schnetzke M, Swartman B, Herrmann P, Woelfl C, Heppert V, Gruetzner PA, Guehring T (2014) Posttraumatic and postoperative osteomyelitis: surgical revision strategy with persisting fistula. Arch Orthop Trauma Surg 134:159–165

Babouee Flury B, Elzi L, Kolbe M, Frei R, Weisser M, Scharen S, Widmer AF, Battegay M (2014) Is switching to an oral antibiotic regimen safe after 2 weeks of intravenous treatment for primary bacterial vertebral osteomyelitis? BMC Infect Dis 14:226

Barrington NA (1988) The radiology of bone and joint infection. Br J Hosp Med 40:464–472

Becker K, Heilmann C, Peters G (2014) Coagulase-negative staphylococci. Clin Microbiol Rev 27:870–926

Berbari EF, Kanj SS, Kowalski TJ, Darouiche RO, Widmer AF, Schmitt SK, Hendershot EF, Holtom PD, Huddleston PM III, Petermann GW, Osmon DR (2015) 2015 Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and treatment of native vertebral osteomyelitis in adults. Clin Infect Dis 61:e26–e46

Bernard A, Kermarrec G, Parize P, Caruba T, Bouvet A, Mainardi JL, Sabatier B, Nich C (2015a) Dramatic reduction of clindamycin serum concentration in staphylococcal osteoarticular infection patients treated with the oral clindamycin-rifampicin combination. J Infect 71:200–206

Bernard L, Dinh A, Ghout I, Simo D, Zeller V, Issartel B, Le M, V, Belmatoug N, Lesprit P, Bru JP, Therby A, Bouhour D, Denes E, Debard A, Chirouze C, Fevre K, Dupon M, Aegerter P, Mulleman D (2015b) Antibiotic treatment for 6 weeks versus 12 weeks in patients with pyogenic vertebral osteomyelitis: an open-label, non-inferiority, randomised, controlled trial. Lancet 385:875–882

Bickels J, Ben-Sira L, Kessler A, Wientroub S (2002) Primary pyomyositis. J Bone Joint Surg Am 84-A:2277–2286

Bischoff M, Dunman P, Kormanec J, Macapagal D, Murphy E, Mounts W, Berger-Bachi B, Projan S (2004) Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. J Bacteriol 186:4085–4099

Blyth MJ, Kincaid R, Craigen MA, Bennet GC (2001) The changing epidemiology of acute and subacute haematogenous osteomyelitis in children. J Bone Joint Surg Br 83:99–102

Boody BS, Jenkins TJ, Maslak J, Hsu WK, Patel AA (2015) Vertebral osteomyelitis and spinal epidural abscess: an evidence-based review. J Spinal Disord Tech 28:E316–E327

Borger MA, Rao V, Weisel RD, Ivanov J, Cohen G, Scully HE, David TE (1998) Deep sternal wound infection: risk factors and outcomes. Ann Thorac Surg 65:1050–1056

Breusch SJ, Kuhn KD (2003) Bone cements based on polymethylmethacrylate. Orthopade 32:41–50

Buchholz HW, Elson RA, Engelbrecht E, Lodenkamper H, Rottger J, Siegel A (1981) Management of deep infection of total hip replacement. J Bone Joint Surg Br 63-B:342–353

Campoccia D, Speziale P, Ravaioli S, Cangini I, Rindi S, Pirini V, Montanaro L, Arciola CR (2009) The presence of both bone sialoprotein-binding protein gene and collagen adhesin gene as a typical virulence trait of the major epidemic cluster in isolates from orthopedic implant infections. Biomaterials 30:6621–6628

Caputo GM, Cavanagh PR, Ulbrecht JS, Gibbons GW, Karchmer AW (1994) Assessment and management of foot disease in patients with diabetes. N Engl J Med 331:854–860

Carragee EJ (1997) The clinical use of magnetic resonance imaging in pyogenic vertebral osteomyelitis. Spine (Phila Pa 1976) 22:780–785

Carragee EJ, Kim D, van der Vlugt T, Vittum D (1997) The clinical use of erythrocyte sedimentation rate in pyogenic vertebral osteomyelitis. Spine (Phila Pa 1976) 22:2089–2093

Ceroni D, Cherkaoui A, Ferey S, Kaelin A, Schrenzel J (2010) *Kingella kingae* osteoarticular infections in young children: clinical features and contribution of a new specific real-time PCR assay to the diagnosis. J Pediatr Orthop 30:301–304

Chauhan S, Jain S, Varma S, Chauhan SS (2004) Tropical pyomyositis (myositis tropicans): current perspective. Postgrad Med J 80:267–270

Chiedozi LC (1979) Pyomyositis. Review of 205 cases in 112 patients. Am J Surg 137:255–259

Christin L, Sarosi GA (1992) Pyomyositis in North America: case reports and review. Clin Infect Dis 15:668–677

Ciampolini J, Harding KG (2000) Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often? Postgrad Med J 76:479–483

Clarke SR, Foster SJ (2006) Surface adhesins of *Staphylococcus aureus*. Adv Microb Physiol 51:187–224

Clerc O, Prod'hom G, Greub G, Zanetti G, Senn L (2011) Adult native septic arthritis: a review of 10 years of experience and lessons for empirical antibiotic therapy. J Antimicrob Chemother 66:1168–1173

Coakley G, Mathews C, Field M, Jones A, Kingsley G, Walker D, Phillips M, Bradish C, McLachlan A, Mohammed R, Weston V (2006) BSR & BHPR, BOA, RCGP and BSAC guidelines for management of the hot swollen joint in adults. Rheumatology (Oxford) 45:1039–1041

Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, Meersseman W, Akova M, Arendrup MC, Arikan-Akdagli S, Bille J, Castagnola E, Cuenca-Estrella M, Donnelly JP, Groll AH, Herbrecht R, Hope WW, Jensen HE, Lass-Florl C, Petrikkos G, Richardson MD, Roilides E, Verweij PE, Viscoli C, Ullmann AJ (2012) ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 18(Suppl 7):19–37

Craigen MA, Watters J, Hackett JS (1992) The changing epidemiology of osteomyelitis in children. J Bone Joint Surg Br 74:541–545

Crum NF (2004) Bacterial pyomyositis in the United States. Am J Med 117:420–428

Czekaj J, Dinh A, Moldovan A, Vaudaux P, Gras G, Hoffmeyer P, Lew D, Bernard L, Uckay I (2011) Efficacy of a combined oral clindamycin-rifampicin regimen for therapy of staphylococcal osteoarticular infections. Scand J Infect Dis 43:962–967

Dartnell J, Ramachandran M, Katchburian M (2012) Haematogenous acute and subacute paediatric osteomyelitis: a systematic review of the literature. J Bone Joint Surg Br 94:584–595

Davis JS (2005) Management of bone and joint infections due to *Staphylococcus aureus*. Intern Med J 35(Suppl 2):S79–S96

Desandre AR, Cottone FJ, Evers ML (1995) Iliopsoas abscess: etiology, diagnosis, and treatment. Am Surg 61:1087–1091

Doss M, Martens S, Wood JP, Wolff JD, Baier C, Moritz A (2002) Vacuum-assisted suction drainage versus conventional treatment in the management of poststernotomy osteomyelitis. Eur J Cardiothorac Surg 22:934–938

Dubost JJ, Fis I, Denis P, Lopitaux R, Soubrier M, Ristori JM, Bussiere JL, Sirot J, Sauvezie B (1993) Polyarticular septic arthritis. Medicine (Baltimore) 72:296–310

Dubost JJ, Soubrier M, De Champs C, Ristori JM, Bussiere JL, Sauvezie B (2002) No changes in the distribution of organisms responsible for septic arthritis over a 20 year period. Ann Rheum Dis 61:267–269

El Oakley RM, Wright JE (1996) Postoperative mediastinitis: classification and management. Ann Thorac Surg 61:1030–1036

Elasri MO, Thomas JR, Skinner RA, Blevins JS, Beenken KE, Nelson CL, Smeltzer MS (2002) *Staphylococcus aureus* collagen adhesin contributes to the pathogenesis of osteomyelitis. Bone 30:275–280

Ellington JK, Reilly SS, Ramp WK, Smeltzer MS, Kellam JF, Hudson MC (1999) Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts. Microb Pathog 26:317–323

Falagas ME, Rosmarakis ES (2006) Recurrent post-sternotomy mediastinitis. J Infect 52:e151–e154

Fuchs T, Schmidmaier G, Raschke MJ, Stange R (2008) Bioactive-coated implants in trauma surgery. Eur J Trauma Emerg Surg 34:60–68

Fuursted K, Arpi M, Lindblad BE, Pedersen LN (2008) Broad-range PCR as a supplement to culture for detection of bacterial pathogens in patients with a clinically diagnosed spinal infection. Scand J Infect Dis 40:772–777

Garzoni C, Kelley WL (2009) *Staphylococcus aureus*: new evidence for intracellular persistence. Trends Microbiol 17:59–65

Gasbarrini AL, Bertoldi E, Mazzetti M, Fini L, Terzi S, Gonella F, Mirabile L, Barbanti BG, Furno A, Gasbarrini A, Boriani S (2005) Clinical features, diagnostic and therapeutic approaches to haematogenous vertebral osteomyelitis. Eur Rev Med Pharmacol Sci 9:53–66

Gold RH, Hawkins RA, Katz RD (1991) Bacterial osteomyelitis: findings on plain radiography, CT, MR, and scintigraphy. AJR Am J Roentgenol 157:365–370

Goldenberg DL (1998) Septic arthritis. Lancet 351:197–202

Goldenberg DL, Reed JI (1985) Bacterial arthritis. N Engl J Med 312:764–771

Gordon BA, Martinez S, Collins AJ (1995) Pyomyositis: characteristics at CT and MR imaging. Radiology 197:279–286

Grammatico L, Baron S, Rusch E, Lepage B, Surer N, Desenclos JC, Besnier JM (2008) Epidemiology of vertebral osteomyelitis (VO) in France: analysis of hospital-discharge data 2002–2003. Epidemiol Infect 136:653–660

Gross T, Kaim AH, Regazzoni P, Widmer AF (2002) Current concepts in posttraumatic osteomyelitis: a diagnostic challenge with new imaging options. J Trauma 52:1210–1219

Grossi EA, Culliford AT, Krieger KH, Kloth D, Press R, Baumann FG, Spencer FC (1985) A survey of 77 major infectious complications of median sternotomy: a review of 7,949 consecutive operative procedures. Ann Thorac Surg 40:214–223

Gummert JF, Barten MJ, Hans C, Kluge M, Doll N, Walther T, Hentschel B, Schmitt DV, Mohr FW, Diegeler A (2002) Mediastinitis and cardiac surgery—an updated risk factor analysis in 10,373 consecutive adult patients. Thorac Cardiovasc Surg 50:87–91

Gupta MN, Sturrock RD, Field M (2001) A prospective 2-year study of 75 patients with adult-onset septic arthritis. Rheumatology (Oxford) 40:24–30

Gupta A, Kowalski TJ, Osmon DR, Enzler M, Steckelberg JM, Huddleston PM, Nassr A, Mandrekar JM, Berbari EF (2014) Long-term outcome of pyogenic vertebral osteomyelitis: a cohort study of 260 patients. Open Forum Infect Dis 1:ofu107

Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F., Dulgheru R, El KG, Erba PA, Iung B, Miro JM, Mulder BJ, Plonska-Gosciniak E, Price S, Roos-Hesselink J, Snygg-Martin U, Thuny F, Tornos MP, Vilacosta I, Zamorano JL (2015) 2015 ESC guidelines for the management of infective endocarditis: the task force for the management of infective endocarditis of the European Society of Cardiology (ESC) endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). Eur Heart J 36:3075–3128

Hariharan P, Kabrhel C (2011) Sensitivity of erythrocyte sedimentation rate and C-reactive protein for the exclusion of septic arthritis in emergency department patients. J Emerg Med 40:428–431

Haslinger-Löffler B, Kahl BC, Grundmeier M, Strangfeld K, Wagner B, Fischer U, Cheung AL, Peters G, Schulze-Osthoff K, Sinha B (2005) Multiple virulence factors are required for *Staphylococcus aureus*-induced apoptosis in endothelial cells. Cell Microbiol 7:1087–1097

Henke PK, Blackburn SA, Wainess RW, Cowan J, Terando A, Proctor M, Wakefield TW, Upchurch GR Jr, Stanley JC, Greenfield LJ (2005) Osteomyelitis of the foot and toe in adults is a surgical disease: conservative management worsens lower extremity salvage. Ann Surg 241:885–892

Hersh RE, Jack JM, Dahman MI, Morgan RF, Drake DB (2001) The vacuum-assisted closure device as a bridge to sternal wound closure. Ann Plast Surg 46:250–254

Horst SA, Hoerr V, Beineke A, Kreis C, Tuchscherr L, Kalinka J, Lehne S, Schleicher I, Köhler G, Fuchs T, Raschke MJ, Rohde M, Peters G, Faber C, Löffler B, Medina E (2012) A novel mouse model of *Staphylococcus aureus* chronic osteomyelitis that closely mimics the human infection: an integrated view of disease pathogenesis. Am J Pathol 181:1206–1214

Howard AW, Viskontas D, Sabbagh C (1999) Reduction in osteomyelitis and septic arthritis related to *Haemophilus influenzae* type B vaccination. J Pediatr Orthop 19:705–709

Howard-Jones AR, Isaacs D (2010) Systematic review of systemic antibiotic treatment for children with chronic and sub-acute pyogenic osteomyelitis. J Paediatr Child Health 46:736–741

Hsueh PR, Hsiue TR, Hsieh WC (1996) Pyomyositis in intravenous drug abusers: report of a unique case and review of the literature. Clin Infect Dis 22:858–860

Hughes JG, Vetter EA, Patel R, Schleck CD, Harmsen S, Turgeant LT, Cockerill FR III (2001) Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J Clin Microbiol 39:4468–4471

Idusuyi OB (2015) Surgical management of Charcot neuroarthropathy. Prosthet Orthot Int 39:61–72

Jagodzinski NA, Kanwar R, Graham K, Bache CE (2009) Prospective evaluation of a shortened regimen of treatment for acute osteomyelitis and septic arthritis in children. J Pediatr Orthop 29:518–525

Jaramillo D (2011) Infection: musculoskeletal. Pediatr Radiol 41(Suppl 1):S127–S134

Join-Lambert O, Ribadeau-Dumas F, Jullien V, Kitzis MD, Jais JP, Coignard-Biehler H, Guet-Revillet H, Consigny PH, Delage M, Nassif X, Lortholary O, Nassif A (2014) Dramatic reduction of clindamycin plasma concentration in hidradenitis suppurativa patients treated with the rifampin-clindamycin combination. Eur J Dermatol 24:94–95

Julian OC, Lopez-Belio M, Dye WS, Javid H, Grove WJ (1957) The median sternal incision in intracardiac surgery with extracorporeal circulation; a general evaluation of its use in heart surgery. Surgery 42:753–761

Jurkiewicz MJ, Bostwick J III, Hester TR, Bishop JB, Craver J (1980) Infected median sternotomy wound. Successful treatment by muscle flaps. Ann Surg 191:738–744

Kaandorp CJ, van Schaardenburg D, Krijnen P, Habbema JD, van de Laar MA (1995) Risk factors for septic arthritis in patients with joint disease. A prospective study. Arthritis Rheum 38:1819–1825

Kaandorp CJ, Dinant HJ, van de Laar MA, Moens HJ, Prins AP, Dijkmans BA (1997a) Incidence and sources of native and prosthetic joint infection: a community based prospective survey. Ann Rheum Dis 56:470–475

Kaandorp CJ, Krijnen P, Moens HJ, Habbema JD, van SD (1997b) The outcome of bacterial arthritis: a prospective community-based study. Arthritis Rheum 40:884–892

Kalinka J, Hachmeister M, Geraci J, Sordelli D, Hansen U, Niemann S, Oetermann S, Peters G, Löffler B, Tuchscherr L (2014) *Staphylococcus aureus* isolates from chronic osteomyelitis are characterized by high host cell invasion and intracellular adaptation, but still induce inflammation. Int J Med Microbiol 304:1038–1049

Kim H, Kim J, Ihm C (2010) The usefulness of multiplex PCR for the identification of bacteria in joint infection. J Clin Lab Anal 24:175–181

Kim CJ, Song KH, Park WB, Kim ES, Park SW, Kim HB, Oh MD, Kim NJ (2012) Microbiologically and clinically diagnosed vertebral osteomyelitis: impact of prior antibiotic exposure. Antimicrob Agents Chemother 56:2122–2124

Klesius AA, Dzemali O, Simon A, Kleine P, Abdel-Rahman U, Herzog C, Wimmer-Greinecker G, Moritz A (2004) Successful treatment of deep sternal infections following open heart surgery by bilateral pectoralis major flaps. Eur J Cardiothorac Surg 25:218–223

Kortekangas P, Aro HT, Lehtonen OP (1995) Synovial fluid culture and blood culture in acute arthritis. A multi-case report of 90 patients. Scand J Rheumatol 24:44–47

Kowalski TJ, Berbari EF, Huddleston PM, Steckelberg JM, Osmon DR (2006) Do follow-up imaging examinations provide useful prognostic information in patients with spine infection? Clin Infect Dis 43:172–179

Kriegeskorte A, König S, Sander G, Pirkl A, Mahabir E, Proctor RA, von Eiff C, Peters G, Becker K (2011) Small colony variants of *Staphylococcus aureus* reveal distinct protein profiles. Proteomics 11:2476–2490

Levin MJ, Gardner P, Waldvogel FA (1971) Tropical pyomyositis—an unusual infection due to staphylococcus aureus. N Engl J Med 284:196–198

Lew DP, Waldvogel FA (2004) Osteomyelitis. Lancet 364:369–379

Loop FD, Lytle BW, Cosgrove DM, Mahfood S, McHenry MC, Goormastic M, Stewart RW, Golding LA, Taylor PC (1990) Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. Ann Thorac Surg 49:179–186

Losanoff JE, Richman BW, Jones JW (2002) Disruption and infection of median sternotomy: a comprehensive review. Eur J Cardiothorac Surg 21:831–839

Love C, Palestro CJ (2016) Nuclear medicine imaging of bone infections. Clin Radiol

Mader JT, Shirtliff M, Calhoun JH (1997) Staging and staging application in osteomyelitis. Clin Infect Dis 25:1303–1309

Malcius D, Trumpulyte G, Barauskas V, Kilda A (2005) Two decades of acute hematogenous osteomyelitis in children: are there any changes? Pediatr Surg Int 21:356–359

Malizos K, Sarma J, Seaton RA, Militz M, Menichetti F, Riccio G, Gaudias J, Trostmann U, Pathan R, Hamed K (2016) Daptomycin for the treatment of osteomyelitis and orthopaedic device infections: real-world clinical experience from a European registry. Eur J Clin Microbiol Infect Dis 35:111–118

Margaretten ME, Kohlwes J, Moore D, Bent S (2007) Does this adult patient have septic arthritis? JAMA 297:1478–1488

Marschall J, Bhavan KP, Olsen MA, Fraser VJ, Wright NM, Warren DK (2011) The impact of prebiopsy antibiotics on pathogen recovery in hematogenous vertebral osteomyelitis. Clin Infect Dis 52:867–872

Masquelet AC, Begue T (2010) The concept of induced membrane for reconstruction of long bone defects. Orthop Clin North Am 41:27–37

Mathews CJ, Weston VC, Jones A, Field M, Coakley G (2010) Bacterial septic arthritis in adults. Lancet 375:846–855

McHenry MC, Easley KA, Locker GA (2002) Vertebral osteomyelitis: long-term outcome for 253 patients from 7 Cleveland-area hospitals. Clin Infect Dis 34:1342–1350

McKee MD, Wild LM, Schemitsch EH, Waddell JP (2002) The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. J Orthop Trauma 16:622–627

McKee MD, Li-Bland EA, Wild LM, Schemitsch EH (2010) A prospective, randomized clinical trial comparing an antibiotic-impregnated bioabsorbable bone substitute with standard antibiotic-impregnated cement beads in the treatment of chronic osteomyelitis and infected nonunion. J Orthop Trauma 24:483–490

Merritt K (1988) Factors increasing the risk of infection in patients with open fractures. J Trauma 28:823–827

Milano CA, Georgiade G, Muhlbaier LH, Smith PK, Wolfe WG (1999) Comparison of omental and pectoralis flaps for poststernotomy mediastinitis. Ann Thorac Surg 67:377–380

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

Miyake H (1904) Beitrag zur Kenntniss des sogenannten Myositis infectiosa. Mitt Grenzgeb Med Chir 13:145–198

Mylona E, Samarkos M, Kakalou E, Fanourgiakis P, Skoutelis A (2009) Pyogenic vertebral osteomyelitis: a systematic review of clinical characteristics. Semin Arthritis Rheum 39:10–17

Nahai F, Rand RP, Hester TR, Bostwick J III, Jurkiewicz MJ (1989) Primary treatment of the infected sternotomy wound with muscle flaps: a review of 211 consecutive cases. Plast Reconstr Surg 84:434–441

Neut D, Kluin OS, Crielaard BJ, van der Mei HC, Busscher HJ, Grijpma DW (2009) A biodegradable antibiotic delivery system based on poly-(trimethylene carbonate) for the treatment of osteomyelitis. Acta Orthop 80:514–519

Novick R (2000) Pathogenicity factors and their regulation. In: Fischetti VA (ed) Gram-positive pathogens. American Society of Microbiology, Washington, D.C., pp 392–407

Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449

Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR (2013) Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56:e1–e25

Otto M (2009) *Staphylococcus epidermidis*—the 'accidental' pathogen. Nat Rev Microbiol 7:555–567

Otto MP, Martin E, Badiou C, Lebrun S, Bes M, Vandenesch F, Etienne J, Lina G, Dumitrescu O (2013) Effects of subinhibitory concentrations of antibiotics on virulence factor expression by community-acquired methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 68:1524–1532

Parsons B, Strauss E (2004) Surgical management of chronic osteomyelitis. Am J Surg 188:57–66

Patzakis MJ, Wilkins J (1989) Factors influencing infection rate in open fracture wounds. Clin Orthop Relat Res 36–40

Peltola H, Pääkkönen M (2014) Acute osteomyelitis in children. N Engl J Med 370:352–360

Peltola H, Pääkkönen M, Kallio P, Kallio MJ (2010) Short- versus long-term antimicrobial treatment for acute hematogenous osteomyelitis of childhood: prospective, randomized trial on 131 culture-positive cases. Pediatr Infect Dis J 29:1123–1128

Peterson TC, Pearson C, Zekaj M, Hudson I, Fakhouri G, Vaidya R (2014) Septic arthritis in intravenous drug abusers: a historical comparison of habits and pathogens. J Emerg Med 47:723–728

Pfeifer R, Kobbe P, Knobe M, Pape HC (2011) The reamer-irrigator-aspirator (RIA) system. Oper Orthop Traumatol 23:446–452

Pigrau C, Almirante B, Flores X, Falco V, Rodriguez D, Gasser I, Villanueva C, Pahissa A (2005) Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med 118:1287

Proctor RA, van Langevelde P, Kristjansson M, Maslow JN, Arbeit RD (1995) Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis 20:95–102

Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305

Raschke MJ, Schmidmaier G (2004) Biological coating of implants in trauma and orthopedic surgery. Unfallchirurg 107:653–663

Rasigade JP, Trouillet-Assant S, Ferry T, Diep BA, Sapin A, Lhoste Y, Ranfaing J, Badiou C, Benito Y, Bes M, Couzon F, Tigaud S, Lina G, Etienne J, Vandenesch F, Laurent F (2013) PSMs of hypervirulent *Staphylococcus aureus* act as intracellular toxins that kill infected osteoblasts. PLoS ONE 8:e63176

Reichert P, Rutowski R, Zimmer K, Gosk J (2007) Application of gentamycini impregnated polymethylmethacrylate (PMMA) (Septopal) in treatment of infected nonunion. Own experiments. Polim Med 37:65–72

Ricci MA, Rose FB, Meyer KK (1986) Pyogenic psoas abscess: worldwide variations in etiology. World J Surg 10:834–843

Riise OR, Kirkhus E, Handeland KS, Flato B, Reiseter T, Cvancarova M, Nakstad B, Wathne KO (2008) Childhood osteomyelitis-incidence and differentiation from other acute onset musculoskeletal features in a population-based study. BMC Pediatr 8:45

Ringelman PR, Vander Kolk CA, Cameron D, Baumgartner WA, Manson PN (1994) Long-term results of flap reconstruction in median sternotomy wound infections. Plast Reconstr Surg 93:1208–1214

Robicsek F, Daugherty HK, Cook JW (1977) The prevention and treatment of sternum separation following open-heart surgery. J Thorac Cardiovasc Surg 73:267–268

Roblot F, Besnier JM, Juhel L, Vidal C, Ragot S, Bastides F, Le MG, Godet C, Mulleman D, Azais I, Becq-Giraudon B, Choutet P (2007) Optimal duration of antibiotic therapy in vertebral osteomyelitis. Semin Arthritis Rheum 36:269–277

Rodgers WB, Yodlowski ML, Mintzer CM (1993) Pyomyositis in patients who have the human immunodeficiency virus. Case report and review of the literature. J Bone Joint Surg Am 75:588–592

Roux S, Valour F, Karsenty J, Gagnieu MC, Perpoint T, Lustig S, Ader F, Martha B, Laurent F, Chidiac C, Ferry T (2016) Daptomycin >6 mg/kg/day as salvage therapy in patients with complex bone and joint infection: cohort study in a regional reference center. BMC Infect Dis 16:83

Rybak MJ, Lomaestro BM, Rotschafer JC, Moellering RC, Craig WA, Billeter M, Dalovisio JR, Levine DP (2009) Vancomycin therapeutic guidelines: a summary of consensus recommendations from the Infectious Diseases Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists. Clin Infect Dis 49:325–327

Salar O, Baker B, Kurien T, Taylor A, Moran C (2014) Septic arthritis in the era of immunosuppressive treatments. Ann R Coll Surg Engl 96:e11–e12

Schaumburg F, Peters G, Alabi A, Becker K, Idelevich EA (2016) Missense mutations of PBP2a are associated with reduced susceptibility to ceftaroline and ceftobiprole in African MRSA. J Antimicrob Chemother 71:41–44

Schmidmaier G, Lucke M, Wildemann B, Haas NP, Raschke M (2006) Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. Injury 37(Suppl 2):S105–S112

Schrenzel J, Harbarth S, Schockmel G, Genne D, Bregenzer T, Flueckiger U, Petignat C, Jacobs F, Francioli P, Zimmerli W, Lew DP (2004) A randomized clinical trial to compare fleroxacin-rifampicin with flucloxacillin or vancomycin for the treatment of staphylococcal infection. Clin Infect Dis 39:1285–1292

Seaton RA, Menichetti F, Dalekos G, Beiras-Fernandez A, Nacinovich F, Pathan R, Hamed K (2015) Evaluation of effectiveness and safety of high-dose daptomycin: results from patients included in the European Cubicin® outcomes registry and experience. Adv Ther 32:1192–1205

Sehn JK, Gilula LA (2012) Percutaneous needle biopsy in diagnosis and identification of causative organisms in cases of suspected vertebral osteomyelitis. Eur J Radiol 81:940–946

Sendi P, Zimmerli W (2012) Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect 18:1176–1184

Shields D, Robinson P, Crowley TP (2012) Iliopsoas abscess—a review and update on the literature. Int J Surg 10:466–469

Shirtliff ME, Mader JT (2002) Acute septic arthritis. Clin Microbiol Rev 15:527–544

Shmerling RH, Delbanco TL, Tosteson AN, Trentham DE (1990) Synovial fluid tests. What should be ordered? JAMA 264:1009–1014

Sinha B, Francois PP, Nusse O, Foti M, Hartford OM, Vaudaux P, Foster TJ, Lew DP, Herrmann M, Krause KH (1999) Fibronectin-binding protein acts as *Staphylococcus aureus* invasin via fibronectin bridging to integrin alpha5beta1. Cell Microbiol 1:101–117

Stengel D, Bauwens K, Sehouli J, Ekkernkamp A, Porzsolt F (2001) Systematic review and meta-analysis of antibiotic therapy for bone and joint infections. Lancet Infect Dis 1:175–188

Strommenger B, Layer F, Klare I, Werner G (2015) Pre-use susceptibility to ceftaroline in clinical *Staphylococcus aureus* isolates from Germany: is there a non-susceptible pool to be selected? PLoS ONE 10:e0125864

Tande AJ, Patel R (2014) Prosthetic joint infection. Clin Microbiol Rev 27:302–345

Tebartz C, Horst SA, Sparwasser T, Huehn J, Beineke A, Peters G, Medina E (2015) A major role for myeloid-derived suppressor cells and a minor role for regulatory T cells in immunosuppression during *Staphylococcus aureus* infection. J Immunol 194:1100–1111

Tegnell A, Arén C, Öhman L (2000) Coagulase-negative staphylococci and sternal infections after cardiac operation. Ann Thorac Surg 69:1104–1109

Tice AD, Hoaglund PA, Shoultz DA (2003) Outcomes of osteomyelitis among patients treated with outpatient parenteral antimicrobial therapy. Am J Med 114:723–728

Torda AJ, Gottlieb T, Bradbury R (1995) Pyogenic vertebral osteomyelitis: analysis of 20 cases and review. Clin Infect Dis 20:320–328

Trampuz A, Zimmerli W (2006a) Antimicrobial agents in orthopaedic surgery: prophylaxis and treatment. Drugs 66:1089–1105

Trampuz A, Zimmerli W (2006b) Diagnosis and treatment of infections associated with fracture-fixation devices. Injury 37(Suppl 2):S59–S66

Tuchscherr L, Löffler B (2016) *Staphylococcus aureus* dynamically adapts global regulators and virulence factor expression in the course from acute to chronic infection. Curr Genet 62:15–17

Tuchscherr L, Heitmann V, Hussain M, Viemann D, Roth J, von Eiff C, Peters G, Becker K, Löffler B (2010) *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. J Infect Dis 202:1031–1040

Tuchscherr L, Medina E, Hussain M, Volker W, Heitmann V, Niemann S, Holzinger D, Roth J, Proctor RA, Becker K, Peters G, Löffler B (2011) *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med 3:129–141

Tuchscherr L, Bischoff M, Lattar SM, Noto Llana M, Pförtner H, Niemann S, Geraci J, Van de Vyver H, Fraunholz MJ, Cheung AL, Herrmann M, Völker U, Sordelli DO, Peters G, Löffler B (2015) Sigma factor SigB is crucial to mediate *Staphylococcus aureus* adaptation during chronic infections. PLoS Pathog 11:e1004870

Tuchscherr L, Kreis CA, Hoerr V, Flint L, Hachmeister M, Geraci J, Bremer-Streck S, Kiehntopf M, Medina E, Kribus M, Raschke M, Pletz M, Peters G, Loffler B (2016) *Staphylococcus aureus* develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. J Antimicrob Chemother 71:438–448

Tung H, Guss B, Hellman U, Persson L, Rubin K, Ryden C (2000) A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. Biochem J 345(Pt 3):611–619

Uckay I, Gariani K, Pataky Z, Lipsky BA (2014) Diabetic foot infections: state-of-the-art. Diabetes Obes Metab 16:305–316

Uckay I, Aragon-Sanchez J, Lew D, Lipsky BA (2015) Diabetic foot infections: what have we learned in the last 30 years? Int J Infect Dis 40:81–91

Valour F, Rasigade JP, Trouillet-Assant S, Gagnaire J, Bouaziz A, Karsenty J, Lacour C, Bes M, Lustig S, Benet T, Chidiac C, Etienne J, Vandenesch F, Ferry T, Laurent F (2015) Delta-toxin production deficiency in *Staphylococcus aureus*: a diagnostic marker of bone and joint infection chronicity linked with osteoblast invasion and biofilm formation. Clin Microbiol Infect 21:568

Van Bambeke F, Barcia-Macay M, Lemaire S, Tulkens PM (2006) Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives. Curr Opin Drug Discov Devel 9:218–230

Venkatesan K (1992) Pharmacokinetic drug interactions with rifampicin. Clin Pharmacokinet 22:47–65

Viale P, Furlanut M, Scudeller L, Pavan F, Negri C, Crapis M, Zamparini E, Zuiani C, Cristini F, Pea F (2009) Treatment of pyogenic (non-tuberculous) spondylodiscitis with tailored high-dose levofloxacin plus rifampicin. Int J Antimicrob Agents 33:379–382

von Eiff C, Bettin D, Proctor RA, Rolauffs B, Lindner N, Winkelmann W, Peters G (1997a) Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. Clin Infect Dis 25:1250–1251

von Eiff C, Heilmann C, Proctor RA, Woltz C, Peters G, Götz F (1997b) A site-directed *Staphylococcus aureus hemB* mutant is a small-colony variant which persists intracellularly. J Bacteriol 179:4706–4712

von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med 344:11–16

Wagner FW Jr (1981) The dysvascular foot: a system for diagnosis and treatment. Foot Ankle 2:64–122

Waldvogel FA, Medoff G, Swartz MN (1970a) Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. N Engl J Med 282:198–206

Waldvogel FA, Medoff G, Swartz MN (1970b) Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. 3. Osteomyelitis associated with vascular insufficiency. N Engl J Med 282:316–322

Weston VC, Jones AC, Bradbury N, Fawthrop F, Doherty M (1999) Clinical features and outcome of septic arthritis in a single UK Health District 1982–1991. Ann Rheum Dis 58:214–219

Wright JA, Nair SP (2010) Interaction of staphylococci with bone. Int J Med Microbiol 300:193–204

Zieger MM, Dorr U, Schulz RD (1987) Ultrasonography of hip joint effusions. Skeletal Radiol 16:607–611

Ziegler C, Goldmann O, Hobeika E, Geffers R, Peters G, Medina E (2011) The dynamics of T cells during persistent *Staphylococcus aureus* infection: from antigen-reactivity to in vivo anergy. EMBO Mol Med 3:652–666

Zimmerli W (2010) Vertebral osteomyelitis. N Engl J Med 362:1022–1029

Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE (1998) Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) study group. JAMA 279:1537–1541

Bacteremia, Sepsis, and Infective Endocarditis Associated with *Staphylococcus aureus*

Stephen P. Bergin, Thomas L. Holland, Vance G. Fowler Jr and Steven Y.C. Tong

Abstract Bacteremia and infective endocarditis (IE) are important causes of morbidity and mortality associated with *Staphylococcus aureus* infections. Increasing exposure to healthcare, invasive procedures, and prosthetic implants has been associated with a rising incidence of *S. aureus* bacteremia (SAB) and IE since the late twentieth century. *S. aureus* is now the most common cause of bacteremia and IE in industrialized nations worldwide and is associated with excess mortality when compared to other pathogens. Central tenets of management include identification of complicated bacteremia, eradicating foci of infection, and, for many, prolonged antimicrobial therapy. Evolving multidrug resistance and limited therapeutic options highlight the many unanswered clinical questions and urgent need for further high-quality clinical research.

Abbreviations

CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
ClfA	Clumping factor A
FnBPA	Fibronectin-binding protein A
IDU	Injection drug use
IE	Infective endocarditis
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCRAMM	Microbial surface component reacting with adhesive matrix molecules
MSSA	Methicillin-sensitive *Staphylococcus aureus*
SAB	*Staphylococcus aureus* bacteremia

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SSTI	Skin and soft tissue infection
TEE	Transesophageal echocardiography
TTE	Transthoracic echocardiography

Contents

1 Introduction

Over the past 20 years, *Staphylococcus aureus* emerged as the leading cause of bacteremia and infective endocarditis (IE) in the industrialized world. Increasing healthcare exposure and utilization of invasive devices accelerated these epidemiologic shifts. The epidemiology of *S. aureus* bacteremia (SAB) and IE has been well described. Nevertheless, optimal management strategies for these lethal clinical entities have not yet been defined. The morbidity and mortality associated with these infections highlight the urgent need for better therapeutic options and stronger clinical evidence.

2 *Staphylococcus aureus* Bacteremia

2.1 Epidemiology

Multinational, population-based surveillance studies estimate the incidence of SAB ranges from 10 to 30 cases per 100,000 person-years (Laupland et al. 2013). Longitudinal trends captured in a Danish national study demonstrated an increasing incidence from 3 per 100,000 person-years in 1957 to 20 per 100,000 person-years in 1990 (Frimodt-Moller et al. 1997). Continued surveillance of the Danish

population from 1995 to 2008, however, demonstrated a subsequent stable incidence of 21.8 per 100,000 person-years despite an increasingly older population with more comorbid medical conditions (Mejer et al. 2012). In other contemporary studies, healthcare-associated infections now account for more than two-thirds of SAB cases (Friedman et al. 2002; Fowler et al. 2003). This escalation in healthcare-associated SAB incidence is most likely linked to increasing rates of hospitalization as well as increasing use of central venous catheters, advanced surgical procedures, implantable cardiac devices, and the growing availability of intensive care.

Though overall rates of SAB have been stable for the past 20 years, the proportion of cases due to methicillin-resistant *S. aureus* (MRSA) varied significantly. From 1990 to 2005, observational studies from Minnesota, USA (El Atrouni et al. 2009), Calgary and Quebec, Canada (Laupland et al. 2008; Allard et al. 2008), and Oxfordshire, UK (Wyllie et al. 2005), documented an increasing proportion of SAB cases caused by MRSA. Once considered a hospital-acquired pathogen, the emergence of community-associated MRSA (CA-MRSA) at the end of the twentieth century dramatically changed the epidemiology of invasive MRSA infections (David and Daum 2010). Before the mid-1990s, MRSA risk was almost exclusively associated with healthcare exposure and chronic illness. Within a decade, the explosion of the USA300 strain of CA-MRSA significantly increased the burden of invasive MRSA disease in previously healthy, community-dwelling individuals in the USA who lacked traditional risk factors for MRSA acquisition. CA-MRSA strains have now been isolated on six continents, yet there is significant geographic variability in the prevalence of CA-MRSA. In the USA, Canada, and Australia, CA-MRSA now causes the majority of invasive MRSA disease. In other regions, healthcare-acquired MRSA continues to cause the majority of SAB (Chi et al. 2007). The basis for this significant variation is poorly understood.

Implementation of rigorous infection control and reporting practices have been associated with decreasing rates of MRSA bacteremia in the hospital setting (Kallen et al. 2010; Jarlier et al. 2010). The most dramatic decreases have been reported in the UK, where MRSA bacteremia incidence declined more than 50 % from 2004 to 2008 following implementation of a nationwide mandatory reporting system (Johnson et al. 2012) and federally funded handwashing initiative (Stone et al. 2012). In some regions, improvements in overall rates of MRSA acquisition have been tempered by the burden of CA-MRSA, which is now a well-established cause of nosocomial MRSA bacteremia (Seybold et al. 2006; Klevens et al. 2007). In resource-limited settings, far less is known about the incidence and longitudinal trends in rates of SAB.

SAB disproportionately affects persons at both extremes of life (Laupland et al. 2013). Population-based studies consistently demonstrate a high incidence in the first year of life, and relatively low and stable incidence through age 40, followed by steadily increasing rates with older age. The incidence of SAB for individuals aged 70 or greater exceeds 100 per 100,000 person-years (Laupland et al. 2013). In contrast, a rate of 4.7 per 100,000 person-years was observed in the younger, healthier US Military population (Landrum et al. 2012). The excessive burden of SAB in the older population may be partially explained by higher rates of

healthcare exposure and comorbid disease. Across numerous studies, male gender is consistently associated with higher rates of SAB, with male-to-female case ratios of approximately 1.5 (Allard et al. 2008; Klevens et al. 2007; Landrum et al. 2012). Similarly, differing rates of SAB have been associated with ethnicity. For example, the incidence of invasive MRSA infections (75 % bacteremia) in the US black population doubles that observed in whites (66.5 vs. 27.7 per 100,000 person-years) (Klevens et al. 2007; Kallen et al. 2010). Similar differences between ethnic groups have been reported in Australia and New Zealand (Tong et al. 2009; Hill et al. 2001). While observed SAB rates were higher among individuals in the lowest socioeconomic groups, discordant rates among ethnic groups persisted across all socioeconomic strata (Tong et al. 2012).

Nasal colonization with *S. aureus* upon hospital admission has been associated with a threefold higher risk of subsequent SAB (Wertheim et al. 2004). Among patients colonized with *S. aureus* who develop SAB, more than 80 % of bloodstream isolates are clonally identical to colonizing strains (Von Eiff et al. 2001). Furthermore, eradication of *S. aureus* nasal colonization with topical mupirocin has been associated with a lower incidence of SAB in hemodialysis patients and surgical site infections among patients undergoing cardiothoracic surgery (Boelaert et al. 1993; Kluytmans et al. 1996). These findings provide insight into the importance of *S. aureus* colonization for these specific patient groups (hospitalized, hemodialysis, and cardiothoracic surgery). It is unclear whether colonization rates in males and lower socioeconomic groups may account for higher rates of SAB reported in these populations (Wertheim et al. 2005; Den Heijer et al. 2013; Graham et al. 2006; Bagger et al. 2004). For example, despite a lower rate of nasal colonization, US blacks suffer from significantly higher rates of SAB (Graham et al. 2006; Klevens et al. 2007). This discrepancy highlights the need for further study of SAB epidemiology.

The presence of prosthetic devices, most prominently central venous catheters, is associated with markedly increased rates of SAB. A recent pooled analysis of five prospective observational studies from North America and Europe identified an intravenous catheter as the primary site of infection in 27.7 % of all SAB cases (Kaasch et al. 2014). The odds of developing nosocomial SAB are almost 7 times higher in patients with central venous catheters (Jensen et al. 1999). Additionally, venous access devices have become an increasingly common source of community-onset SAB (Steinberg et al. 1996). The type of catheter, access location, and therapy delivered, as well as catheter-care practices (i.e., use of full-barrier precautions for catheter care or access), may influence the relative risk of this increasingly common cause of community-onset SAB. Importantly, improved central line insertion practices and infection control efforts appear to have been effective in reducing the incidence of central line-associated bloodstream infections due to *S. aureus* (Burton et al. 2009).

Hemodialysis is associated with increased risk of SAB. In the USA, the incidence of SAB in the hemodialysis population ranges from 4045 to 5015 per 100,000 person-years, more than 100 times the risk observed in the healthy US population (Kallen et al. 2010). The type of vascular access utilized, particularly

tunneled and cuffed intravascular catheters, significantly influences SAB risk. In a 6-year population-based study of invasive MRSA infections among chronic dialysis patients captured in the Active Bacterial Core Surveillance program, 1543 of 2489 (62 %) cases of MRSA bacteremia were associated with a hemodialysis catheter (Nguyen et al. 2013). Large, prospective studies confirm that intravenous catheters are associated with a substantial portion of SAB cases, even though they are used in relatively few chronic dialysis patients (Allon et al. 2003). For those patients without other viable hemodialysis access options, the annual rate of bacteremia is exceedingly high (Lee et al. 2005). Additional factors increasing risk of SAB in chronic hemodialysis patients include higher rates of *S. aureus* colonization (Zimakoff et al. 1996) and impaired host immunity secondary to reduced neutrophil function (Vanholder et al. 1991) and iron overload (Boelaert et al. 1990). Finally, infrequent vancomycin dosing regimens used in combination with high-flux hemodialysis commonly result in low trough levels, increasing the risk of inadequately treated or relapsed SAB (Barth and DeVincenzo 1996; Jeremiah et al. 2014).

The incidence of SAB is significantly higher among patients with HIV. Among patients in a US metropolitan HIV clinic, the incidence of SAB was 1960 per 100,000 person-years (Burkey et al. 2008). Injection drug use (IDU), end-stage renal disease, and CD4 count <200 cells/microliter were independently associated with higher rates of MRSA bacteremia. A Danish population-based study reported a SAB incidence of 494 per 100,000 person-years, more than 24 times higher than the non-HIV-infected population (Larsen et al. 2012). SAB incidence rates for individuals acquiring HIV via IDU were 5 times higher than the remainder of the HIV-infected cohort. Among all HIV-infected patients in the cohort, immunosuppression was the strongest predictor of SAB. SAB rates for individuals with a CD4 count <100 cells/microliter were 10 times higher than those with CD4 count >350 cells/microliter. It is likely that the more severely immunocompromised group had greater exposure to healthcare interventions and hospitalization, resulting in increased risk of SAB. Additionally, multiple studies comparing HIV-infected and non-infected cohorts have demonstrated higher rates of nasal and extranasal *S. aureus* colonization and higher colonization burdens among HIV-infected individuals, likely increasing the risk of SAB (Hidron et al. 2005; Miller et al. 2007; Popovich et al. 2013).

2.2 Clinical Manifestations and Outcomes

SAB is associated with a variety of clinical manifestations. Non-specific findings of fever and hypotension are common, but there are no historical or examination features that are pathognomonic of SAB. Presenting symptoms may be associated with a primary focus of infection from which SAB results as a complication. Alternatively, symptoms may result from hematogenous spread to a previously sterile site (IE, vertebral osteomyelitis). The most common primary clinical foci

across numerous cohorts are intravascular catheter-related infections, skin and soft tissue infections (SSTIs), osteoarticular infections, pleuro-pulmonary infections, and IE (Table 1) (Laupland et al. 2008; Tong et al. 2012; Hewagama et al. 2012; Bishara et al. 2012; Lewis et al. 2011).

The frequency of specific symptoms or clinical syndromes varies with the population studied and continues to evolve with changing clinical practice and emergence of epidemic strains of the bacterium. For example, the incidence of central line-associated SAB in US ICUs significantly declined in association with newly implemented standards for catheter insertion and care (Burton et al. 2009). With the emergence of the MRSA USA300 SSTI epidemic in the San Francisco area, rates of community-acquired MRSA bacteremia associated with SSTI substantially increased (Tattevin et al. 2012). Primary SAB, in which no focus of infection is identified, is common and associated with poor outcomes. In a recent pooled analysis of 5 prospective cohort studies, no focus of infection was identified in 18.9 % of cases and was associated with a 3.45-fold higher 90-day mortality compared to those with a defined focus (Kaasch et al. 2014).

The classification of SAB as "complicated" or "uncomplicated" is significantly associated with prognosis and should inform the diagnostic evaluation and duration of antimicrobial therapy prescribed. In a single-center prospective study of 724 cases of SAB, complicated SAB was defined as infection resulting in attributable mortality, recurrent infection within 12 weeks, central nervous system involvement, embolic phenomenon, or metastatic sites of infection (Fowler et al. 2003). Community-acquired disease, positive follow-up blood cultures at 48–96 h, persistent fever at 72 h, or skin findings suggesting acute systemic infection (petechiae, vasculitis, infarcts, ecchymoses, or pustules) were significantly associated with complicated SAB. The SAB score generated from these findings predicted the likelihood of complicated bacteremia. One point was assigned for each positive finding of community-acquired infection, skin findings of acute systemic infection, persistent fever at 72 h, and 2 points for positive follow-up blood cultures at 48–96 h. The likelihood of complicated infection with a SAB score of 0 was 16 %, increasing linearly to 90 % for a score of 5.

Fatality rates associated with SAB exceeded 80 % in the preantibiotic era (Skinner 1941). The introduction of penicillin dramatically reduced associated mortality (Spink 1945). Despite improvements in the understanding of SAB, overall mortality rates of 20–30 % are still reported in contemporary studies. Accordingly, 30-day all-cause mortality from SAB results in estimated 2–10 deaths per 100,000 person-years annually, exceeding estimates for HIV, tuberculosis, and viral hepatitis (Van Hal et al. 2012). Improvements in mortality attributed to SAB have plateaued in part as a result of an aging population with more extensive comorbid disease. In a comprehensive review of SAB mortality, factors associated with risk of death included advanced age, comorbid medical problems (diabetes mellitus, congestive heart failure, chronic kidney disease, malignancy, and cirrhosis), primary source of infection, complicated infection, and inadequate source control. Observed 30-day mortality rates are highest for SAB associated with pulmonary

Table 1 Foci of infection associated with *Staphylococcus aureus* bacteremia

Region(s)	Total# cases	IV catheter-associated	SSTI	Pulmonary and pleural	Osteoarticular	Infective endocarditis	No identifiable focus	Other	% MRSA cases	% Healthcare-associated cases	Reference
Australia	7231	1387 (19)	1415 (20)	519 (7)	956 (13)	433 (6)	1100 (15)	1421 (20)	24.8	79.1	Turnidge et al. (2009)
Multiple	3395	942 (28)	502 (15)	178 (5)	456 (13)	282 (8)	641 (19)	394 (12)	20.6	40.7	Kaasch et al. (2014)
Calgary, Canada	1440	NR	224 (16)	220 (15)	227 (16)	79 (6)	586 (41)	104 (7)	11.3	75.3	Laupland et al. (2008)
Israel	1261	172 (14)	294 (23)	144 (11)	71 (6)	55 (4)	298 (24)	227 (18)	42.8	100	Bishara et al. (2012)
New York, USA	652	302 (46)	112 (17)	55 (8)	72 (11)	91 (14)	0	20 (3)	100	97.9	Pastagia et al. (2012)
Sydney, Australia	399	140 (35)	80 (20)	52 (13)	37 (9)	15 (4)	40 (10)	35 (9)	100	92	Van Hal et al. (2011)
South Korea	268	132 (49)	35 (13)	24 (9)	16 (6)	9 (3)	36 (13)	16 (6)	100	95.1	Park et al. (2012b)
Birmingham, UK	195	73 (37)	37 (19)	0	3 (2)	6 (3)	68 (35)	8 (4)	100	99.5	Lewis et al. (2011)
Missouri, USA	163	37 (23)	39 (24)	0	0	0	70 (43)	17 (10)	100	92.6	Honda et al. (2011)
Italy	151	23 (15)	14 (9)	7 (5)	0	0	104 (69)	3 (2)	53.9	85.5	Bassetti et al. (2012)
Central Australia	125	9 (7)	42 (34)	11 (9)	20 (16)	9 (7)	30 (24)	4 (3)	21.6	25.6	Hewagama et al. (2012)
Japan	115	27 (23)	17 (15)	10 (9)	0	0	23 (20)	38 (33)	100	NR	Isobe et al. (2012)
Thailand	73	10 (14)	20 (27)	16 (22)	9 (12)	8 (11)	0	10 (14)	27.6	55.1	Nickerson et al. (2009)
Totals (%)	15,468	3254 (21)	2831 (18)	1236 (8)	1867 (12)	987 (6)	2996 (19)	2297 (15)			

Number of cases associated with each focus of infection (%). *IV* intravenous, *SSTI* skin and soft tissue infection, *MRSA* methicillin-resistant *Staphylococcus aureus*, *NR* not reported

infections (39–67 %), IE (25–60 %), and primary bacteremia without an identified focus of infection (22–48 %) (Van Hal et al. 2012).

2.3 Management

Clinical guidelines for the management of SAB have been published (Gould et al. 2009; Mitchell and Howden 2005; Liu et al. 2011). A comprehensive review of SAB management published in 2011 highlighted the limited evidence available to guide management decisions, noting less than 1500 patients had been enrolled in only 16 clinical trials of SAB antimicrobial therapy (Thwaites et al. 2011). Similarly, a systematic review of evidence evaluating the role of transesophageal echocardiography (TEE) and antimicrobial therapy strategies in SAB management identified only one study meeting Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) standards (Guyatt et al. 2008) for high-quality evidence (Holland et al. 2014). Despite the limitations of currently available evidence, consensus recommendations include (1) early identification of complicated infection, (2) eradication of infection foci, and (3) treatment with an optimal dose, frequency, and duration of a proven antimicrobial agent.

Optimal management of SAB begins with a careful history and physical examination focused on identifying primary sources of infection and metastatic complications, which occur in approximately 30 % of cases. Even among patients with catheter-related SAB, metastatic complications have been observed in 14 % (Fowler et al. 2003). Symptoms suggesting complicated disease may be minimal or absent at the time of presentation, as exemplified by a series of 133 patients with SAB complicated by vertebral osteomyelitis. In this series, only 39 % were noted to have a diagnosis related to the spine upon admission (Jensen et al. 1998).

All cases of SAB should be classified as complicated or uncomplicated. Several definitions of uncomplicated SAB have been published (Fowler et al. 1998; Jenkins et al. 2008; Naber et al. 2009). The Infectious Diseases Society of America (IDSA) 2011 clinical practice guidelines for the treatment of MRSA infections define uncomplicated SAB as (1) exclusion of endocarditis, (2) no implanted prostheses, (3) negative follow-up blood cultures obtained 48–96 h after initial positive cultures, (4) defervescence within 72 h of initiating effective antimicrobial therapy, and (5) no evidence of metastatic sites of infection (Liu et al. 2011). Complicated disease is defined as any presentation not fulfilling all 5 criteria for uncomplicated disease. Appropriate classification of SAB informs the extent of further diagnostic evaluation and duration of therapy and minimizes the risk of recurrent disease.

Because IE complicates 25–32 % of SAB cases (Fowler et al. 1997; Abraham et al. 2004) and clinical prediction rules are insufficiently sensitive for exclusion of IE (Sullenberger et al. 2005), echocardiography is recommended for all patients with SAB (Liu et al. 2011). The superior sensitivity of TEE has been well described (Fowler et al. 1997; Van Hal et al. 2005; Khatib and Sharma 2013; Sullenberger et al. 2005). In these studies, up to 19 % of patients with SAB and negative

transthoracic echocardiography (TTE) were diagnosed with IE via TEE. Whether TEE is required for all patients with SAB, however, remains unclear. For patients at low risk for complicated disease, the excess procedural risk and cost of TEE must be weighed against the benefit of enhanced sensitivity in a relatively lower prevalence population. Criteria identifying patients with otherwise uncomplicated SAB who may not require TEE have been proposed (Van Hal et al. 2005; Khatib and Sharma 2013; Joseph et al. 2013; Rasmussen et al. 2011; Kaasch et al. 2011; Heriot et al. 2015). Each study proposed one or more of the following criteria: (1) negative TTE, (2) negative follow-up blood cultures, (3) nosocomial acquisition of SAB, (4) absence of an intracardiac device, (5) absence of hemodialysis dependence, and (6) no clinical signs of metastatic disease or endocarditis. Application of these criteria has been limited by risk of verification bias, as a minority of patients in each study underwent TEE and some received longer courses of antimicrobial therapy for other indications. To address these limitations, clinical prediction rules applied on days 1 and 5 of hospitalization have been proposed (Palraj et al. 2015). Using data from a single-center retrospective cohort of 678 patients, a day 1 score prioritizing specificity is calculated to identify high-risk patients who should undergo early TEE and be considered for repeat examination if the first TEE is negative and follow-up blood cultures remain positive. Points are assigned for the presence and type of existing intracardiac devices and location of SAB acquisition. For patients not undergoing early TEE, a day 5 score incorporating the results of follow-up blood cultures is calculated. In the population studied, a day 5 score <2 was associated with a 98.5 % negative predictive value for IE. In summary, the results suggest that patients with healthcare-associated or nosocomial SAB, no intracardiac devices, and negative follow-up blood cultures do not require TEE to rule out IE.

Infectious disease (ID) consultation is now a well-established tenet of SAB management. Observational studies consistently demonstrate that ID consultation is associated with higher rates of compliance with critical components of high-quality SAB care including obtaining follow-up blood cultures to ensure clearance of bacteremia (Lahey et al. 2009; Fries et al. 2014; Bai et al. 2015), appropriate use of echocardiography (Fowler et al. 2003; Nagao et al. 2010; Bai et al. 2015), eradication of infected foci (Lahey et al. 2009), longer duration of antimicrobial therapy for complicated SAB (Fries et al. 2014; Honda et al. 2010; Bai et al. 2015), and appropriate use of β-lactam antibiotics for methicillin-sensitive *S. aureus* (MSSA) bacteremia (Nagao et al. 2010; Robinson et al. 2012). ID consultation has been associated with lower patient mortality in numerous studies of SAB (Pastagia et al. 2012; Isobe et al. 2012; Lahey et al. 2009; Nagao et al. 2010; Choi et al. 2011; Robinson et al. 2012; Fries et al. 2014; Tissot et al. 2014; Bai et al. 2015). Where available, bedside ID consultation should be considered a standard component of SAB management (Forsblom et al. 2013).

Effective antimicrobial therapy for SAB requires careful selection of the optimal agent and duration of therapy. For patients with MSSA bacteremia, limited data suggest that β-lactam therapy is superior to the glycopeptides. Two small randomized trials compared cloxacillin plus gentamicin versus either teicoplanin

monotherapy (Fortun et al. 1995) or a glycopeptide plus gentamicin (Fortun et al. 2001) in patients with IDU-associated SAB or right-sided endocarditis. In both studies, higher rates of clinical and microbiologic treatment failure were observed in patients treated with glycopeptides. A third randomized study comparing flucloxacillin and teicoplanin was terminated early because of high rates of treatment failure in the teicoplanin arm. Notably, teicoplanin doses employed were significantly lower than currently recommended (Calain et al. 1987). A number of observational studies comparing β-lactam and glycopeptides have reported faster bacteriologic clearance, lower mortality, and less risk of treatment failure associated with β-lactam therapy (Stryjewski et al. 2007; Chang et al. 2003; Chan et al. 2012; Khatib et al. 2006). For patients empirically treated with vancomycin monotherapy, improved mortality has also been associated with de-escalation to β-lactam therapy once cultures demonstrate MSSA (Schweizer et al. 2011). Importantly, initial β-lactam therapy, as compared to empiric vancomycin monotherapy with subsequent de-escalation to β-lactam treatment once culture results are available, has been associated with lower rates of persistent SAB and reduced mortality (Khatib et al. 2006; Lodise et al. 2007). Inferior outcomes associated with empiric vancomycin monotherapy have prompted recommendations for initial treatment with an antistaphylococcal β-lactam in combination with vancomycin if MRSA bacteremia is suspected (McConeghy et al. 2013).

Only two antimicrobial agents are approved by the US Food and Drug Administration (FDA) for the treatment of MRSA bacteremia or right-sided IE: vancomycin and daptomycin. These two agents were compared in a single high-quality randomized controlled trial of SAB and IE. In the MRSA subgroup, daptomycin 6 mg/kg IV once daily resulted in treatment success for 20/45 (44 %) of patients, which was non-inferior to vancomycin plus low-dose gentamicin 14/44 (32 %) (absolute difference = 12.6 %, 95 % CI [−7.4, 32.6 %], $p = 0.28$) (Fowler et al. 2006).

Open-label randomized controlled trials have compared vancomycin to teicoplanin in the empiric treatment of neutropenic fever (Menichetti et al. 1994), trimethoprim–sulfamethoxazole (TMP-SMX) in the treatment of IDU-associated SAB (Markowitz et al. 1992), and dalbavancin in the treatment of catheter-related bacteremia (23 episodes of SAB) (Raad et al. 2005). In each of these studies, comparator drugs achieved similar cure rates to vancomycin, but none were adequately powered to evaluate superiority. In a meta-analysis of 5 randomized studies comparing vancomycin and linezolid, 53 clinically evaluable patients with MRSA bacteremia were identified (Shorr et al. 2005). No significant differences in clinical response, as defined by rates of improvement in presenting symptoms at the test of cure visit, were noted: 14/25 (56 %) treated with linezolid versus 13/28 (46 %) treated with vancomycin, OR = 1.47 (0.50–4.34). Additionally, linezolid was compared to vancomycin in the treatment of catheter-related SAB (Wilcox et al. 2009). No significant differences in mortality were noted among patients with gram-positive infections.

Vancomycin treatment failure has been associated with higher baseline vancomycin minimum inhibitory concentrations (MICs), even at values below the

Clinical Laboratory Standards Institute (CLSI) susceptibility breakpoint of ≤2 μg/dL (Soriano et al. 2008; Sakoulas et al. 2004; Hidayat et al. 2006). Accordingly, consensus guidelines recommend use of an alternative agent for the treatment of bacteremia due to MRSA with a vancomycin MIC >1 μg/dL (Rybak et al. 2009). No randomized trial has compared vancomycin to an alternative agent for the treatment of MRSA bacteremia with high vancomycin MICs. The single randomized trial comparing vancomycin and daptomycin for the treatment of MRSA bacteremia did not evaluate this population, as vancomycin minimum inhibitory concentration (MIC) of baseline isolates was ≤1 μg/dL for all patients randomized to vancomycin therapy (Rehm et al. 2008). Case–control (Moore et al. 2012; Falcone et al. 2012; Cheng et al. 2013) and cohort studies (Murray et al. 2013; Carugati et al. 2013) have associated daptomycin therapy with superior treatment response and survival as compared to vancomycin for the treatment of MRSA bacteremia with high vancomycin MICs. As compared to vancomycin, daptomycin treatment both at the FDA-approved dose of 6 mg/kg/day (Murray et al. 2013) and at median doses as high as 9.2 mg/kg/day (Carugati et al. 2013; Cheng et al. 2013) has been associated with superior outcomes and low rates of adverse drug events. Successful treatment of persistent MRSA bacteremia with ceftaroline salvage therapy has been described (Paladino et al. 2014; Casapao et al. 2014), but there are currently no studies directly comparing this agent to vancomycin or daptomycin. Linezolid was compared to glycopeptide-based therapy in a trial of 90 patients with persistent (≥7 days) MRSA bacteremia (Park et al. 2012a). Median duration of MRSA bacteremia was significantly longer in patients treated with linezolid: 16 versus 10 days, $p = 0.008$. No significant difference in mortality was noted. Until further high-quality clinical evidence is available, vancomycin and daptomycin remain the standard of care for MRSA bacteremia. For MRSA bacteremia with an elevated vancomycin MIC, daptomycin may be considered in preference to vancomycin. Table 2 summarizes important properties of antimicrobial agents studied for the treatment of MRSA bacteremia.

Currently, a minimum two-week course of intravenous antimicrobial therapy is recommended for uncomplicated SAB (Gould et al. 2009; Liu et al. 2011). Data evaluating the optimal length of treatment are limited, as studies have been constrained by the infrequency of uncomplicated disease among all SAB cases and difficulty accurately identifying uncomplicated SAB at the time of trial enrollment (Stryjewski et al. 2014). A number of observational studies assessing the efficacy of shorter courses of therapy for uncomplicated bacteremia have been reviewed (Thwaites et al. 2011). Though some authors conclude that as little as 7 days of antimicrobial therapy may be sufficient for highly selected cases of uncomplicated SAB, risk of selection and allocation bias in these observational studies cannot be quantified, limiting generalizability of these results. A meta-analysis of 11 observational studies of SAB treatment identified a 6 % rate of relapse or metastatic complication associated with less than 2 weeks of treatment (Jernigan and Farr 1993). These data are consistent with findings from a recent prospective cohort of 111 patients with uncomplicated SAB (Chong et al. 2013). 3/38 (8 %) of patients receiving fewer than 14 days of intravenous antibiotics experienced relapse. 0/73

Table 2 Antimicrobial agents studied in the treatment of MRSA bacteremia

Antibiotic	Dose	Renal/hepatic impairment dose adjustments	Monitoring	Notable adverse effects	Comments
Vancomycin[a]	15–20 mg/kg/every 8–12 h	Renal	Serum trough levels, renal function, and complete blood counts with prolonged use	Red man syndrome	Bactericidal, adjust dose and frequency to maintain serum trough levels ≥15 mg/L, variable tissue penetration
Teicoplanin	12 mg/kg/day	Renal	Serum trough levels	Lower frequency of rash, flushing versus vancomycin	Bactericidal, poor outcomes associated with serum trough levels <20 mg/L for invasive infections
Daptomycin[a]	6 mg/kg/day, 8–10 mg/kg/day recommended for complicated SAB or IE	Renal	Weekly creatine kinase levels	Peripheral neuropathy, myopathy, eosinophilic pneumonia	Bactericidal, biofilm penetration, treatment failure associated with deep infection foci and 6 mg/kg/day dosing
Linezolid	600 mg every 12 h	None recommended	Weekly complete blood counts	Thrombocytopenia, leukopenia and neuropathy with extended use, serotonin crisis in patients taking additional serotonergic agents	Bacteriostatic, excellent tissue penetration
Ceftaroline	600 mg every 12 h	Renal			Bactericidal, limited experience
Rifampin	300–450 mg every 12 h	None recommended	Liver function panel every 2 weeks during extended therapy	Liver function test abnormalities, reddish-discolored tears, saliva, urine	Bactericidal, biofilm penetration. Very frequent drug–drug interactions. Rapid development of rifampin resistance with monotherapy

(continued)

Table 2 (continued)

Antibiotic	Dose	Renal/hepatic impairment dose adjustments	Monitoring	Notable adverse effects	Comments
Trimethoprim–sulfamethoxazole	320 mg TMP every 12 h	Renal	Complete blood counts, renal function, and electrolytes	Rash, hyperkalemia, elevated serum creatinine, leukopenia	Combination bactericidal, limited clinical experience
Dalbavancin	1000 mg initial dose, followed by 500 mg weekly	Renal		Nausea, diarrhea, elevated liver transaminase levels	Long elimination half-life permits once-weekly dosing, limited experience in catheter-associated SAB only
Quinupristin–dalfopristin	7.5 mg/kg every 8 h	None recommended	Liver function panel	Infusion site reactions, myalgia and arthralgia very common, nausea, hyperbilirubinemia	Infusion via central venous access recommended, limited clinical experience
Tigecycline	100 mg initial dose, followed by 50 mg every 12 h	Hepatic	Liver function panel	Nausea, vomiting, photosensitivity	Low serum concentrations, associated with excess mortality compared to alternative agents, not recommended for the treatment of SAB/IE

[a]Drugs approved by the US Food and Drug Administration for the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia

Renal or hepatic dosing adjustment and monitoring information abstracted from manufacturers' prescribing information. *SAB Staphylococcus aureus* bacteremia, *IE* infective endocarditis, *TMP* trimethoprim

(0 %) receiving at least 14 days of therapy experienced relapse, $p = 0.036$. A randomized controlled trial of 14 days of intravenous antimicrobial therapy versus early switch to oral therapy is ongoing (ClinicalTrials.gov identifier: NCT01792804). Until higher-quality evidence is available, all patients should receive at least 2 weeks of intravenous antimicrobial therapy.

Two-week courses of antimicrobial therapy for IDU-associated right-sided IE have been evaluated in prospective observational (Chambers et al. 1988; Torres-Tortosa et al. 1994) and small randomized (Ribera et al. 1996; Fortun et al. 1995) studies. β-lactam regimens both with and without aminoglycosides were effective. As noted previously, two-week glycopeptide regimens, by comparison, were associated with worse outcomes (Chambers et al. 1988; Fortun et al. 2001). For patients with complicated SAB, little data are available to inform treatment duration and 4–6 weeks of antimicrobial therapy remains the standard of care (Gould et al. 2009; Liu et al. 2011).

Combinations of antimicrobial agents to improve suboptimal clinical outcomes and address inherent limitations of currently recommended first-line antimicrobial agents (i.e., emergence of resistance, slow bactericidal effect, and poor tissue penetration associated with vancomycin monotherapy) have been evaluated and recently reviewed (Davis et al. 2015). Combinations of vancomycin with daptomycin (Tsuji and Rybak 2006), linezolid (Mulazimoglu et al. 1996; Jacqueline et al. 2003), tigecycline (Petersen et al. 2006), and quinupristin–dalfopristin (Brown and Freeman 2004) have been evaluated in vitro with conflicting or negative results. Clinical data evaluating these combinations are limited.

β-lactams combined with either vancomycin or daptomycin have consistently demonstrated synergy during in vitro testing (Davis et al. 2015). The combination of a β-lactam and vancomycin was associated with higher rates of microbiologic eradication of MRSA bacteremia versus vancomycin alone in an observational study (Dilworth et al. 2014). The effect of combining an antistaphylococcal β-lactam with standard therapy for MRSA bacteremia is currently being evaluated in randomized clinical trials (ClinicalTrials.gov identifier: NCT02365493; Australian New Zealand Clinical Trials Registry identifier: ACTRN12610000940077).

In vitro studies of rifampin added to standard therapy have yielded mixed results (Perlroth et al. 2008), and current IDSA MRSA treatment guidelines advise against routine use of rifampin in combination with vancomycin for the treatment of SAB (Liu et al. 2011). Nevertheless, a recent retrospective cohort study of 357 patients with SAB and a deep focus of infection reported significantly lower odds of mortality at 90 days for patients treated with early (within 7 days of initial positive blood culture) rifampin therapy for at least 14 days as compared to no rifampin therapy (OR = 0.33, $p<0.01$) (Forsblom et al. 2015). A randomized trial evaluating the impact of rifampicin adjunctive therapy for SAB (MSSA and MRSA) is currently being conducted (Thwaites et al. 2012).

3 *Staphylococcus aureus* Infective Endocarditis

3.1 Epidemiology

S. aureus, a virulent pathogen associated with fulminant disease and excess mortality, is now the most common cause of IE in the industrialized world (Fowler et al. 2005). A systematic review of national population-based studies of IE published prior to 2000 estimated overall IE incidence ranged from 1.5 to 6 per 100,000 person-years (Tleyjeh et al. 2007). The proportion of cases caused by *S. aureus* from 1970 to 2000 ranged from 16 to 34 %, but no temporal trends in overall microbiologic etiology were detected. Over the past two decades, however, the development of large international collaborations such as the International Collaboration on Endocarditis Prospective Cohort Study (ICE-PCS) and publication of several contemporary population-based studies have enhanced our modern understanding of IE (Murdoch et al. 2009).

Analysis of data from the US National Inpatient Sample (NIS) identified an increasing IE incidence of 11.4 per 100,000 person-years in 1999 to 16.6 per 100,000 person-years by 2006 (Federspiel et al. 2012). Rising *S. aureus* IE incidence accounted for much of the change and was associated with excess mortality when compared to other pathogens. A second analysis of US NIS data through 2009 reported an overall IE incidence of 12.9 per 100,000 person-years and confirmed rising rates of *S. aureus* IE over the study period: 24 % of cases in 1999 increasing to 32 % of cases in 2009 (Bor et al. 2013). MRSA caused 53 % of all *S. aureus* IE cases by 2009.

In contrast, IE incidence rates in other parts of the industrialized world are reportedly lower. Nevertheless, *S. aureus* remains the most commonly isolated pathogen in contemporary studies. For example, the proportion of *S. aureus* IE has increased from 16 to 26 % in France despite a stable overall incidence of approximately 3.5 per 100,000 person-years (Duval et al. 2012). In Italy, *S. aureus* is now responsible for 40 % of IE and has been associated with an increasing overall IE incidence from 4.1 to 4.9 per 100,000 person-years from 2000 to 2008 (Fedeli et al. 2011). Similar findings from the population of New South Wales, Australia, have been published (Sy and Kritharides 2010). In summary, the preponderance of evidence from industrialized settings confirms that *S. aureus* is the most common cause of IE. Even in countries with a high burden of rheumatic heart disease, where viridans streptococci have traditionally been the predominant pathogen, *S. aureus* has become increasingly common (Kanafani et al. 2002; Trabelsi et al. 2008; Mirabel et al. 2015; Fayyaz et al. 2014).

IE in industrialized settings is now increasingly acquired as a healthcare-associated or nosocomial infection (Selton-Suty et al. 2012; Fowler et al. 2005). A study involving over 1600 patients from 28 countries in the ICE-PCS cohort found that 34 % of all IE cases were healthcare-associated, of which 54 % were nosocomial infections (Benito et al. 2009). Notably, *S. aureus* accounted for 47 % of nosocomial and 42 % of non-nosocomial healthcare-associated IE, findings

that are consistent with other large cohorts (Selton-Suty et al. 2012). Thus, the growing burden of healthcare-associated IE significantly contributed to the emergence of *S. aureus* as the most common cause of IE in the industrialized world.

3.2 Prosthetic Valve Endocarditis

For patients with prosthetic valves, the burden of IE is exceedingly high. A study of 1032 patients from the Veterans Affairs Health Cooperative with an average follow-up of 7.7 years reported an annual rate of prosthetic valve IE of 0.8 % (Grover et al. 1994). As with native valve IE, *S. aureus* is now the most common cause of prosthetic valve IE. Among 556 patients with prosthetic valve IE in the ICE-PCS cohort from 2000 to 2005, 128 (23 %) cases were caused by *S. aureus* and 94 (16.9 %) were caused by coagulase-negative staphylococci (Wang et al. 2007). *S. aureus* is a leading cause of the emerging clinical entity of transcatheter valve endocarditis (Amat-Santos et al. 2015a, b). In critically ill patients, the proportion of prosthetic valve IE caused by *S. aureus* is likely even higher (Wolff et al. 1995).

The changing microbiology of prosthetic valve IE reflects risk associated with increasing healthcare exposure. Hospitalization and healthcare exposure increase risk for SAB, which results in hematogenous seeding of prosthetic valves in 44–51 % of cases (Fang et al. 1993; El-Ahdab et al. 2005). The risk for the development of *S. aureus* prosthetic valve IE is highest in the first year after surgery, also likely a reflection of healthcare exposure (Wang et al. 2007). Incomplete endothelialization may increase the risk of early prosthetic valve IE. Accordingly, higher rates of early prosthetic valve IE have been observed with mechanical valves, yet the 5-year risk of IE is similar (Ivert et al. 1984; Calderwood et al. 1985). For patients with SAB, however, neither valve composition (bioprosthetic vs. mechanical) nor location (mitral vs. aortic) alters the risk of hematogenous seeding (El-Ahdab et al. 2005). In summary, the risk of IE is exceedingly high for all patients with prosthetic valves and SAB, necessitating the evaluation for IE with echocardiogram (preferably TEE) for all bacteremic patients.

3.3 Pathophysiology

Damage to the cardiac endothelium, resulting in the deposition of extracellular matrix proteins, fibrin, and platelets, forms a sterile thrombotic lesion, which is a prerequisite to the development of IE (Que and Moreillon 2011). Proposed mechanisms of endothelial damage include direct trauma from intravascular catheters/devices, injected particulate matter, turbulent blood flow from prosthetic valves or congenital heart disease, or inflammation from rheumatic or degenerative

valvular pathology. The resultant sterile thrombotic endocarditis provides an excellent culture medium in the bacteremic patient.

S. aureus and other gram-positive organisms commonly causing IE utilize a number of adhesins (collectively called microbial surface component reacting with adhesive matrix molecules or MSCRAMMs) to bind to host extracellular matrix proteins (Patti et al. 1994). The MSCRAMMs clumping factor A (ClfA, also named fibrinogen-binding protein A) and fibronectin-binding protein A (FnBPA) are essential for *S. aureus* colonization of cardiac valves. *S. aureus* binds to sterile thrombotic endocarditis foci via ClfA and subsequently invades endothelial cells via FnBPA (Piroth et al. 2008). Local endothelial inflammation also triggers fibronectin expression, promoting adherence and vegetation growth of *S. aureus* via fibronectin-binding proteins (Fitzgerald et al. 2006). Staphylococcal superantigens may also cause endothelial damage that facilitates vegetation development. High circulating superantigen levels were essential to the development of IE in animal models (Salgado-Pabon et al. 2013). These mechanisms are supported by a growing number of studies linking distinct bacterial genotypes from clinical isolates to disease phenotypes. For example, *S. aureus* isolates of clonal complex 30 (CC30) are more likely to express adhesion and superantigen genes linked to hematogenous disease and cause IE (Fowler et al. 2007; Nienaber et al. 2011). *S. aureus* isolates are capable of producing biofilms, which adhere to prosthetic or even healthy heart valves. The resulting microenvironment is relatively devoid of nutrients and oxygen, creating a state of slow microbial growth and relative antibiotic resistance. The biofilm also confers protection from host immune cells and limits penetration of some antibiotics (Patel 2005). Defective electron transport has been demonstrated in subpopulations of the *S. aureus* colony, creating small colony variants with low rates of metabolic activity, altered biochemical profiles, and often significant antibiotic resistance (Proctor et al. 1998). These small colony variants may be resistant to antibiotics prescribed on the basis of traditional *in vitro* susceptibility testing and serve as reservoirs for late infection recurrence.

3.4 Clinical Manifestations and Outcomes

Clinical presentation, complications, and outcomes associated with *S. aureus* IE have been well defined in the ICE-PCS cohort (Fowler et al. 2005), Danish national cohort (Roder et al. 1999), and several single-center series (Fernandez Guerrero et al. 2009; Nadji et al. 2005; Rogers et al. 2009). In the ICE-PCS cohort, median age was 57, 61 % of patients were male, and 39 % were recently exposed to the healthcare system. These findings were consistent across all cohorts. Common comorbidities include diabetes mellitus (20–30 %) and hemodialysis dependence (8–14 %). As compared to other types of IE, patients with *S. aureus* IE tended to present with a shorter duration of symptoms and were more likely to have prolonged bacteremia.

Native valvular disease comprises 70–80 % of *S. aureus* IE cases. Left-sided IE is most common, with mitral valve disease occurring approximately 1.5 times more often than aortic disease. Although right-sided IE was the most common site associated with IE in IDU, left-sided IE represents 30 % of *S. aureus* IE in this patient group (Fowler et al. 2005; Ruotsalainen et al. 2006). Complications from left-sided *S. aureus* IE are particularly devastating, as systemic emboli are common. Cerebrovascular events (18–35 %) and heart failure (15–53 %) frequently complicate *S. aureus* IE.

For patients with prosthetic valves and SAB, fever persisting more than 72 h after initiation of appropriate antimicrobial therapy (OR = 4.4, 95 % CI 1.0–19.1) or persistently positive blood cultures 2–4 days after initial positive culture (OR 11.7, 2.9–47.7) strongly suggest IE and require evaluation with echocardiography (El-Ahdab et al. 2005; Palraj et al. 2015). Splenomegaly, peripheral emboli, and new regurgitant murmurs are classically associated with *S. aureus* prosthetic valve IE (Ben Ismail et al. 1987), although new murmurs are actually more common in patients with native valve IE, perhaps because patients typically present after a longer duration of symptoms (Fernandez Guerrero et al. 2009). Stroke is a frequent manifestation of systemic embolism, complicating 23–33 % of *S. aureus* IE cases, and is associated with significant excess mortality (Chirouze et al. 2004; John et al. 1998).

Overall mortality associated with *S. aureus* IE ranges from 22 to 66 % and is consistently higher than IE mortality rates associated with other pathogens (Fowler et al. 2005; Nadji et al. 2005). Higher mortality associated with *S. aureus* endocarditis is likely a consequence of infection with a pathogen that is more virulent than other common bacterial causes of IE in a patient population that suffers from a higher burden of comorbid illness. Several characteristics of *S. aureus* IE have consistently demonstrated prognostic significance. For example, healthcare-associated *S. aureus* IE and prosthetic valve IE are associated with poorer outcomes than community-onset and native valve IE, respectively. Right-sided *S. aureus* IE is associated with better outcomes than left-sided disease. *S. aureus* IE associated with IDU carries a more favorable prognosis than with non-IDU populations. Complications universally associated with excess mortality include older age, stroke, and heart failure (Roder et al. 1999).

3.5 Management

The central tenets of *S. aureus* IE management are prolonged intravenous antibiotics and evaluating the need for early surgical management. Comprehensive management guidelines have been published by European and American professional societies (Liu et al. 2011; Gould et al. 2012; Habib et al. 2009; Baddour et al. 2005). For MSSA IE, β-lactam therapy with nafcillin or oxacillin (flucloxacillin per British guidelines) is preferred first-line therapy. For MRSA IE, vancomycin is recommended as first-line therapy. European guidelines recommend daptomycin for MRSA IE resistant to vancomycin (vancomycin minimum inhibitory concentration >2 mg/L), or for

vancomycin intolerance. IDSA guidelines for the treatment of MRSA IE now include both vancomycin and daptomycin as first-line therapy options.

These recommendations are based on a randomized controlled non-inferiority trial of daptomycin versus standard therapy for SAB with or without IE (Fowler et al. 2006). All patients randomized to standard therapy (β-lactam for MSSA or vancomycin for MRSA) and all patients with left-sided endocarditis were also treated with low-dose gentamicin. Overall, daptomycin met non-inferiority criteria and is FDA-approved for the treatment of SAB and right-sided IE. Notably, only 18 patients with left-sided IE were included in the trial, preventing definitive conclusions about the efficacy of daptomycin in this setting. Interestingly, the most recent IDSA guidelines extend the recommendation for the use of daptomycin 6 mg/kg IV once daily beyond the FDA-approved indication of right-sided IE to include all MRSA IE (Liu et al. 2011). Given these broader recommendations, further considerations for clinicians are the development of treatment-emergent non-susceptibility to daptomycin and whether higher doses of daptomycin should be used.

The development of treatment-emergent non-susceptibility to daptomycin was noted in 5/45 (11 %) patients with MRSA in the original non-inferiority trial. Subsequent reports of treatment-emergent non-susceptibility have since been published: 6/54 (11 %) patients receiving daptomycin salvage therapy for MRSA IE (Kullar et al. 2013), 6/10 (60 %) patients with persistent SAB (Sharma et al. 2008), and 7/18 (39 %) patients with persistent SAB (Gasch et al. 2014). Non-susceptibility may have resulted from inadequate source control (Fowler et al. 2006) and suboptimal antimicrobial dosing (Sharma et al. 2008) in highly selected cohorts of patients with persistent SAB. In an effort to minimize the risk of treatment-emergent resistance, a number of antimicrobial agents have been combined with daptomycin. Successful treatment has been described with rifampin (Rose et al. 2013), TMP/SMX (Di Carlo et al. 2013), fosfomycin (Miro et al. 2012), and β-lactams (Dhand et al. 2011; Moise et al. 2013). These observational studies are limited by small sample size and lack of comparator groups. Additional studies are needed to conclusively define the role of daptomycin combination therapy.

Higher daptomycin dosing regimens (8–10 mg/kg IV once daily) have been advocated to reduce the risk of treatment-emergent resistance. These recommendations are included in IDSA guidelines for MRSA treatment (Liu et al. 2011) and have been associated with treatment success without increased toxicity. In a registry of 312 patients treated with daptomycin as salvage therapy for IE, 72 patients were treated with doses >8 mg/kg/day. Clinical success rates for right (91 %) and left (89 %) IE in the high-dose group were significant. Overall, clinical success was achieved in 81 % MRSA IE cases (Dohmen et al. 2013). Similarly, a retrospective cohort analysis of 126 patients receiving high-dose daptomycin (median dose 8.9 mg/kg/day) for MRSA bacteremia, many for salvage therapy, reported an overall clinical response rate of 83 % (Kullar et al. 2011). In a 2008–2010 ICE-PCS cohort, outcomes for 29 patients receiving high-dose daptomycin (2/3 as salvage therapy) were compared to 149 patients receiving standard antimicrobial therapy (Carugati et al. 2013). Time to clearance of MRSA bacteremia was significantly shorter for patients receiving daptomycin versus standard therapy (1.0 vs. 5.0 days).

For prosthetic valve MRSA IE, American and European guidelines recommend combination antimicrobial therapy with vancomycin, gentamicin, and rifampin (Liu et al. 2011; Gould et al. 2012; Habib et al. 2009; Baddour et al. 2005). Recommendations are largely extrapolated from two small retrospective studies of methicillin-resistant *Staphylococcus epidermidis* of 23 (Karchmer et al. 1983a) and 75 patients (Karchmer et al. 1983b). In each study, the addition of rifampin to vancomycin was associated with higher rates of clinical success. Subsequently, rifampin has been studied in combination with standard therapy for native valve IE caused by *S. aureus* (Riedel et al. 2008). Rifampin resistance developed during therapy in 9/42 cases. Mortality was higher among patients receiving rifampin (21 % vs. 5 % for controls, $p = 0.048$), and adverse effects were common: 9/42 patients developed significant transaminase elevation and drug interactions developed in more than 50 % of patients. Patients treated with rifampin were sicker at baseline, limiting the interpretation of this retrospective study. In summary, these data highlight the need for continued research to define optimal antimicrobial regimens for prosthetic valve IE.

Despite published guidelines from European and American professional societies (Gould et al. 2012; Habib et al. 2009; Baddour et al. 2005), numerous questions regarding the optimal timing and indications for native valvular surgery persist. The benefit of early surgery (defined as valvular replacement or repair during the initial hospitalization) for native valve IE was evaluated in 1552 patients from the ICE-PCS cohort. Propensity-based matching was utilized to address many of the biases limiting prior observational studies. Survival benefit from early surgery was demonstrated for patients with *S. aureus* IE, paravalvular complications, stroke, and systemic embolization (Lalani et al. 2010). Subsequently, the effects of early surgery were evaluated in a trial of 76 patients with left-sided IE, severe valvular disease, and large vegetations (10 mm or larger). Patients were randomized to early surgery (within 48 h of enrollment) or standard care. Notably, 27/30 patients in the standard care arm underwent surgery during initial hospitalization. Surgery within 48 h was associated with a substantial decrease in the composite outcome of all-cause mortality, systemic embolization, or recurrent endocarditis at 6 months (3 % vs. 28 %, HR 0.08, 95 % CI 0.01, 0.65) (Kang et al. 2012). Eight patients included in the study had *S. aureus* IE, limiting definitive conclusions about this specific population.

Although early surgery reduces the risk of subsequent embolization, the optimal timing of surgery for patients who have already suffered a stroke remains controversial. A number of observational studies evaluating the timing of surgery after ischemic stroke have been summarized (Rossi et al. 2012) and suggest that delay is not necessary if clear indications for valvular surgery are present. The optimal timing of surgery was evaluated in 198 patients with definite IE and ischemic stroke from the ICE-PCS cohort. Outcomes for patients undergoing surgery within 7 days of ischemic stroke were compared to those undergoing delayed surgery. There were increased odds of in-hospital mortality for patients undergoing early surgery that did not reach statistical significance (OR = 2.3, 95 % CI 0.94, 5.7) (Barsic et al. 2013). Further detailed observational or randomized studies assessing optimal timing of surgery are required.

Numerous observational studies have documented improved mortality rates associated with surgical management of *S. aureus* prosthetic valve endocarditis (Fernandez Guerrero et al. 2009; El-Ahdab et al. 2005; Wolff et al. 1995). The optimal timing of valvular surgery for *S. aureus* prosthetic valve IE, however, has not been well defined. The role of early surgery was evaluated in 1025 patients with prosthetic valve IE from the ICE-PCS cohort. Though early surgery was associated with improved mortality in unadjusted analyses, no significant differences were noted after adjustment for treatment selection and survivor bias (Lalani et al. 2013). The effect of early surgery for *S. aureus* prosthetic valve IE was evaluated in 168 patients from the ICE-PCS cohort (Chirouze et al. 2015). 74/168 (44 %) underwent early valve surgery, which was associated with improved mortality in unadjusted analyses (33.8 % vs. 59.1 %, $p = 0.001$). In multivariable propensity-adjusted models, however, no survival advantage was associated with early valvular surgery. Notably, for the subset of patients in the highest surgical propensity quintile, there was an association between early surgery and improved mortality in the propensity-adjusted model (Lalani et al. 2013). Altogether, these results suggest that a single approach cannot be applied to all patients with *S. aureus* prosthetic valve endocarditis, and decisions regarding timing of surgical management should incorporate an individualized assessment of risk.

4 Conclusions

Staphylococcus aureus is a key pathogen causing bacteremia and IE in the industrialized world. Through the creation of large multinational collaborations, the epidemiology of these fulminant infections has been well described. Nevertheless, morbidity and mortality associated with SAB and IE remain excessively high. Antimicrobial resistance and limitations of current clinical evidence highlight the urgent need for high-quality research designed to limit the burden of this deadly disease.

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References

Abraham J, Mansour C, Veledar E, Khan B, Lerakis S (2004) *Staphylococcus aureus* bacteremia and endocarditis: the Grady Memorial Hospital experience with methicillin-sensitive S aureus and methicillin-resistant S aureus bacteremia. Am Heart J 147:536–539

Allard C, Carignan A, Bergevin M, Boulais I, Tremblay V, Robichaud P, Duperval R, Pepin J (2008) Secular changes in incidence and mortality associated with *Staphylococcus aureus* bacteraemia in Quebec, Canada, 1991–2005. Clin Microbiol Infect 14:421–428

Allon M, Depner TA, Radeva M, Bailey J, Beddhu S, Butterly D, Coyne DW, Gassman JJ, Kaufman AM, Kaysen GA, Lewis JA, Schwab SJ (2003) Impact of dialysis dose and membrane on infection-related hospitalization and death: results of the HEMO Study. J Am Soc Nephrol 14:1863–1870

Amat-Santos IJ, Messika-Zeitoun D, Eltchaninoff H, Kapadia S, Lerakis S, Cheema AN, Gutierrez-Ibanes E, Munoz-Garcia AJ, Pan M, Webb JG, Herrmann HC, Kodali S, Nombela-Franco L, Tamburino C, Jilaihawi H, Masson JB, De Brito FS, Jr Ferreira MC, Lima VC, Mangione JA, Iung B, Vahanian A, Durand E, Tuzcu EM, Hayek SS, Angulo-Llanos R, Gomez-Doblas JJ, Castillo JC, Dvir D, Leon MB, Garcia E, Cobiella J, Vilacosta I, Barbanti M, Makkar R, Ribeiro HB, Urena M, Dumont E, Pibarot P, Lopez J, Roman AS, Rodes-Cabau J (2015a) Infective endocarditis after transcatheter aortic valve implantation: results from a large multicenter registry. Circulation, 131:1566–1574

Amat-Santos IJ, Ribeiro HB, Urena M, Allende R, Houde C, Bedard E, Perron J, Delarochelliere R, Paradis JM, Dumont E, Doyle D, Mohammadi S, Cote M, San Roman JA, Rodes-Cabau J (2015b) Prosthetic valve endocarditis after transcatheter valve replacement: a systematic review. JACC Cardiovasc Interv 8:334–346

Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Bolger AF, Levison ME, Ferrieri P, Gerber MA, Tani LY, Gewitz MH, Tong DC, Steckelberg JM, Baltimore RS, Shulman ST, Burns JC, Falace DA, Newburger JW, Pallasch TJ, Takahashi M, Taubert KA (2005) Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the committee on rheumatic fever, endocarditis, and Kawasaki disease, council on cardiovascular disease in the young, and the councils on clinical cardiology, stroke, and cardiovascular surgery and anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation 111:e394–e434

Bagger JP, Zindrou D, Taylor KM (2004) Postoperative infection with meticillin-resistant *Staphylococcus aureus* and socioeconomic background. Lancet 363:706–708

Bai AD, Showler A, Burry L, Steinberg M, Ricciuto DR, Fernandes T, Chiu A, Raybardhan S, Science M, Fernando E, Tomlinson G, Bell CM, Morris AM (2015) Impact of infectious disease consultation on quality of care, mortality, and length of stay in *staphylococcus aureus* bacteremia: results from a large multicenter cohort study. Clin Infect Dis 60:1451–1461

Barsic B, Dickerman S, Krajinovic V, Pappas P, Altclas J, Carosi G, Casabe JH, Chu VH, Delahaye F, Edathodu J, Fortes CQ, Olaison L, Pangercic A, Patel M, Rudez I, Tamin SS, Vincelj J, Bayer AS, Wang A (2013) Influence of the timing of cardiac surgery on the outcome of patients with infective endocarditis and stroke. Clin Infect Dis 56:209–217

Barth RH, Devincenzo N (1996) Use of vancomycin in high-flux hemodialysis: experience with 130 courses of therapy. Kidney Int 50:929–936

Bassetti M, Trecarichi EM, Mesini A, Spanu T, Giacobbe DR, Rossi M, Shenone E, Pascale GD, Molinari MP, Cauda R, Viscoli C, Tumbarello M (2012) Risk factors and mortality of healthcare-associated and community-acquired *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 18:862–869

Ben Ismail M, Hannachi N, Abid F, Kaabar Z, Rouge JF (1987) Prosthetic valve endocarditis. A survey. Br Heart J 58:72–77

Benito N, Miro JM, De Lazzari E, Cabell CH, Del Rio A, Altclas J, Commerford P, Delahaye F, Dragulescu S, Giamarellou H, Habib G, Kamarulzaman A, Kumar AS, Nacinovich FM, Suter F, Tribouilloy C, Venugopal K, Moreno A, Fowler VG Jr (2009) Health care-associated native valve endocarditis: importance of non-nosocomial acquisition. Ann Intern Med 150:586–594

Bishara J, Goldberg E, Leibovici L, Samra Z, Shaked H, Mansur N, Paul M (2012) Healthcare-associated vs. hospital-acquired *Staphylococcus aureus* bacteremia. Int J Infect Dis 16:e457–e463

Boelaert JR, Daneels RF, Schurgers ML, Matthys EG, Gordts BZ, Van Landuyt HW (1990) Iron overload in haemodialysis patients increases the risk of bacteraemia: a prospective study. Nephrol Dial Transplant 5:130–134

Boelaert JR, Van Landuyt HW, Godard CA, Daneels RF, Schurgers ML, Matthys EG, De Baere YA, Gheyle DW, Gordts BZ, Herwaldt LA (1993) Nasal mupirocin ointment decreases the incidence of *Staphylococcus aureus* bacteraemias in haemodialysis patients. Nephrol Dial Transplant 8:235–239

Bor DH, Woolhandler S, Nardin R, Brusch J, Himmelstein DU (2013) Infective endocarditis in the U.S., 1998–2009: a nationwide study. PLoS ONE 8:e60033

Brown J, Freeman BB 3rd (2004) Combining quinupristin/dalfopristin with other agents for resistant infections. Ann Pharmacother 38:677–685

Burkey MD, Wilson LE, Moore RD, Lucas GM, Francis J, Gebo KA (2008) The incidence of and risk factors for MRSA bacteraemia in an HIV-infected cohort in the HAART era. HIV Med 9:858–862

Burton DC, Edwards JR, Horan TC, Jernigan JA, Fridkin SK (2009) Methicillin-resistant *Staphylococcus aureus* central line-associated bloodstream infections in US intensive care units, 1997–2007. JAMA 301:727–736

Calain P, Krause KH, Vaudaux P, Auckenthaler R, Lew D, Waldvogel F, Hirschel B (1987) Early termination of a prospective, randomized trial comparing teicoplanin and flucloxacillin for treating severe staphylococcal infections. J Infect Dis 155:187–191

Calderwood SB, Swinski LA, Waternaux CM, Karchmer AW, Buckley MJ (1985) Risk factors for the development of prosthetic valve endocarditis. Circulation 72:31–37

Carugati M, Bayer AS, Miro JM, Park LP, Guimaraes AC, Skoutelis A, Fortes CQ, Durante-Mangoni E, Hannan MM, Nacinovich F, Fernandez-Hidalgo N, Grossi P, Tan RS, Holland T, Fowler VG Jr, Corey RG, Chu VH (2013) High-dose daptomycin therapy for left-sided infective endocarditis: a prospective study from the international collaboration on endocarditis. Antimicrob Agents Chemother 57:6213–6222

Casapao AM, Davis SL, Barr VO, Klinker KP, Goff DA, Barber KE, Kaye KS, Mynatt RP, Molloy LM, Pogue JM, Rybak MJ (2014) Large retrospective evaluation of the effectiveness and safety of *Ceftaroline fosamil* therapy. Antimicrob Agents Chemother 58:2541–2546

Chambers HF, Miller RT, Newman MD (1988) Right-sided *Staphylococcus aureus* endocarditis in intravenous drug abusers: two-week combination therapy. Ann Intern Med 109:619–624

Chan KE, Warren HS, Thadhani RI, Steele DJ, Hymes JL, Maddux FW, Hakim RM (2012) Prevalence and outcomes of antimicrobial treatment for *Staphylococcus aureus* bacteremia in outpatients with ESRD. J Am Soc Nephrol 23:1551–1559

Chang FY, Peacock JE, Jr Musher DM, Triplett P, Macdonald BB, Mylotte JM, O'donnell A, Wagener MM, Yu VL (2003) *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. Medicine (Baltimore) 82:333–339

Cheng CW, Hsu PC, Yang CC, Chang HJ, Siu LK, Wu TL, Huang CT, Lee MH (2013) Influence of early daptomycin therapy on treatment outcome of meticillin-resistant *Staphylococcus aureus* bacteraemia with high vancomycin minimum inhibitory concentrations. Int J Antimicrob Agents 41:293–294

Chi CY, Ho MW, Ho CM, Lin PC, Wang JH, Fung CP (2007) Molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* bacteremia in a teaching hospital. J Microbiol Immunol Infect 40:310–316

Chirouze C, Cabell CH, Fowler VG Jr, Khayat N, Olaison L, Miro JM, Habib G, Abrutyn E, Eykyn S, Corey GR, Selton-Suty C, Hoen B (2004) Prognostic factors in 61 cases of *Staphylococcus aureus* prosthetic valve infective endocarditis from the International Collaboration on Endocarditis merged database. Clin Infect Dis 38:1323–1327

Chirouze C, Alla F, Fowler VG Jr, Sexton DJ, Corey GR, Chu VH, Wang A, Erpelding ML, Durante-Mangoni E, Fernandez-Hidalgo N, Giannitsioti E, Hannan MM, Lejko-Zupanc T, Miro JM, Munoz P, Murdoch DR, Tattevin P, Tribouilloy C, Hoen B (2015) Impact of early valve surgery on outcome of *Staphylococcus aureus* prosthetic valve infective endocarditis: analysis in the International Collaboration of Endocarditis-Prospective Cohort Study. Clin Infect Dis 60:741–749

Choi SH, Cho SY, Park JH, Chung JW (2011) Impact of infectious-disease specialist consultations on outcomes of *Staphylococcus aureus* bacteremia in a hospital with a low volume of patients with S. aureus bacteremia. J Infect 62:181–185

Chong YP, Moon SM, Bang KM, Park HJ, Park SY, Kim MN, Park KH, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS (2013) Treatment duration for uncomplicated *Staphylococcus aureus* bacteremia to prevent relapse: analysis of a prospective observational cohort study. Antimicrob Agents Chemother 57:1150–1156

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23:616–687

Davis JS, Hal SV, Tong SY (2015) Combination antibiotic treatment of serious methicillin-resistant *Staphylococcus aureus* infections. Semin Respir Crit Care Med 36:3–16

Den Heijer CD, Van Bijnen EM, Paget WJ, Pringle M, Goossens H, Bruggeman CA, Schellevis FG, Stobberingh EE (2013) Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant S aureus, in nine European countries: a cross-sectional study. Lancet Infect Dis 13:409–415

Dhand A, Bayer AS, Pogliano J, Yang SJ, Bolaris M, Nizet V, Wang G, Sakoulas G (2011) Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. Clin Infect Dis 53:158–163

Di Carlo P, D'alessandro N, Guadagnino G, Bonura C, Mammina C, Lunetta M, Novo S, Giarratano A (2013) High dose of trimethoprim-sulfamethoxazole and daptomycin as a therapeutic option for MRSA endocarditis with large vegetation complicated by embolic stroke: a case report and literature review. Infez Med 21:45–49

Dilworth TJ, Ibrahim O, Hall P, Sliwinski J, Walraven C, Mercier RC (2014) beta-Lactams enhance vancomycin activity against methicillin-resistant *Staphylococcus aureus* bacteremia compared to vancomycin alone. Antimicrob Agents Chemother 58:102–109

Dohmen PM, Guleri A, Capone A, Utili R, Seaton RA, Gonzalez-Ramallo VJ, Pathan R, Heep M, Chaves RL (2013) Daptomycin for the treatment of infective endocarditis: results from a European registry. J Antimicrob Chemother 68:936–942

Duval X, Delahaye F, Alla F, Tattevin P, Obadia JF, Le Moing V, Doco-Lecompte T, Celard M, Poyart C, Strady C, Chirouze C, Bes M, Cambau E, Iung B, Selton-Suty C, Hoen B (2012) Temporal trends in infective endocarditis in the context of prophylaxis guideline modifications: three successive population-based surveys. J Am Coll Cardiol 59:1968–1976

El Atrouni WI, Knoll BM, Lahr BD, Eckel-Passow JE, Sia IG, Baddour LM (2009) Temporal trends in the incidence of *Staphylococcus aureus* bacteremia in Olmsted County, Minnesota, 1998 to 2005: a population-based study. Clin Infect Dis 49:e130–e138

El-Ahdab F, Benjamin DK Jr, Wang A, Cabell CH, Chu VH, Stryjewski ME, Corey GR, Sexton DJ, Reller LB, Fowler VG Jr (2005) Risk of endocarditis among patients with prosthetic valves and *Staphylococcus aureus* bacteremia. Am J Med 118:225–229

Falcone M, Russo A, Pompeo ME, Vena A, Marruncheddu L, Ciccaglioni A, Grossi P, Mancini C, Novelli A, Stefani S, Venditti M (2012) Retrospective case-control analysis of patients with staphylococcal infections receiving daptomycin or glycopeptide therapy. Int J Antimicrob Agents 39:64–68

Fang G, Keys TF, Gentry LO, Harris AA, Rivera N, Getz K, Fuchs PC, Gustafson M, Wong ES, Goetz A, Wagener MM, Yu VL (1993) Prosthetic valve endocarditis resulting from nosocomial bacteremia. A prospective, multicenter study. Ann Intern Med 119:560–567

Fayyaz I, Rasheed MA, Ashraf M, Bukhsh A, Wadood A (2014) Determination of bacterial etiological agents, sensitivity pattern and clinical outcome of patients with bacterial endocarditis at Punjab Institute of Cardiology, Lahore. J Pak Med Assoc 64:1384–1388

Fedeli U, Schievano E, Buonfrate D, Pellizzer G, Spolaore P (2011) Increasing incidence and mortality of infective endocarditis: a population-based study through a record-linkage system. BMC Infect Dis 11:48

Federspiel JJ, Stearns SC, Peppercorn AF, Chu VH, Fowler VG Jr (2012) Increasing US rates of endocarditis with *Staphylococcus aureus*: 1999–2008. Arch Intern Med 172:363–365

Fernandez Guerrero ML, Gonzalez Lopez JJ, Goyenechea A, Fraile J, De Gorgolas M (2009) Endocarditis caused by *Staphylococcus aureus*: A reappraisal of the epidemiologic, clinical, and pathologic manifestations with analysis of factors determining outcome. Medicine (Baltimore) 88:1–22

Fitzgerald JR, Loughman A, Keane F, Brennan M, Knobel M, Higgins J, Visai L, Speziale P, Cox D, Foster TJ (2006) Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcgammaRIIa receptor. Mol Microbiol 59:212–230

Forsblom E, Ruotsalainen E, Ollgren J, Jarvinen A (2013) Telephone consultation cannot replace bedside infectious disease consultation in the management of *Staphylococcus aureus* Bacteremia. Clin Infect Dis 56:527–535

Forsblom E, Ruotsalainen E, Jarvinen A (2015) Improved Outcome with Early Rifampicin Combination Treatment in Methicillin-Sensitive *Staphylococcus aureus* Bacteraemia with a Deep Infection Focus - A Retrospective Cohort Study. PLoS ONE 10:e0122824

Fortun J, Perez-Molina JA, Anon MT, Martinez-Beltran J, Loza E, Guerrero A (1995) Right-sided endocarditis caused by *Staphylococcus aureus* in drug abusers. Antimicrob Agents Chemother 39:525–528

Fortun J, Navas E, Martinez-Beltran J, Perez-Molina J, Martin-Davila P, Guerrero A, Moreno S (2001) Short-course therapy for right-side endocarditis due to *Staphylococcus aureus* in drug abusers: cloxacillin versus glycopeptides in combination with gentamicin. Clin Infect Dis 33:120–125

Fowler VG Jr, Li J, Corey GR, Boley J, Marr KA, Gopal AK, Kong LK, Gottlieb G, Donovan CL, Sexton DJ, Ryan T (1997) Role of echocardiography in evaluation of patients with *Staphylococcus aureus* bacteremia: experience in 103 patients. J Am Coll Cardiol 30:1072–1078

Fowler VG Jr, Sanders LL, Sexton DJ, Kong L, Marr KA, Gopal AK, Gottlieb G, Mcclelland RS, Corey GR (1998) Outcome of *Staphylococcus aureus* bacteremia according to compliance with recommendations of infectious diseases specialists: experience with 244 patients. Clin Infect Dis 27:478–486

Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, Cheng AC, Dudley T, Oddone EZ (2003) Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med 163:2066–2072

Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, Pappas PA, Anstrom KJ, Wray D, Fortes CQ, Anguera I, Athan E, Jones P, Van Der Meer JT, Elliott TS, Levine DP, Bayer AS (2005) Staphylococcus aureus endocarditis: a consequence of medical progress. JAMA 293:3012–3021

Fowler VG Jr, Boucher HW, Corey GR, Abrutyn E, Karchmer AW, Rupp ME, Levine DP, Chambers HF, Tally FP, Vigliani GA, Cabell CH, Link AS, Demeyer I, Filler SG, Zervos M, Cook P, Parsonnet J, Bernstein JM, Price CS, Forrest GN, Fatkenheuer G, Gareca M, Rehm SJ, Brodt HR, Tice A, Cosgrove SE (2006) Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. N Engl J Med 355:653–665

Fowler VG Jr, Nelson CL, Mcintyre LM, Kreiswirth BN, Monk A, Archer GL, Federspiel J, Naidich S, Remortel B, Rude T, Brown P, Reller LB, Corey GR, Gill SR (2007) Potential associations between hematogenous complications and bacterial genotype in *Staphylococcus aureus* infection. J Infect Dis 196:738–747

Friedman ND, Kaye KS, Stout JE, Mcgarry SA, Trivette SL, Briggs JP, Lamm W, Clark C, Macfarquhar J, Walton AL, Reller LB, Sexton DJ (2002) Health care–associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 137:791–797

Fries BL, Licitra C, Crespo A, Akhter K, Busowski MT, Salazar D, Wallace MR (2014) Infectious diseases consultation and the management of *Staphylococcus aureus* bacteremia. Clin Infect Dis 58:598–599

Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT (1997) Epidemiology of *Staphylococcus aureus* bacteremia in Denmark from 1957 to 1990. Clin Microbiol Infect 3:297–305

Gasch O, Camoez M, Dominguez MA, Padilla B, Pintado V, Almirante B, Martin C, Lopez-Medrano F, De Gopegui ER, Blanco JR, Garcia-Pardo G, Calbo E, Montero M, Granados A, Jover A, Duenas C, Pujol M (2014) Emergence of resistance to daptomycin in a cohort of patients with methicillin-resistant *Staphylococcus aureus* persistent bacteraemia treated with daptomycin. J Antimicrob Chemother 69:568–571

Gould FK, Denning DW, Elliott TS, Foweraker J, Perry JD, Prendergast BD, Sandoe JA, Spry MJ, Watkin RW, Working Party of the British Society for Antimicrobial (2012) Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 67:269–289

Gould FK, Brindle R, Chadwick PR, Fraise AP, Hill S, Nathwani D, Ridgway GL, Spry MJ, Warren RE (2009) Guidelines (2008) for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United Kingdom. J Antimicrob Chemother 63:849–861

Graham PL 3rd, Lin SX, Larson EL (2006) A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med 144:318–325

Grover FL, Cohen DJ, Oprian C, Henderson WG, Sethi G, Hammermeister KE (1994) Determinants of the occurrence of and survival from prosthetic valve endocarditis. Experience of the Veterans Affairs Cooperative Study on Valvular Heart Disease. J Thorac Cardiovasc Surg 108:207–214

Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336:924–926

Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I, Moreillon P, De Jesus Antunes M, Thilen U, Lekakis J, Lengyel M, Muller L, Naber CK, Nihoyannopoulos P, Moritz A, Zamorano JL (2009) Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J 30:2369–2413

Heriot G, Yeoh J, Street A, Ratnam I (2015) Echocardiography has minimal yield and may not be warranted in *Staphylococcus aureus* bacteremia without clinical risk factors for endocarditis. Eur J Clin Microbiol Infect Dis 34:1231–1236

Hewagama S, Spelman T, Einsiedel LJ (2012) *Staphylococcus aureus* bacteraemia at Alice Springs Hospital, Central Australia, 2003-2006. Intern Med J 42:505–512

Hidayat LK, Hsu DI, Quist R, Shriner KA, Wong-Beringer A (2006) High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. Arch Intern Med 166:2138–2144

Hidron AI, Kourbatova EV, Halvosa JS, Terrell BJ, Mcdougal LK, Tenover FC, Blumberg HM, King MD (2005) Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. Clin Infect Dis 41:159–166

Hill PC, Birch M, Chambers S, Drinkovic D, Ellis-Pegler RB, Everts R, Murdoch D, Pottumarthy S, Roberts SA, Swager C, Taylor SL, Thomas MG, Wong CG, Morris AJ (2001) Prospective study of 424 cases of *Staphylococcus aureus* bacteraemia: determination of factors affecting incidence and mortality. Intern Med J 31:97–103

Holland TL, Arnold C, Fowler VG Jr (2014) Clinical management of *Staphylococcus aureus* bacteremia: a review. JAMA 312:1330–1341

Honda H, Krauss MJ, Jones JC, Olsen MA, Warren DK (2010) The value of infectious diseases consultation in *Staphylococcus aureus* bacteremia. Am J Med 123:631–637

Honda H, Doern CD, Michael-Dunne W Jr, Warren DK (2011) The impact of vancomycin susceptibility on treatment outcomes among patients with methicillin resistant *Staphylococcus aureus* bacteremia. BMC Infect Dis 11:335

Isobe M, Uejima E, Seki M, Yamagishi Y, Miyawaki K, Yabuno K, Masaoka M, Hamaguchi S, Yoshioka N, Tomono K (2012) Methicillin-resistant *Staphylococcus aureus* bacteremia at a university hospital in Japan. J Infect Chemother 18:841–847

Ivert TS, Dismukes WE, Cobbs CG, Blackstone EH, Kirklin JW, Bergdahl LA (1984) Prosthetic valve endocarditis. Circulation 69:223–232

Jacqueline C, Caillon J, Le Mabecque V, Miegeville AF, Donnio PY, Bugnon D, Potel G (2003) In vitro activity of linezolid alone and in combination with gentamicin, vancomycin or rifampicin against methicillin-resistant *Staphylococcus aureus* by time-kill curve methods. J Antimicrob Chemother 51:857–864

Jarlier V, Trystram D, Brun-Buisson C, Fournier S, Carbonne A, Marty L, Andremont A, Arlet G, Buu-Hoi A, Carlet J, Decre D, Gottot S, Gutmann L, Joly-Guillou ML, Legrand P, Nicolas-Chanoine MH, Soussy CJ, Wolf M, Lucet JC, Aggoune M, Brucker G, Regnier B (2010) Curbing methicillin-resistant *Staphylococcus aureus* in 38 French hospitals through a 15-year institutional control program. Arch Intern Med 170:552–559

Jenkins TC, Price CS, Sabel AL, Mehler PS, Burman WJ (2008) Impact of routine infectious diseases service consultation on the evaluation, management, and outcomes of *Staphylococcus aureus* bacteremia. Clin Infect Dis 46:1000–1008

Jensen AG, Espersen F, Skinhoj P, Frimodt-Moller N (1998) Bacteremic *Staphylococcus aureus* spondylitis. Arch Intern Med 158:509–517

Jensen AG, Wachmann CH, Poulsen KB, Espersen F, Scheibel J, Skinhoj P, Frimodt-Moller N (1999) Risk factors for hospital-acquired *Staphylococcus aureus* bacteremia. Arch Intern Med 159:1437–1444

Jeremiah CJ, Wills C, Bayly A, Perry GJ, Davis JS, Tong SY, Currie BJ (2014) Vancomycin dosing nomogram for haemodialysis patients. Nephrology (Carlton) 19:513–514

Jernigan JA, Farr BM (1993) Short-course therapy of catheter-related *Staphylococcus aureus* bacteremia: a meta-analysis. Ann Intern Med 119:304–311

John MD, Hibberd PL, Karchmer AW, Sleeper LA, Calderwood SB (1998) *Staphylococcus aureus* prosthetic valve endocarditis: optimal management and risk factors for death. Clin Infect Dis 26:1302–1309

Johnson AP, Davies J, Guy R, Abernethy J, Sheridan E, Pearson A, Duckworth G (2012) Mandatory surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in England: the first 10 years. J Antimicrob Chemother 67:802–809

Joseph JP, Meddows TR, Webster DP, Newton JD, Myerson SG, Prendergast B, Scarborough M, Herring N (2013) Prioritizing echocardiography in *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother 68:444–449

Kaasch AJ, Fowler VG Jr, Rieg S, Peyerl-Hoffmann G, Birkholz H, Hellmich M, Kern WV, Seifert H (2011) Use of a simple criteria set for guiding echocardiography in nosocomial *Staphylococcus aureus* bacteremia. Clin Infect Dis 53:1–9

Kaasch AJ, Barlow G, Edgeworth JD, Fowler VG Jr, Hellmich M, Hopkins S, Kern WV, Llewelyn MJ, Rieg S, Rodriguez-Bano J, Scarborough M, Seifert H, Soriano A, Tilley R, Torok ME, Weiss V, Wilson AP, Thwaites GE (2014) *Staphylococcus aureus* bloodstream infection: a pooled analysis of five prospective, observational studies. J Infect 68:242–251

Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gershman K, Ray SM, Harrison LH, Lynfield R, Dumyati G, Townes JM, Schaffner W, Patel PR, Fridkin SK (2010) Health care-associated invasive MRSA infections, 2005–2008. JAMA 304:641–648

Kanafani ZA, Mahfouz TH, Kanj SS (2002) Infective endocarditis at a tertiary care centre in Lebanon: predominance of streptococcal infection. J Infect 45:152–159

Kang DH, Kim YJ, Kim SH, Sun BJ, Kim DH, Yun SC, Song JM, Choo SJ, Chung CH, Song JK, Lee JW, Sohn DW (2012) Early surgery versus conventional treatment for infective endocarditis. N Engl J Med 366:2466–2473

Karchmer AW, Archer GL, Dismukes WE (1983a) Rifampin treatment of prosthetic valve endocarditis due to Staphylococcus epidermidis. Rev Infect Dis 5(Suppl 3):S543–S548

Karchmer AW, Archer GL, Dismukes WE (1983b) Staphylococcus epidermidis causing prosthetic valve endocarditis: microbiologic and clinical observations as guides to therapy. Ann Intern Med 98:447–455

Khatib R, Sharma M (2013) Echocardiography is dispensable in uncomplicated *Staphylococcus aureus* bacteremia. Medicine (Baltimore) 92:182–188

Khatib R, Johnson LB, Fakih MG, Riederer K, Khosrovaneh A, Shamse Tabriz M, Sharma M, Saeed S (2006) Persistence in *Staphylococcus aureus* bacteremia: incidence, characteristics of patients and outcome. Scand J Infect Dis 38:7–14

Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, Mcdougal LK, Carey RB, Fridkin SK (2007) Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298:1763–1771

Kluytmans JA, Mouton JW, Vandenbergh MF, Manders MJ, Maat AP, Wagenvoort JH, Michel MF, Verbrugh HA (1996) Reduction of surgical-site infections in cardiothoracic surgery by elimination of nasal carriage of *Staphylococcus aureus*. Infect Control Hosp Epidemiol 17:780–785

Kullar R, Davis SL, Levine DP, Zhao JJ, Crank CW, Segreti J, Sakoulas G, Cosgrove SE, Rybak MJ (2011) High-dose daptomycin for treatment of complicated gram-positive infections: a large, multicenter, retrospective study. Pharmacotherapy 31:527–536

Kullar R, Casapao AM, Davis SL, Levine DP, Zhao JJ, Crank CW, Segreti J, Sakoulas G, Cosgrove SE, Rybak MJ (2013) A multicentre evaluation of the effectiveness and safety of high-dose daptomycin for the treatment of infective endocarditis. J Antimicrob Chemother 68:2921–2926

Lahey T, Shah R, Gittzus J, Schwartzman J, Kirkland K (2009) Infectious diseases consultation lowers mortality from *Staphylococcus aureus* bacteremia. Medicine (Baltimore) 88:263–267

Lalani T, Cabell CH, Benjamin DK, Lasca O, Naber C, Fowler VG Jr, Corey GR, Chu VH, Fenely M, Pachirat O, Tan RS, Watkin R, Ionac A, Moreno A, Mestres CA, Casabe J, Chipigina N, Eisen DP, Spelman D, Delahaye F, Peterson G, Olaison L, Wang A (2010) Analysis of the impact of early surgery on in-hospital mortality of native valve endocarditis: use of propensity score and instrumental variable methods to adjust for treatment-selection bias. Circulation 121:1005–1013

Lalani T, Chu VH, Park LP, Cecchi E, Corey GR, Durante-Mangoni E, Fowler VG Jr, Gordon D, Grossi P, Hannan M, Hoen B, Munoz P, Rizk H, Kanj SS, Selton-Suty C, Sexton DJ, Spelman D, Ravasio V, Tripodi MF, Wang A (2013) In-hospital and 1-year mortality in patients undergoing early surgery for prosthetic valve endocarditis. JAMA Intern Med 173:1495–1504

Landrum ML, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, Murray CK (2012) Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005–2010. JAMA 308:50–59

Larsen MV, Harboe ZB, Ladelund S, Skov R, Gerstoft J, Pedersen C, Larsen CS, Obel N, Kronborg G, Benfield T (2012) Major but differential decline in the incidence of *Staphylococcus aureus* bacteraemia in HIV-infected individuals from 1995 to 2007: a nationwide cohort study*. HIV Med 13:45–53

Laupland KB, Ross T, Gregson DB (2008) *Staphylococcus aureus* bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000–2006. J Infect Dis 198:336–343

Laupland KB, Lyytikainen O, Sogaard M, Kennedy KJ, Knudsen JD, Ostergaard C, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schonheyder HC (2013) The changing epidemiology of *Staphylococcus aureus* bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471

Lee T, Barker J, Allon M (2005) Tunneled catheters in hemodialysis patients: reasons and subsequent outcomes. Am J Kidney Dis 46:501–508

Lewis T, Chaudhry R, Nightingale P, Lambert P, Das I (2011) Methicillin-resistant *Staphylococcus aureus* bacteremia: epidemiology, outcome, and laboratory characteristics in a tertiary referral center in the UK. Int J Infect Dis 15:e131–e135

Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, Rybak MJ, Talan DA, Chambers HF (2011) Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin Infect Dis 52:e18–e55

Lodise TP Jr, Mckinnon PS, Levine DP, Rybak MJ (2007) Impact of empirical-therapy selection on outcomes of intravenous drug users with infective endocarditis caused by methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother 51:3731–3733

Markowitz N, Quinn EL, Saravolatz LD (1992) Trimethoprim-sulfamethoxazole compared with vancomycin for the treatment of *Staphylococcus aureus* infection. Ann Intern Med 117:390–398

Mcconeghy KW, Bleasdale SC, Rodvold KA (2013) The empirical combination of vancomycin and a beta-lactam for Staphylococcal bacteremia. Clin Infect Dis 57:1760–1765

Mejer N, Westh H, Schonheyder HC, Jensen AG, Larsen AR, Skov R, Benfield T (2012) Stable incidence and continued improvement in short term mortality of *Staphylococcus aureus* bacteraemia between 1995 and 2008. BMC Infect Dis 12:260

Menichetti F, Martino P, Bucaneve G, Gentile G, D'antonio D, Liso V, Ricci P, Nosari AM, Buelli M, Carotenuto M, et al (1994) Effects of teicoplanin and those of vancomycin in initial empirical antibiotic regimen for febrile, neutropenic patients with hematologic malignancies. Gimema Infection Program. Antimicrob Agents Chemother 38:2041–2046

Miller M, Cespedes C, Bhat M, Vavagiakis P, Klein RS, Lowy FD (2007) Incidence and persistence of *Staphylococcus aureus* nasal colonization in a community sample of HIV-infected and -uninfected drug users. Clin Infect Dis 45:343–346

Mirabel M, Andre R, Barsoum Mikhail P, Colboc H, Lacassin F, Noel B, Robert J, Nadra M, Braunstein C, Gervolino S, Marijon E, Iung B, Jouven X (2015) Infective endocarditis in the Pacific: clinical characteristics, treatment and long-term outcomes. Open Heart 2:e000183

Miro JM, Entenza JM, Del Rio A, Velasco M, Castaneda X, Garcia De La Maria C, Giddey M, Armero Y, Pericas JM, Cervera C, Mestres CA, Almela M, Falces C, Marco F, Moreillon P, Moreno A (2012) High-dose daptomycin plus fosfomycin is safe and effective in treating methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* endocarditis. Antimicrob Agents Chemother 56:4511–4515

Mitchell DH, Howden BP (2005) Diagnosis and management of *Staphylococcus aureus* bacteraemia. Intern Med J 35(Suppl 2):S17–S24

Moise PA, Amodio-Groton M, Rashid M, Lamp KC, Hoffman-Roberts HL, Sakoulas G, Yoon MJ, Schweitzer S, Rastogi A (2013) Multicenter evaluation of the clinical outcomes of daptomycin with and without concomitant beta-lactams in patients with *Staphylococcus aureus* bacteremia and mild to moderate renal impairment. Antimicrob Agents Chemother 57:1192–1200

Moore CL, Osaki-Kiyan P, Haque NZ, Perri MB, Donabedian S, Zervos MJ (2012) Daptomycin versus vancomycin for bloodstream infections due to methicillin-resistant *Staphylococcus aureus* with a high vancomycin minimum inhibitory concentration: a case-control study. Clin Infect Dis 54:51–58

Mulazimoglu L, Drenning SD, Yu VL (1996) In vitro activities of two novel oxazolidinones (U100592 and U100766), a new fluoroquinolone (trovafloxacin), and dalfopristin-quinupristin against *Staphylococcus aureus* and Staphylococcus epidermidis. Antimicrob Agents Chemother 40:2428–2430

Murdoch DR, Corey GR, Hoen B, Miro JM, Fowler VG Jr, Bayer AS, Karchmer AW, Olaison L, Pappas PA, Moreillon P, Chambers ST, Chu VH, Falco V, Holland DJ, Jones P, Klein JL, Raymond NJ, Read KM, Tripodi MF, Utili R, Wang A, Woods CW, Cabell CH (2009) Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. Arch Intern Med 169:463–473

Murray KP, Zhao JJ, Davis SL, Kullar R, Kaye KS, Lephart P, Rybak MJ (2013) Early use of daptomycin versus vancomycin for methicillin-resistant *Staphylococcus aureus* bacteremia with vancomycin minimum inhibitory concentration >1 mg/L: a matched cohort study. Clin Infect Dis 56:1562–1569

Naber CK, Baddour LM, Giamarellos-Bourboulis EJ, Gould IM, Herrmann M, Hoen B, Karchmer AW, Kobayashi Y, Kozlov RS, Lew D, Miro JM, Moellering RC Jr, Moreillon P, Peters G, Rubinstein E, Seifert H, Corey GR (2009) Clinical consensus conference: survey on Gram-positive bloodstream infections with a focus on *Staphylococcus aureus*. Clin Infect Dis 48(Suppl 4):S260–S270

Nadji G, Remadi JP, Coviaux F, Mirode AA, Brahim A, Enriquez-Sarano M, Tribouilloy C (2005) Comparison of clinical and morphological characteristics of *Staphylococcus aureus* endocarditis with endocarditis caused by other pathogens. Heart 91:932–937

Nagao M, Iinuma Y, Saito T, Matsumura Y, Shirano M, Matsushima A, Takakura S, Ito Y, Ichiyama S (2010) Close cooperation between infectious disease physicians and attending physicians can result in better management and outcome for patients with *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 16:1783–1788

Nguyen DB, Lessa FC, Belflower R, Mu Y, Wise M, Nadle J, Bamberg WM, Petit S, Ray SM, Harrison LH, Lynfield R, Dumyati G, Thompson J, Schaffner W, Patel PR (2013) Invasive methicillin-resistant *Staphylococcus aureus* infections among patients on chronic dialysis in the United States, 2005–2011. Clin Infect Dis 57:1393–1400

Nickerson EK, Hongsuwan M, Limmathurotsakul D, Wuthiekanun V, Shah KR, Srisomang P, Mahavanakul W, Wacharaprechasgul T, Fowler VG, West TE, Teerawatanasuk N, Becher H, White NJ, Chierakul W, Day NP, Peacock SJ (2009) *Staphylococcus aureus* bacteraemia in a tropical setting: patient outcome and impact of antibiotic resistance. PLoS ONE 4:e4308

Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthon S, Park L, Rude TH, Barriere S, Woods CW, Chu VH, Marin M, Bukovski S, Garcia P, Corey GR, Korman T, Doco-Lecompte T, Murdoch DR, Reller LB, Fowler VG Jr (2011) Methicillin-susceptible *Staphylococcus aureus* endocarditis isolates are associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and adhesins. J Infect Dis 204:704–713

Paladino JA, Jacobs DM, Shields RK, Taylor J, Bader J, Adelman MH, Wilton GJ, Crane JK, Schentag JJ (2014) Use of ceftaroline after glycopeptide failure to eradicate meticillin-resistant *Staphylococcus aureus* bacteraemia with elevated vancomycin minimum inhibitory concentrations. Int J Antimicrob Agents 44:557–563

Palraj BR, Baddour LM, Hess EP, Steckelberg JM, Wilson WR, Lahr BD, Sohail MR (2015) Predicting Risk of Endocarditis Using a Clinical Tool (PREDICT): scoring system to guide use of echocardiography in the management of *Staphylococcus aureus* bacteremia. Clin Infect Dis

Park HJ, Kim SH, Kim MJ, Lee YM, Park SY, Moon SM, Park KH, Chong YP, Lee SO, Choi SH, Woo JH, Kim YS (2012a) Efficacy of linezolid-based salvage therapy compared with glycopeptide-based therapy in patients with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. J Infect 65:505–512

Park KH, Kim ES, Kim HS, Park SJ, Bang KM, Park HJ, Park SY, Moon SM, Chong YP, Kim SH, Lee SO, Choi SH, Jeong JY, Kim MN, Woo JH, Kim YS (2012b) Comparison of the clinical features, bacterial genotypes and outcomes of patients with bacteraemia due to heteroresistant vancomycin-intermediate *Staphylococcus aureus* and vancomycin-susceptible S. aureus. J Antimicrob Chemother 67:1843–1849

Pastagia M, Kleinman LC, Lacerda De La Cruz EG, Jenkins SG (2012) Predicting risk for death from MRSA bacteremia. Emerg Infect Dis 18:1072–1080

Patel R (2005) Biofilms and antimicrobial resistance. Clin Orthop Relat Res 437:41–47

Patti JM, Allen BL, Mcgavin MJ, Hook M (1994) MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585–617

Perlroth J, Kuo M, Tan J, Bayer AS, Miller LG (2008) Adjunctive use of rifampin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. Arch Intern Med 168:805–819

Petersen PJ, Labthavikul P, Jones CH, Bradford PA (2006) In vitro antibacterial activities of tigecycline in combination with other antimicrobial agents determined by chequerboard and time-kill kinetic analysis. J Antimicrob Chemother 57:573–576

Piroth L, Que YA, Widmer E, Panchaud A, Piu S, Entenza JM, Moreillon P (2008) The fibrinogen- and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein a synergistically promote endothelial invasion and experimental endocarditis. Infect Immun 76:3824–3831

Popovich KJ, Hota B, Aroutcheva A, Kurien L, Patel J, Lyles-Banks R, Grasso AE, Spec A, Beavis KG, Hayden MK, Weinstein RA (2013) Community-associated methicillin-resistant *Staphylococcus aureus* colonization burden in HIV-infected patients. Clin Infect Dis 56:1067–1074

Proctor RA, Kahl B, Von Eiff C, Vaudaux PE, Lew DP, Peters G (1998) Staphylococcal small colony variants have novel mechanisms for antibiotic resistance. Clin Infect Dis 27(Suppl 1): S68–S74

Que YA, Moreillon P (2011) Infective endocarditis. Nat Rev Cardiol 8:322–336

Raad I, Darouiche R, Vazquez J, Lentnek A, Hachem R, Hanna H, Goldstein B, Henkel T, Seltzer E (2005) Efficacy and safety of weekly dalbavancin therapy for catheter-related bloodstream infection caused by gram-positive pathogens. Clin Infect Dis 40:374–380

Rasmussen RV, Host U, Arpi M, Hassager C, Johansen HK, Korup E, Schonheyder HC, Berning J, Gill S, Rosenvinge FS, Fowler VG Jr, Moller JE, Skov RL, Larsen CT, Hansen TF, Mard S, Smit J, Andersen PS, Bruun NE (2011) Prevalence of infective endocarditis in patients with *Staphylococcus aureus* bacteraemia: the value of screening with echocardiography. Eur J Echocardiogr 12:414–420

Rehm SJ, Boucher H, Levine D, Campion M, Eisenstein BI, Vigliani GA, Corey GR, Abrutyn E (2008) Daptomycin versus vancomycin plus gentamicin for treatment of bacteraemia and endocarditis due to *Staphylococcus aureus*: subset analysis of patients infected with methicillin-resistant isolates. J Antimicrob Chemother 62:1413–1421

Ribera E, Gomez-Jimenez J, Cortes E, Del Valle O, Planes A, Gonzalez-Alujas T, Almirante B, Ocana I, Pahissa A (1996) Effectiveness of cloxacillin with and without gentamicin in short-term therapy for right-sided *Staphylococcus aureus* endocarditis. A randomized, controlled trial. Ann Intern Med 125:969–974

Riedel DJ, Weekes E, Forrest GN (2008) Addition of rifampin to standard therapy for treatment of native valve infective endocarditis caused by *Staphylococcus aureus*. Antimicrob Agents Chemother 52:2463–2467

Robinson JO, Pozzi-Langhi S, Phillips M, Pearson JC, Christiansen KJ, Coombs GW, Murray RJ (2012) Formal infectious diseases consultation is associated with decreased mortality in *Staphylococcus aureus* bacteraemia. Eur J Clin Microbiol Infect Dis 31:2421–2428

Roder BL, Wandall DA, Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT (1999) Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. Arch Intern Med 159:462–469

Rogers BA, Drake AK, Spelman D (2009) Methicillin resistant *Staphylococcus aureus* endocarditis in an Australian tertiary hospital: 1991–2006. Heart Lung Circ 18:208–213

Rose WE, Berti AD, Hatch JB, Maki DG (2013) Relationship of in vitro synergy and treatment outcome with daptomycin plus rifampin in patients with invasive methicillin-resistant *Staphylococcus aureus* infections. Antimicrob Agents Chemother 57:3450–3452

Rossi M, Gallo A, De Silva RJ, Sayeed R (2012) What is the optimal timing for surgery in infective endocarditis with cerebrovascular complications? Interact CardioVasc Thorac Surg 14:72–80

Ruotsalainen E, Sammalkorpi K, Laine J, Huotari K, Sarna S, Valtonen V, Jarvinen A (2006) Clinical manifestations and outcome in *Staphylococcus aureus* endocarditis among injection drug users and nonaddicts: a prospective study of 74 patients. BMC Infect Dis 6:137

Rybak M, Lomaestro B, Rotschafer JC, Moellering R Jr, Craig W, Billeter M, Dalovisio JR, Levine DP (2009) Therapeutic monitoring of vancomycin in adult patients: a consensus review

of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Am J Health Syst Pharm 66:82–98
Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM (2004) Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol 42:2398–2402
Salgado-Pabon W, Breshears L, Spaulding AR, Merriman JA, Stach CS, Horswill AR, Peterson ML, Schlievert PM (2013) Superantigens are critical for *Staphylococcus aureus* Infective endocarditis, sepsis, and acute kidney injury. MBio 4
Schweizer ML, Furuno JP, Harris AD, Johnson JK, Shardell MD, Mcgregor JC, Thom KA, Cosgrove SE, Sakoulas G, Perencevich EN (2011) Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible *Staphylococcus aureus* bacteremia. BMC Infect Dis 11:279
Selton-Suty C, Celard M, Le Moing V, Doco-Lecompte T, Chirouze C, Iung B, Strady C, Revest M, Vandenesch F, Bouvet A, Delahaye F, Alla F, Duval X, Hoen B (2012) Preeminence of *Staphylococcus aureus* in infective endocarditis: a 1-year population-based survey. Clin Infect Dis 54:1230–1239
Seybold U, Kourbatova EV, Johnson JG, Halvosa SJ, Wang YF, King MD, Ray SM, Blumberg HM (2006) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. Clin Infect Dis 42:647–656
Sharma M, Riederer K, Chase P, Khatib R (2008) High rate of decreasing daptomycin susceptibility during the treatment of persistent *Staphylococcus aureus* bacteremia. Eur J Clin Microbiol Infect Dis 27:433–437
Shorr AF, Kunkel MJ, Kollef M (2005) Linezolid versus vancomycin for *Staphylococcus aureus* bacteraemia: pooled analysis of randomized studies. J Antimicrob Chemother 56:923–929
Skinner DKC (1941) Significance of bacteremia caused by *Staphylococcus aureus*: A study of one hundred and twenty-two cases and a review of the literature concerned with experimental infection in animals. Arch Intern Med 68:851–875
Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, Alamo D, Ortega M, Lopez J, Mensa J (2008) Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 46:193–200
Spink Ww HW (1945) Penicillin therapy at the University of Minnesota hospitals: 1942–1944. Ann Intern Med 22:510–525
Steinberg JP, Clark CC, Hackman BO (1996) Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin Infect Dis 23:255–259
Stone SP, Fuller C, Savage J, Cookson B, Hayward A, Cooper B, Duckworth G, Michie S, Murray M, Jeanes A, Roberts J, Teare L, Charlett A (2012) Evaluation of the national Cleanyourhands campaign to reduce *Staphylococcus aureus* bacteraemia and Clostridium difficile infection in hospitals in England and Wales by improved hand hygiene: four year, prospective, ecological, interrupted time series study. BMJ 344:e3005
Stryjewski ME, Szczech LA, Benjamin DK Jr, Inrig JK, Kanafani ZA, Engemann JJ, Chu VH, Joyce MJ, Reller LB, Corey GR, Fowler VG Jr (2007) Use of vancomycin or first-generation cephalosporins for the treatment of hemodialysis-dependent patients with methicillin-susceptible *Staphylococcus aureus* bacteremia. Clin Infect Dis 44:190–196
Stryjewski ME, Lentnek A, O'riordan W, Pullman J, Tambyah PA, Miro JM, Fowler VG, Jr Barriere SL, Kitt MM, Corey GR (2014) A randomized Phase 2 trial of telavancin versus standard therapy in patients with uncomplicated *Staphylococcus aureus* bacteremia: the ASSURE study. BMC Infect Dis 14:289
Sullenberger AL, Avedissian LS, Kent SM (2005) Importance of transesophageal echocardiography in the evaluation of *Staphylococcus aureus* bacteremia. J Heart Valve Dis 14:23–28
Sy RW, Kritharides L (2010) Health care exposure and age in infective endocarditis: results of a contemporary population-based profile of 1536 patients in Australia. Eur Heart J 31:1890–1897

Tattevin P, Schwartz BS, Graber CJ, Volinski J, Bhukhen A, Bhukhen A, Mai TT, Vo NH, Dang DN, Phan TH, Basuino L, Perdreau-Remington F, Chambers HF, Diep BA (2012) Concurrent epidemics of skin and soft tissue infection and bloodstream infection due to community-associated methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 55:781–788

Thwaites GE, Edgeworth JD, Gkrania-Klotsas E, Kirby A, Tilley R, Torok ME, Walker S, Wertheim HF, Wilson P, Llewelyn MJ (2011) Clinical management of *Staphylococcus aureus* bacteraemia. Lancet Infect Dis 11:208–222

Thwaites G, Auckland C, Barlow G, Cunningham R, Davies G, Edgeworth J, Greig J, Hopkins S, Jeyaratnam D, Jenkins N, Llewelyn M, Meisner S, Nsutebu E, Planche T, Read RC, Scarborough M, Soares M, Tilley R, Torok ME, Williams J, Wilson P, Wyllie S, Walker AS (2012) Adjunctive rifampicin to reduce early mortality from *Staphylococcus aureus* bacteraemia (ARREST): study protocol for a randomised controlled trial. Trials 13:241

Tissot F, Calandra T, Prod'hom G, Taffe P, Zanetti G, Greub G, Senn L (2014) Mandatory infectious diseases consultation for MRSA bacteremia is associated with reduced mortality. J Infect 69:226–234

Tleyjeh IM, Abdel-Latif A, Rahbi H, Scott CG, Bailey KR, Steckelberg JM, Wilson WR, Baddour LM (2007) A systematic review of population-based studies of infective endocarditis. Chest 132:1025–1035

Tong SY, Bishop EJ, Lilliebridge RA, Cheng AC, Spasova-Penkova Z, Holt DC, Giffard PM, Mcdonald MI, Currie BJ, Boutlis CS (2009) Community-associated strains of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus* in indigenous Northern Australia: epidemiology and outcomes. J Infect Dis 199:1461–1470

Tong SY, Van Hal SJ, Einsiedel L, Currie BJ, Turnidge JD (2012) Impact of ethnicity and socio-economic status on *Staphylococcus aureus* bacteremia incidence and mortality: a heavy burden in Indigenous Australians. BMC Infect Dis 12:249

Torres-Tortosa M, De Cueto M, Vergara A, Sanchez-Porto A, Perez-Guzman E, Gonzalez-Serrano M, Canueto J (1994) Prospective evaluation of a two-week course of intravenous antibiotics in intravenous drug addicts with infective endocarditis. Grupo de Estudio de Enfermedades Infecciosas de la Provincia de Cadiz. Eur J Clin Microbiol Infect Dis 13:559–564

Trabelsi I, Rekik S, Znazen A, Maaloul I, Abid D, Maalej A, Kharrat I, Ben Jemaa M, Hammemi A, Kammoun S (2008) Native valve infective endocarditis in a tertiary care center in a developing country (Tunisia). Am J Cardiol 102:1247–1251

Tsuji BT, Rybak MJ (2006) Etest synergy testing of clinical isolates of *Staphylococcus aureus* demonstrating heterogeneous resistance to vancomycin. Diagn Microbiol Infect Dis 54:73–77

Turnidge JD, Kotsanas D, Munckhof W, Roberts S, Bennett CM, Nimmo GR, Coombs GW, Murray RJ, Howden B, Johnson PD, Dowling K (2009) *Staphylococcus aureus* bacteraemia: a major cause of mortality in Australia and New Zealand. Med J Aust 191:368–373

Van Hal SJ, Mathur G, Kelly J, Aronis C, Cranney GB, Jones PD (2005) The role of transthoracic echocardiography in excluding left sided infective endocarditis in *Staphylococcus aureus* bacteraemia. J Infect 51:218–221

Van Hal SJ, Jones M, Gosbell IB, Paterson DL (2011) Vancomycin heteroresistance is associated with reduced mortality in ST239 methicillin-resistant *Staphylococcus aureus* blood stream infections. PLoS ONE 6:e21217

Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB (2012) Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clin Microbiol Rev 25:362–386

Vanholder R, Ringoir S, Dhondt A, Hakim R (1991) Phagocytosis in uremic and hemodialysis patients: a prospective and cross sectional study. Kidney Int 39:320–327

Von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med 344:11–16

Wang A, Athan E, Pappas PA, Fowler VG Jr, Olaison L, Pare C, Almirante B, Munoz P, Rizzi M, Naber C, Logar M, Tattevin P, Iarussi DL, Selton-Suty C, Jones SB, Casabe J, Morris A, Corey GR, Cabell CH (2007) Contemporary clinical profile and outcome of prosthetic valve endocarditis. JAMA 297:1354–1361

Wertheim HF, Vos MC, Ott A, Van Belkum A, Voss A, Kluytmans JA, Van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA (2004) Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364:703–705

Wertheim HF, Melles DC, Vos MC, Van Leeuwen W, Van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762

Wilcox MH, Tack KJ, Bouza E, Herr DL, Ruf BR, Ijzerman MM, Croos-Dabrera RV, Kunkel MJ, Knirsch C (2009) Complicated skin and skin-structure infections and catheter-related bloodstream infections: noninferiority of linezolid in a phase 3 study. Clin Infect Dis 48:203–212

Wolff M, Witchitz S, Chastang C, Regnier B, Vachon F (1995) Prosthetic valve endocarditis in the ICU. Prognostic factors of overall survival in a series of 122 cases and consequences for treatment decision. Chest 108:688–694

Wyllie DH, Peto TE, Crook D (2005) MRSA bacteraemia in patients on arrival in hospital: a cohort study in Oxfordshire 1997–2003. BMJ 331:992

Zimakoff J, Bangsgaard Pedersen F, Bergen L, Baago-Nielsen J, Daldorph B, Espersen F, Gahrn Hansen B, Hoiby N, Jepsen OB, Joffe P, Kolmos HJ, Klausen M, Kristoffersen K, Ladefoged J, Olesen-Larsen S, Rosdahl VT, Scheibel J, Storm B, Tofte-Jensen P (1996) *Staphylococcus aureus* carriage and infections among patients in four haemo- and peritoneal-dialysis centres in Denmark. The Danish Study Group of Peritonitis in Dialysis (DASPID). J Hosp Infect 33:289–300

Amphixenosic Aspects of *Staphylococcus aureus* Infection in Man and Animals

Giacomo Rossi, Matteo Cerquetella and Anna Rita Attili

Abstract According to the mode of transmission, *Staphylococcus aureus* infection between hosts is classified as "*direct zoonoses,*" or infection that is transmitted from an infected vertebrate host to a susceptible host (man) by direct contact, by contact with a fomite or by a mechanical vector. The agent itself undergoes little or no propagative or developmental changes during transmission. According to the reservoir host, staphylococcosis is most precisely defined as "zooanthroponoses" or infections transmitted from man to lower vertebrate animals (e.g., streptococci, diphtheria, Enterobacteriaceae, human tuberculosis in cattle and parrots), but also "anthropozoonoses" or infections transmitted to man from lower vertebrate animals. In particular, actually, the correct definition of *S. aureus* infections between humans and animals is "amphixenoses" or infections maintained in both man and lower vertebrate animals and transmitted in either direction. *S. aureus* exhibits tropisms to many distinct animal hosts. While spillover events can occur wherever there is an interface between host species, changes in host tropism only occur with the establishment of sustained transmission in the new host species, leading to clonal expansion. Although the genomic variation underpinning adaptation in *S. aureus* genotypes infecting bovids and poultry has been well characterized, the frequency of switches from one host to another remains obscure. In this review, we sought to identify the sustained switches in host tropism in the *S. aureus* population, both anthroponotic and zoonotic, and their distribution over the species phylogeny. *S. aureus* is an organism with the capacity to switch into and adapt to novel hosts, even after long periods of isolation in a single host species. Based on this evidence, animal-adapted *S. aureus* lineages exhibiting resistance to antibiotics must be considered a major threat to public health, as they can adapt to the human population.

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1 Introduction

The word "zoonoses" was introduced by Rudolf Virchow in 1880 to include collectively the diseases shared in nature by man and animals. Later, WHO in 1959 defined that zoonoses are "*those diseases and infections which are naturally transmitted between vertebrate animals and man.*" Zoonoses include only those infections where there is either a proof or a strong circumstantial evidence for transmission between animals and man. Historically, zoonotic diseases had a tremendous impact on the evolution of man, especially those cultures and societies that domesticated and bred animals for food and clothing (Joint WHO/FAO Expert Committee 1967). Zoonoses are among the most frequent and dreaded risks to which mankind is exposed. Zoonoses occur throughout the world transcending the natural boundaries. Their important effect on global economy and health is well known, extending from the international movement of animals and importation of diseases to bans on importation of all animal products and restrictions on other international trade practices (Pal 1997). So, zoonoses no longer are solely a national problem. For effective control of zoonoses, global surveillance is necessary.

Over the last two decades, there has been considerable change in the importance of certain zoonotic diseases in many parts of the world, resulting from ecological changes such as urbanization, industrialization, and diminishing proportion of persons working in the so-called primary sector. We do not know with what challenge nature will confront us in the world of constant interference with ecology. Most of the infections of man that have been discovered in the last twenty years are shared with lower animals, and a number of other diseases previously thought to be limited to man have now been found to be zoonoses. Reference may be made to various types of encephalitis, eosinophilic meningitis, capillariasis, anisakiasis, Lyme disease, Monkeypox diseases in humans, Lassa fever, Marburg disease, and Ebola for all of which an animal link has been established. Among those zoonoses recognized today as particularly important are anthrax, plague, brucellosis, bovine tuberculosis, leptospirosis, salmonellosis, spotted fever caused by rickettsiae,

rabies, several common arthropod borne viral infections (arboviral infection), certain parasitic diseases, especially cysticercosis, hydatid disease, trypanosomiasis, and toxoplasmosis (WHO 1979, 1982). According to the etiological agents, bacterial zoonoses are actually the most numerous diseases transmitted from animals to humans. Among these, *Staphylococcus aureus* infections are considered as relevant on the basis of their diffusion. *S. aureus* is a commensal and pathogen of both humans and many animal species (Enright et al. 2002; Sung et al. 2008; Sakwinska et al. 2011). Distinct lineages have been identified within the global population of *S. aureus* that associate closely with specific hosts (Smyth et al. 2009; Sung et al. 2008; Wiles et al. 2006; Weinert et al. 2012). While the mechanism of adaptation to host environments is not fully understood, it has been shown that such host tropisms are associated with adaptive evolution, in particular at immunologically relevant genes such as those encoding proteins determining virulence and cell adhesion (Smyth et al. 2009; Guinane et al. 2010; Viana et al. 2010; Price et al. 2012). However, adaptation to one host species does not prevent occasional infection of other species (Wiles et al. 2006; Rasigade et al. 2010; Armand-Lefevre et al. 2005; van Loo et al. 2007; Wolfe et al. 2007; Lloyd-Smith et al. 2009). The majority of cases where genotypes have been isolated outside of their typical host species probably represent spillover events, transient infections from one host species to another which do not last long, and die out without establishing transmission within the new host population (van Loo et al. 2007; Wolfe et al. 2007; Didelot et al. 2010). These are distinct from rarer interspecies transmission events that lead to sustained transmission and establishment within the new host species (Lowder et al. 2009; Weinert et al. 2012). According to the mode of transmission, *S. aureus* infections between hosts are classified as "*direct zoonoses,*" or infections that are transmitted from an infected vertebrate host to a susceptible host (man) by direct contact, by contact with a fomite or by a mechanical vector. The agent itself undergoes little or no propagative or developmental changes during transmission (as observed for anthrax, brucellosis, leptospirosis, or toxoplasmosis). According to the reservoir host, staphylococcosis is most precisely defined as "anthropozoonoses", or infections transmitted to man from lower vertebrate animals (e.g., rabies, leptospirosis, plague, arboviral infections, brucellosis, and Q fever) but also "zooanthroponoses" or infections transmitted from man to lower vertebrate animals (e.g., streptococci, staphylococci, diphtheria, Enterobacteriaceae, and human tuberculosis in cattle and parrots).

In particular, actually, the correct definition of *S. aureus* infections between man and animals is "amphixenoses" or infections maintained in both man and lower vertebrate animals and transmitted in either direction (e.g., salmonellosis, *E. coli,* and other bacterial infections) (Mitchell 1998).

As asymptomatic carriers serve as reservoirs for amphixenoses, and asymptomatic food-producing animal carriers and their meat may also represent potential reservoirs for human infections, *S. aureus* infections pose a significant health burden (Fig. 1).

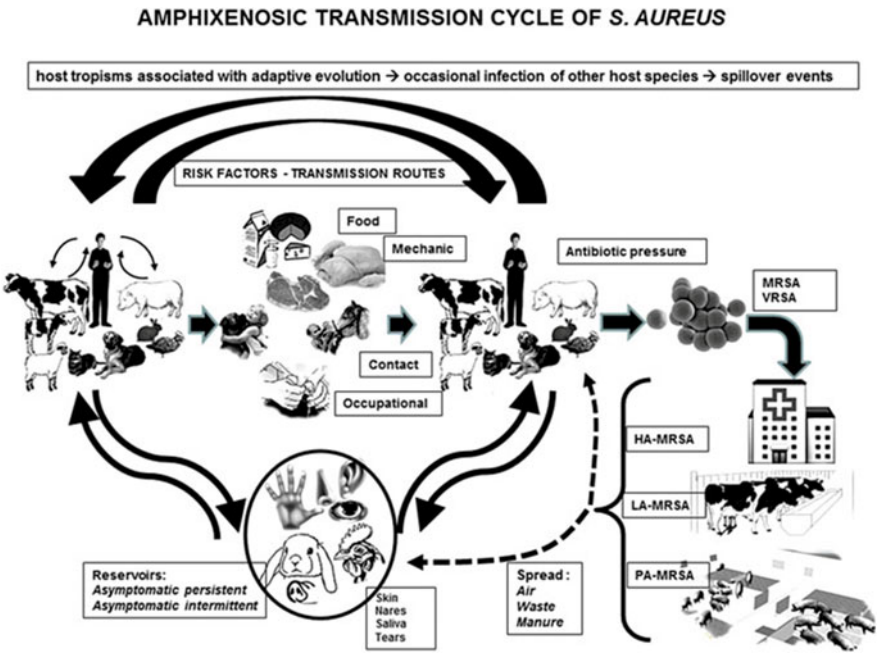

Fig. 1 Schematic representation of amphixenosic transmission cycle of *S. aureus*; this term indicates infections between man and animals or infections maintained in both man and lower vertebrate animals and transmitted in either direction. *Legend MRSA* methicillin-resistant *S. aureus*, *VRSA* vancomycin-resistant *S. aureus*, *HA-MRSA* healthcare-associated methicillin-resistant *S. aureus*, *LA-MRSA* livestock-associated methicillin-resistant *S. aureus*, *PA-MRSA* pig-associated methicillin-resistant *S. aureus*

2 Factors Influencing Prevalence of Staphylococcal Amphixenoses and Related Risks

Handling animal by-products and wastes (occupational hazards) is the most important factors that influencing amphixenoses, as staphylococcosis (Fig. 1). There is significantly higher exposition rates in workers during the course of their occupation than the rest of the population, e.g., listeriosis in agricultural workers, erysipeloid in butchers and fish merchants, tularemia and trypanosomiasis in hunters, and creeping eruptions in plumbers and trench diggers. Other examples of zoonoses as occupational hazards are Q fever in abattoir and rendering plant workers, salmonellosis in food processors, bovine tuberculosis in farmers, etc. (Lai et al. 2015; Podolak et al. 2010; Ameni et al. 2001).

Zoonotic transfer of bacterial pathogens, either through contact or the food chain, represents a serious threat to public health. In particular, potentially zoonotic pathogens that display resistance to antimicrobials used in humans, such as *S. aureus* and *Escherichia coli,* are a matter of serious concern (de Boer et al. 2009;

Leverstein-van Hall et al. 2011; García-Álvarez et al. 2011; Sung and Lindsay 2007).

S. aureus is a bacterium which belongs to the commensal flora of humans and various animal species (Vanderhaeghen et al. 2010b). Human population can represent a persistent or intermittent carrier. Multiple body sites can be colonized, but the anterior nares are the most frequent ones (Wertheim et al. 2005). Moreover, the skin is often contaminated in nasal carriers, and transmission occurs principally by means of hands (Fig. 1). As *S. aureus* is also an important pathogen in the food industry, carriers can contaminate the food and *S. aureus*, in opportune environmental conditions, can produce toxins resulting in human intoxication (Lowy 1998). Since its discovery during the 1880s, *S. aureus* has emerged as a potentially pathogenic bacterium that can cause a broad spectrum of diseases, ranging from minor infections of the skin to postoperative wound infections, life-threatening bacteremia, infections associated with foreign bodies, and necrotizing pneumonia (Lowy 1998; Deurenberg et al. 2007; Kluytmans and Struelens 2009).

Approximately 20 % of healthy human individuals is persistent *S. aureus* carrier, about 30 % is intermittent carrier, and around 50 % is never colonized with *S. aureus* (Kluytmans and Struelens 2009).

S. aureus exhibits tropisms to many distinct animal hosts. In animals, *S. aureus* resulted one of the three major pathogenic *Staphylococcus* species, together with *S. hyicus* and *S. intermedius* group—SIG (*S. pseudintermedius*, *S. intermedius*, and *S. delphini*). *S. aureus* can cause intramammary infections (Fig. 2), especially in dairy-producing animals, including cattle and small ruminants (Goni et al. 2004; Vanderhaeghen et al. 2010b), mastitis in rabbit (Goni et al. 2004), "bumblefoot" and joint problems in chickens (Fig. 3) (McNamee and Smyth 2000; Butterworth et al. 2001), and being identified as a pathogen of farmed rabbits (Viana et al. 2007). It is increasingly reported in surgical site infections in small companion animals and horses (Catry et al. 2010). In rabbits, *S. aureus* can infect small dermal lesions and invade subcutaneous tissue generating pododermatitis, subcutaneous abscesses, and

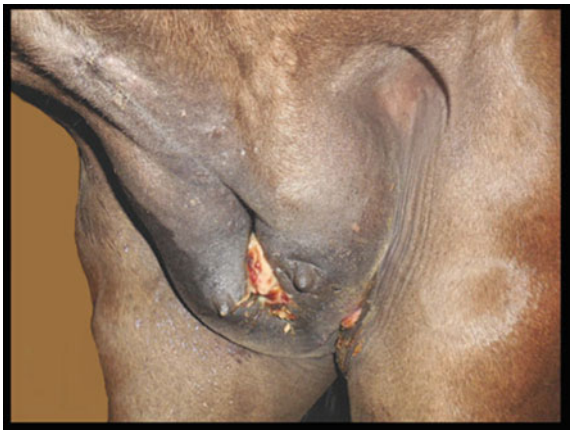

Fig. 2 *S. aureus* can cause intramammary infections in different species of animals. In this picture note a typical *S. aureus* associated suppurative mastitis in a mare (courtesy of Prof. Giuseppe Catone)

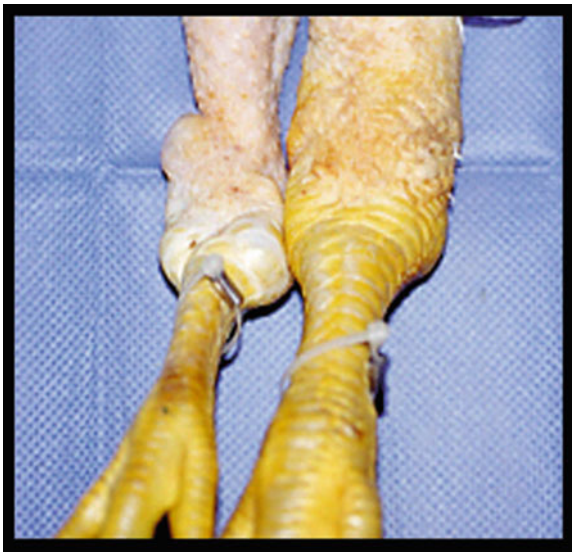

Fig. 3 *S. aureus* generally cause "*bumblefoot*" and joint problems in chickens. In this photograph, a characteristic bilateral arthrosynovitis in a broiler is shown. Note the alterations of joint profiles and a severe enlargement of the left tarso-metatarsus also involved

mastitis (Fig. 4). Sporadically, internal organ abscesses are observed, predominantly in lungs, liver, and uterus leading to poor production results, infertility and death (Hagen 1963; Okerman et al. 1984; Carolan 1986; Holliman and Girvan 1986; Rossi et al. 1995; Devriese et al. 1987).

S. aureus is the most common bacterial species isolated from conjunctival microbial flora in donkeys and sheep (Foti et al. 2013; Bonelli et al. 2014). On a total of 46 healthy donkeys, of the 52 *Staphylococcus* spp. isolates, 14 (26.9 %)

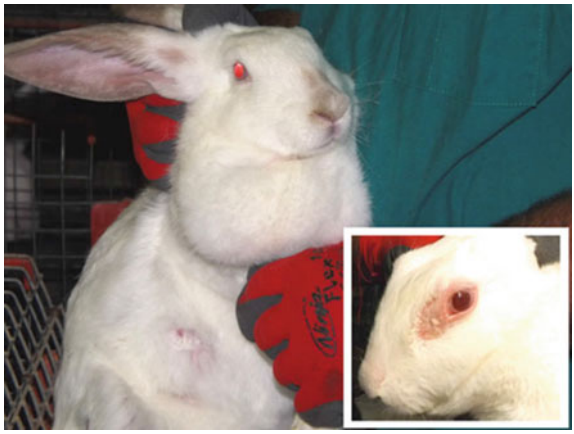

Fig. 4 In rabbits, *S. aureus* can infect small dermal lesions and invade subcutaneous tissue generating pododermatitis, and large subcutaneous abscesses as evidenced in the throat region of the rabbit in the photograph. *S. aureus* is the most common bacterial species isolated from conjunctival microbial flora. In some cases, rabbits showed nasolacrimal infections resulting in purulent discharge (see *insert*). Nasolacrimal conduct and nares represent the most important sites of *S. aureus* colonization also in asymptomatic persistent or intermittent reservoirs

strains were oxacillin/methicillin resistant and the mecA gene was detected in 6/52 (11.5 %) strains (Foti et al. 2013).

Most reports characterizing animal-associated *S. aureus* have demonstrated that strains affecting animals are distinct from those infecting humans, suggesting that there are host-specific lineages which only rarely cross species boundaries (Shepheard et al. 2013). Nonetheless, it is documented the zoonotic transfer of bacterial pathogens, either through contact or the food chain, representing a serious threat to public health (Smith 2015).

While spillover events can occur wherever there is an interface between host species, changes in host tropism only occur with the establishment of sustained transmission in the new host species, leading to clonal expansion. Although the genomic variation underpinning adaptation in *S. aureus* genotypes infecting bovids and poultry has been well characterized, the frequency of switches from one host to another remains obscure. *S. aureus* can switch into and adapt to novel hosts, even after long periods of isolation in a single host species. It can also adapt rapidly to the selective pressure of antibiotics, resulting in the emergence and spread of antibiotic-resistant *S. aureus* that is a growing public health concern too. It has been shown that one lineage of bovine staphylococci is hypersusceptible to the acquisition of vancomycin resistance from enterococci, posing the risk by vancomycin-resistant *S. aureus* (VRSA) for interspecies transmission (Sung and Lindsay 2007).

Antimicrobial resistance can generate during animal husbandry, because of the use of antibiotics as feed additives for growth promotion in industrial livestock and poultry (Silbergeld et al. 2008), for prevention of disease within a herd, or for the treatment of an existing disease outbreak. As agricultural-use antibiotics include many classes that are relevant for human health: tetracyclines, macrolides, penicillin, and sulfonamides, the antimicrobial resistant *S. aureus* may be spread to the general human population, into communities and the environment in different manners: contact with contaminated meat products (via handling or ingestion); occupational contact (farmers, meat packers, butchers, etc.) and potential secondary spread into the larger community from those who are occupationally exposed; entry into and transmission via hospitals or other health care facilities; or spread via environmental routes including air, water, or manure in areas in proximity to live animal farms or crop farms where manure has been used as a fertilizer (Smith 2015).

Until the introduction of penicillin, the mortality rate of patients infected with *S. aureus* was about 80 % (Deurenberg et al. 2007). In the early 1940s, *S. aureus* infections were treated with penicillin, but the first strains resistant to this antibiotic were isolated in 1942, first in hospitals, and later in the community. This resistance resulted from the acquisition of a plasmid that encoded a penicillin-hydrolyzing enzyme, i.e., penicillinase. In the early 1960s, the adaptive power of *S. aureus* to antibiotics leaded to the emergence of methicillin-resistant *S. aureus* (MRSA) by the acquisition of the *mecA* gene and, during the 1970s, MRSA emerged as a

therapeutic problem in humans to become, by the 1990s, a serious nosocomial infection worldwide. MRSA probably originated through the transfer of SCC*mec* into a limited number of methicillin-sensitive *S. aureus* (MSSA) lineages. In addition, *S. aureus* strains have developed resistance to vancomycin through the acquisition of *vanA* gene (Deurenberg et al. 2007; Lowy 2003). The cause of resistance to methicillin and all other beta-lactam antibiotics is the *mecA* gene, which is situated on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Seven major variants of SCC*mec*, type I to VII, have been distinguished.

The epidemiology of MRSA is changing. Numerous MRSA clones have emerged and disseminated worldwide. The prevalence of MRSA ranges from 0.6 % in the Netherlands to 66.8 % in Japan (Smith 2015). The early MRSA clones were hospital-associated (HA-MRSA), and from the late 1990s, community-associated MRSA (CA-MRSA) clones emerged worldwide becoming important public health problem because they have been associated with high morbidity and mortality in the community (Okesola 2011). CA-MRSA harbors SCC*mec* type IV, V, or VII, the majority belong to other *S. aureus* lineages compared to HA-MRSA, and CA-MRSA was often associated with the presence of the toxin Panton–Valentine leukocidin (PVL). However, during recent years, the distinction between HA/CA-MRSA has started to disappear, and CA-MRSA is now endemic in many hospitals (Deurenberg et al. 2007; Deurenberg and Stobberingh 2008).

3 The Role of Companion Animals in the Amphixenosic Transmission of *S. aureus*

Generally, companion animal strains of MRSA differ from those in livestock and meat production animals, probably because in companion animals, MRSA acquisition is primarily a humanosis, the strains carried by human owners being passed on to their animals. Traditional animal husbandry involved far less close contact between animals than today's intensively farmed livestock intended for human consumption.

Also, the potential involvement of domestic pets, and horses, in the dissemination of MRSA to men and vice versa has been investigated and is considered possible (Duquette and Nuttal 2004; Vitale et al. 2006; Gosbell 2011; Petinaki and Spiliopoulou 2012). Many studies have been performed in these directions with interesting results, assuming that humans are an important source for dogs (Heller et al. 2011), as also recognizing the role of animals as sources of recolonization or reinfection (Weese et al. 2006; Bender and Minicucci 2007; Loeffler and Lloyd 2010). It is very interesting to notice that, frequently, MRSA isolated in veterinary patients are those typically found in nosocomial infections in human facilities as reported in Germany (e.g., lineages CC22 and CC5) (Vincze et al. 2014). Again in

Germany, 16 MRSA isolates obtained from infected dogs and cats, hospitalized in the same veterinary facility, resulted closely related among them and to the ST22 MRSA reference strain, that is, frequently associated to nosocomial infections in men in Germany (Strommenger et al. 2006). In the Midwestern and Northeastern USA, a study performed on 533 coagulase-positive staphylococci isolated from different animals (dogs, cats, horses, pigs, hamster, rabbit, mink, and rat) treated in different veterinary facilities, showed the presence of *S. aureus*, inter alia, in 32 out of 487 dogs (6.6 %), 19 out of 48 cats (39.6 %), and 10 out of 12 horses (83.3 %), and of these, 12 (of 32: 37 %), 6 (of 19: 32 %), and 5 (of 10: 50 %) were MRSA, respectively. Remarkably, four MRSA clones included all the 24 MRSA isolates [USA100 (12 isolates—10 dogs and 2 cats), USA300 (4 isolates—2 cats, 1 dog and 1 pig), USA800 (3 isolates—2 cats and 1 dog), and USA500 (5 isolates—all from horses)], and all of them are either CA- or HA-MRSA strains (Yihan et al. 2011). Indistinguishable isolates of USA300 MRSA were also found in the USA in swabs from the anterior nares of a cat and of its owner, but it was not possible to determine who represented the source (Vitale et al. 2006). An interesting study performed on 99 pets (47 dogs and 52 cats) coming from 66 households in which MRSA patients with active or recent infection were present and showed that 11 (11.5 %) animals were MRSA positive, even in the absence of MRSA-related signs of infection, allowing to consider a possible role for these animals in harboring the pathogen; however, the carrier state has been hypothesized to be transitory. The study also underlined that in 6 out of 9 households with pets related positivity, the MRSA strains were genetically related to the human's one of the same household; in general, the USA 100 strain most frequently isolated from pets and the USA 300 from men (Morris et al. 2012). USA100 strain was also isolated in 2 resident cats of a long-term care facility, in which human MRSA cases were present (Coughlan et al. 2010). USA100 was also the main isolate (32/37: 86.5 %) in a study performed on a large cohort of dogs (435 of which 25 resulted MRSA positive); the other isolates that were identified were USA500 (1/37: 2.7 %), and USA800 (2/37: 5.4 %). With regard to the clinical condition of positive dogs, 4 were healthy, 20 were ill independently from MRSA, and 1 presented a deep pyoderma associated with MSRA (Hoet et al. 2013) (Fig. 5). In 2006, a case study of Weese et al. (2006) suggested concomitant colonization/infection, with Canadian epidemic MRSA-2 (CMRSA) indistinguishable isolates (within individual cases) of dogs and cats and their owners or personnel of the veterinary facilities receiving these animals. CMRSA-2 (similar to USA100) and CMRSA-5 (similar to USA500) were also found in persons attending a veterinary conference in USA in 2005. More precisely, of 27 isolates (6.5 % considering 417 subjects), CMRSA-2 was found in 13 persons, of which 11 coming from small animal practice and 2 from large animal (except 1 coming from Germany, they were all coming from USA), as CMRSA-5 that was also found in 13 persons, all working in large animal practice (coming 10 from USA, 2 from UK, and 1 from Denmark) (Hanselman et al. 2006). In another conference in the USA (veterinary surgeons), 59 MRSA isolations resulted from 341 participants sampled; 32 isolates (23, i.e., 72 % working with small animals; 6, i.e., 19 % with horses; 3, i.e., 9.4 % with large animals) were associated with

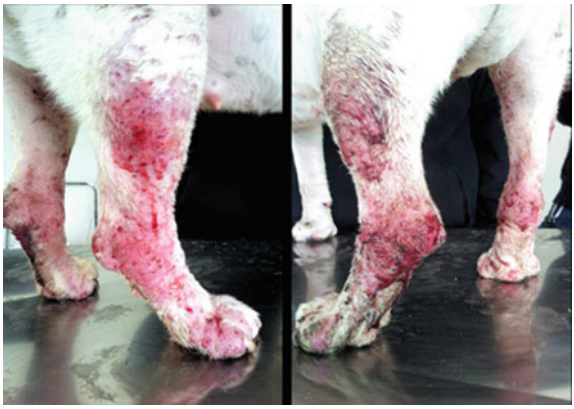

Fig. 5 Dog with severe deep pyoderma associated with MSRA (courtesy of Prof. Andrea Spaterna)

CMRSA-2 and 16 isolates (9, i.e., 56 % were working with horses; 4, i.e., 25 % with small animal; 2, i.e., 13 % with large animal; 1, i.e., 6.3 % coming from mixed animal practice) were associated with CMRSA-5, while the only one positive for CMRSA-10/USA300 was working with horses (Burstiner et al. 2010). In Canada, on a total of 122 household, 535 subjects (242 humans, 132 dogs, and 161 cats) were sampled, and *S. aureus* was isolated from 67 humans, 19 dogs, and 7 cats, while MRSA colonization was found in 8 humans and 2 dogs. Interestingly, *S. aureus* was indistinguishable in 4 out of 8 households with isolates coming from humans and dogs, and in 1 out of 2 households with isolates coming from humans and cats (Hanselman et al. 2009). A study performed in the UK on dogs, cats, and horses found that 3 clinically ill dogs (out of 55 dogs' samples) were carriers of MRSA and that a student and 2 staff members of the University of Liverpool's Small Animal Hospital were harboring the same strain, moreover matching to the human epidemic MRSA-15 (EMRSA) (Baptiste et al. 2005). MRSA isolates indistinguishable from EMRSA-15 were found also in another study in the UK in 56 % of isolates (15 out of 27 isolates from veterinary staff, dogs, and environmental surfaces). More generally, in the same study, 14 out of 78 staff members (17.9 %), 4 out of 45 dogs (8.9 %), 3 out of 30 (10 %) environmental surfaces, and 0 out of 12 cats resulted positive for MRSA (Loeffler et al. 2005). With regard to the epidemic clone EMRSA-15, a further study made in the UK and in Ireland on dogs (n.27), cats (n.6), horses (n.9), human (n.22), and environmental (n.3) isolates demonstrated that in all cats, in 96 % of dogs and in 82 % of men, the isolates were indistinguishable or closely related to EMRSA-15. With regard to horses, only one presented an isolate related to EMRSA-15, while the others were related to CC8 (Moodley et al. 2006). EMRSA-15 was also isolated in 7 out of 724 (1 %) dogs in a further study in the UK (Wedley et al. 2014). In Ireland, in a small animal practice, 5 dogs and 1 veterinary surgeon resulted positive for an MRSA similar to the one most commonly isolated in human in Ireland and were indistinguishable from EMRSA-15 (Leonard et al. 2006). Likewise, EMRSA-15 were found both on 2 out of 64 staff members (3.1 %) and on 3 samples from environmental sites (in two

cases, the sample was from the same site, a corridor door handle, repeated after 14 days) in a study performed in Scotland (University of Glasgow Small Animal Hospital) (Heller et al. 2009).

In a study, Wettstein Rosenkranz et al. (2014) found MRSA isolates (n = 14) in 3.8 % (95 % CI 2.1-6.3 %) of the 340 veterinarians (including general practitioners, small animal practitioners, large animal practitioners, and veterinarians working in different veterinary services or industry) and 29 veterinary assistants screened.

Large animal practitioners were carriers of livestock-associated MRSA (LA-MRSA) ST398-t011-V (n = 2), ST398-t011-IV (n = 4), and ST398-t034-V (n = 1). On the other hand, participants working with small animals harbored human healthcare-associated MRSA (HCA-MRSA) which belonged to epidemic lineages ST225-t003-II (n = 2), ST225-t014-II (n = 1), ST5-t002-II (n = 2), ST5-t283-IV (n = 1), and ST88-t186-IV (n = 1). HCA-MRSA harbored virulence factors such as enterotoxins, β-hemolysin converting phage and leukocidins, while none of the MRSA isolates carried PVL (Wettstein Rosenkranz et al. 2014). In the same study, in addition to the methicillin-resistant gene mecA, LA-MRSA ST398 isolates generally contained additional antibiotic resistance genes conferring resistance to tetracycline [tet(M) and tet(K)], trimethoprim [dfrK, dfrG], and the aminoglycosides gentamicin and kanamycin [aac(6′)-Ie-aph(2′)-Ia]. On the other hand, HCA-MRSA ST5 and ST225 mainly contained genes conferring resistance to the macrolide, lincosamide, and streptogramin B antibiotics [erm(A)], to spectinomycin [ant(9)-Ia], amikacin, and tobramycin [ant(4′)-Ia], and to fluoroquinolones [amino acid substitutions in GrlA (S84L) and GyrA (S80F and S81P)] (Wettstein Rosenkranz et al. 2014).

Garcia-Graells et al. (2012) highlighted that LA-MRSA was significantly associated with veterinarians in contact with livestock (P = 0.046) examining the prevalence and risk factors associated with LA-MRSA carriage in Danish and Belgian veterinarians. The MRSA and LA-MRSA carriage rates were 9.5 % (95 % CI 5.3-15.6) and 7.5 % (95 % CI 3.8-13.1) for MRSA and LA-MRSA, respectively, in Belgium and 1.4 % (95 % CI: 0.17-5.05) in Denmark. Moreover, all LA-MRSA isolates were resistant to tetracycline and 53.4 % (7/13) showed a multi-resistant phenotype (Garcia-Graells et al. 2012).

Another study executed in Ireland on different animal species (dogs, horses, a cat, a rabbit, and a seal), showed, in some cases, similar isolates within these animals and veterinary personnel of respective practices. Furthermore, the prevalent MRSA pattern found in a high percentage of sampled dogs and in a rabbit was indistinguishable from the pattern of the most commonly isolated MRSA in human patients; on the contrary, in the same study, isolates from horses were different from all formerly reported Irish human isolates (O'Mahony et al. 2005).Resistance to antibiotics such as methicillin or aminoglycoside-modifying enzymes (AGMEs) production in *S. aureus* isolated from mastitis in rabbit could indicate possible human origin of the strains or a possible source of resistant strains for human beings (Goni et al. 2004). Returning to the UK, 27 out of 220 (12.3 %) veterinary staff being in contact with MRSA infected pets (dogs and cats) and 9 out of 120 (7.5 %) owners of infected animals, resulted carriers of MRSA, and interestingly, almost all

(149 out of 150) the identified MRSA isolates from both pets and human were CC22 or CC30 (typical hospital-associated strains) (Loeffler et al. 2010). A recent study performed in France on a 5-year period reported a positivity for MRSA in 16 and 7 ill dogs and cats, respectively. Interestingly, 20 out of 23 isolates (87.0 %) were human-related clones (noteworthy one was a USA300) and the remaining three were CC398 (Haenni et al. 2012). In Sweden, together with 7 dogs diagnosed with MRSA in 3 different small animal hospital, 20 out of 152 staff members were found positive for MRSA, almost all with indistinguishable isolates (the remaining 2 isolates were, however, closely related) (Andersson et al. 2014). A study performed in 2012 in Egypt, showed that in 70 sampled dogs (either healthy or ill), three positivity from two dogs (one ill, one healthy) resulted (two isolates were found in the ill dog), while none cats (out of 48 sampled) were positive; only 1 out of 28 apparently healthy persons resulted positive and interestingly he was in intimate contact with the healthy dog, and furthermore the isolate were phenotypically similar (Abdel-moein et al. 2012). Two studies performed in Japan investigated the relation between MRSA and veterinary staff both in an academic veterinary hospital and in private veterinary clinics. In the first case, MRSA were isolated from veterinarians, staff members, and students with different percentages in different times; interestingly, two factors were associated with carriage state and precisely the contact with an MRSA animal case and the fact of working in the veterinary hospital instead of being a student (Ishihara et al. 2010). In the second case, 22 out of 96 veterinarians resulted positive as well as 7 out of 70 veterinary technicians, while only 3 (1 dog with hepatic disease and 2 blood donor dogs) out of 292 dogs (1 %) led to positive isolates; all these three dogs were been in clinics in which at least one staff member resulted positive for the same MRSA isolate (Ishihara et al. 2014). The diffusion of MRSA in dogs was also studied in Korea, showing that on 157 hospitalized patients (presenting skin pyoderma, extern otitis, conjunctivitis, and urinary tract infections) 3 were infected by MRSA (2 affected by skin pyoderma and 1 by extern otitis) (Kwon et al. 2006). In support of the fact that animals (in this case dogs and horses) are not always carriers of MRSA, there is a study performed in Slovenia on 300 horses and 200 dogs, all clinically normal, in which no animals resulted positive for MRSA, on samples collected from the nose in both species and from the perineal area/last 5 cm of the anus in dogs (Vengust et al. 2006).

Nevertheless, a study performed on veterinary surgeons/staff with a persistent MRSA nasal colonization showed that they did not represent a consistent risk for patients undergoing orthopedic or spinal surgery (McLean and Ness 2008). While, on 128 small animal dermatologists participating in Italy to a National veterinary conference, 34 positive nasal swabs were found for S. aureus (2 MRSA and 32 methicillin-susceptible *S. aureus*—MSSA) (Paul et al. 2011). Similarly, in Denmark, a study including 702 participants showed that veterinary practitioners were significantly more likely to carry MRSA than professionally unexposed persons and that, in this study, a risk factor was the species with whom they were in touch (yes: small animals, cattle, and horses; no: pigs) (Moodley et al. 2008).

The role of companion animals in the dissemination of *S. aureus* should not be neglected; on the contrary, it could be interesting to further investigate the dissemination of the bacteria in these animals.

4 The Amphixenosic Transmission of *S. aureus*: Human Versus Pet Animals and Vice Versa

Different reports of zoonotic or inverse zoonotic transmission are reported in the literature. As example the case of a man (ill), his wife (ill) and their dog (healthy) in which were isolated indistinguishable MRSA; considering that the dog had never been subjected to antibiotic therapy neither been ill or hospitalized, unlike the owner, the hypothesis is that the dog went in contact with the pathogen from its owner, and then acted as the MRSA source both for him and for his wife (Manian 2003). Two case reports from Germany also refer of the cross-contamination regarding MRSA ST398 and MRSA ST225, and in both cases, the source for the two pets (two dogs) was hypothesized to be the owner. More precisely, in the case involving the MRSA ST398, a strain typically housed by pigs (but also by other animals and human), the owner was a specialist in swine medicine who had access to MRSA ST398-positive breeding, while in the other case, it has to be underlined that MRSA ST225 is very frequent in men in Germany but not in dogs (Nienhoff et al. 2009). MRSA ST398 with similar characteristics were also found in a wound infection of a dog and in the staff of the veterinary facility in which the dog was treated, in Germany (Witte et al. 2007). Another case report is from the Netherlands, where in a woman (a nurse previously identified as MRSA carrier), in her daughter, and in their dog, three indistinguishable MRSA isolates were found; interestingly, the woman, after initial negativities following the treatment, turned positive again, and only after treating the three subjects, and achieving a negativity in all three, they remained negative over time (van Duijkeren et al. 2004). Similarly, always in the Netherlands, a diabetic woman presenting recurrent infections related to MRSA eradicated the MRSA carrier state only after treating, at the same time, the husband, the son, and the dog, all healthy and resulted carriers of the same indistinguishable MRSA isolate (van Duijkeren et al. 2005). Likewise, a nurse, his wife (also a nurse), and their dog were all positive for EMRSA 1, and they had to be all treated to eliminate the carrier state from the dog (Cefai et al. 1991). Even if dogs could carry asymptomatically MRSA when contaminated by their owners, as in the previous cases, there may also be cases in which it does not happen, as in the case reported by Rutland et al. (2009), in which the suggested contamination of a dog from its owner resulted in the euthanasia of the dog. A similar situation has also been reported in the cat, in a case in which a woman continued to be positive for MRSA, even after her husband and her children became negative; one of three family cats also resulted positive for the same MRSA, and only after successfully treating the cat that became negative, the whole family also became negative

(Sing et al. 2008). A MRSA-positive ward cat has also been involved in a MRSA outbreak in a rehabilitation geriatric ward in 1988, where after appropriate control measures (e.g., hygienic and environmental), and the cat's removal, no other cases occurred (Scott et al. 1988). Nevertheless, there are always many difficulties to exactly identify the direction of the transmission between species, even if it is reported in several cases. An interesting study performed between USA and Canada, starting from households in which infected persons were present and other household in which infected dogs or cats were present, showed that in the first case, in the 8 households included, in one case 2 cohabiting dogs were also colonized (in all cases USA 300 strains), while in the second case in 6 out of the 22 included households, one or more colonized persons were present (in the 4 cases in which the strain was identified, it was USA 100 in 3 cases and USA 300 in 1 case) (Faires et al. 2009).

Noteworthy are also studies in which other *S. aureus*, such as enterotoxigenic *S. aureus*, are studied because they also represent a concern for human and animal health. For example, in Egypt, samples from 70 dogs, 47 cats, and 27 men in intimate contact with them (owners or veterinarians) were collected, and results showed that enterotoxigenic *S. aureus* was found in 10 % (7 out of 70), 2.1 % (1 out of 47), and 7.7 % (2 out of 26) of the three species, respectively; also remarkable is that animal isolates were almost all from healthy subjects (Abdel-moein and Samir 2011).

With regard particularly to horses, as partially anticipated and as previously reported in other species, there are evidences of concomitant isolates from human. As example, in 1999 in the USA, 11 horses presented infections resulted positive for MRSA that were closely related to isolates (4 isolates from 3 persons) from members of the veterinary staff; moreover, in this case, it was hypothesized that the source was represented by the staff members (Seguin et al. 1999).

Different sources have been hypothesized for such bacteria, in the case series of Weese et al. (2006a) MRSA were isolated from nasal swabs, postoperative infections, and lower urinary tract infections but, also, in a larger cohort of animals (dogs, cats, and horses) from the same sites and from skin, perianal area, coat, eyes, ears, saliva, synovial fluid, blood, milk, or fomites (Baptiste et al. 2005; Middleton et al. 2005; Snyder et al. 2008; Hoet et al. 2013; Muniz et al. 2013; Vincze et al. 2014). Interestingly, MRSA was also isolated in 3 out of 418 (0.7 %) canine stool samples collected directly on the sidewalks and streets of different areas of Bari, Italy (Cinquepalmi et al. 2013). Furthermore, as previously showed, also environmental sites could represent a source of MRSA as underlined by a recent study, in which it was isolated from 9 out of 101 southern Ontario community veterinary hospitals, by sampling different sites; interestingly, different pathogens were isolated and among these the Canadian epidemic strains MRSa-2 and MRSA-5 (Murphy et al. 2010).

These data suggest that in case of isolation of *S. aureus*, from men or animals, it may be indicated to systematically investigate also cohabiting men or animals.

5 The Epidemiology of Livestock-Associated *S. aureus*: The Role of Bovine Milk and Dairy Cattle

The emergence of antibiotic-resistant *S. aureus* strains in farm animal environments poses a potential public health concern. *S. aureus* is a common cause of mastitis in dairy cows (Trinidad et al. 1990; Turkyilmaz et al. 2010; Vanderhaeghen et al. 2010a; Varshney et al. 2009; Virgin et al. 2009; Waage et al. 1999), a primary reason for antibiotic use on farms. However, the proportion of contagious pathogens (such as *S. aureus* and *Streptococcus agalactiae*) has decreased relative to that of environmental pathogens in the recent past (from 1994 to 2001) (Makovec and Ruegg 2003). The use of antimicrobial agents on dairy farms as well as in other food animal production systems is a major concern in the emergence of resistant zoonotic bacterial pathogens (Piddock 1996) (Fig. 1). Although different antibiotic classes of drugs are used in animal health management and in human medicine, the selection of resistance to one drug class may lead to cross-resistance to another (Piddock 1996). Antibiotics on dairy operations are used to treat highly prevalent infections, such as subclinical mastitis, and as a preventive measure during dry cow therapy. Monitoring the emergence of resistant pathogens in animal reservoirs is important particularly for those with zoonotic potential. MRSA has emerged as a major cause of healthcare-associated (HA) and community-associated (CA) infections (Klein et al. 2007) (Fig. 1). The presence of MRSA in bovine milk and dairy environments poses potential risk to farm workers, veterinarians, and farm animals that are exposed to contaminated cattle. In facts, MRSA carriage may represent an occupational risk and veterinarians should be aware of possible MRSA colonization and potential for developing infection or for transmitting these strains. MRSA transmission between humans and animals, and the spread of MRSA both in the community, and to animal and human hospitals could be. A small number of human MRSA mastitis cases and outbreaks in maternity or neonatal units have been reported which are generally the result of CA-MRSA (Holmes and Zadocks 2011). The establishment of the sequence type 398 (ST398) in farm animals, primarily pigs, in the early 2000s, has provided a reservoir of infection for humans and dairy cattle, particularly in continental Europe, described as LA-MRSA. Livestock-associated *S. aureus* is an emerging category of *S. aureus* throughout the world. Prior to the emergence of ST398, there were sporadic reports of MRSA in bovine milk and cases of mastitis, often caused by strains from human-associated lineages. Subsequently, there have been several reports describing bovine udder infections caused by ST-398 MRSA (Benić et al. 2012) Another group of LA-MRSA strains was discovered in humans and dairy cattle in Europe. This group carries a divergent *mecA* gene and includes a number of *S. aureus* lineages (CC130, ST425, and CC1943) that were hitherto thought to be bovine-specific but are now also found as carriage or clinical isolates in humans. The emergence of MRSA in dairy cattle may be associated with contact with other host species, as in the case of ST398, or with the exchange of genetic material between *S. aureus* and coagulase-negative *Staphylococcus* species, which are the

most common species associated with bovine intramammary infections and commonly carry antimicrobial resistance determinants (Holmes and Zadoks 2011).

In another study carried out in Turkey on bovine milk with mastitis, Turkyilmaz et al. (2010) observed the presence of hospital-related MRSA strains by supposing transmission of these strains between humans and animals. Among 93 *S. aureus* strains, 16 were resistant to methicillin. The MRSA strains were multi-drug resistant. All tetracycline- and gentamicin-resistant strains carried tet(M) and aac(6)-aph (2) gene, respectively, and among macrolide-resistant isolates, nine had erm(A) and seven had both erm(A) and erm(B) genes. The molecular characterization by pulsed-field gel electrophoresis showed the presence of three pulsotypes with their variants. The pulsotype B strains were type IV with SCC*mec* typing, and representative of pulsotype B was t190 by spa typing and ST8 by MLST typing. The strains with pulsotype A and C were SCCmec III, and representative of these pulsotypes was t030 by spa typing. The MLST type of pulsotype A was ST239, and pulsotype C was one allele variant of ST239, while none of the isolates harbored the PVL gene (Turkyilmaz et al. 2010).

Prevalence and molecular characteristics of MRSA in milk of cows with mastitis were analyzed in Brazil (Silva et al. 2014). The four MRSA isolates were typed as t011-ST398-agr1-SCCmecV and presented two different pulsed-field-gel-electrophoresis-ApaI patterns. They showed resistance to tetracycline, streptomycin, and ciprofloxacin, carried the mecA, blaZ, tet(K), and tet(M) resistance genes, and presented the S84L and S80F amino acid substitutions in GyrA and GrlA proteins, respectively. Two ST398 isolates exhibited resistance to gentamicin and tobramycin (with aac(6)-aph(2″) and ant(4)-Ia genes) and one isolate resistance to clindamycin (with lnu(B) and lsa(E) genes); this latter isolate also carried the spectinomycin-/streptomycin-resistant genes spw and aadE. MRSA of lineage ST398 is worldwide spread, normally multi-drug resistant, and may be responsible for bovine mastitis (Silva et al. 2014).

6 Livestock-Associated *S. aureus*: The Role of Swine and Chickens

In the early part of the twenty-first century, a novel pig-associated MRSA clone emerged in pig farms (Armand-Lefevre et al. 2005; Voss et al. 2005) (Fig. 1): sequence type 398 (ST398) and related strains (collectively grouped into clonal complex 398, or CC398 (Fluit 2012). CC398 was first identified in pigs and swine workers but has since been found in other animals (including cattle, poultry, and dogs as well as humans) in a number of countries in Europe (EFSA 2008), Asia, and North and South America (Khanna et al. 2008; Smith et al. 2009), as well as Australia. The analysis of LA-MRSA isolates identified four features that are characteristic for LA-MRSA: (i) They are non-typable by pulsed-field gel electrophoresis (PFGE) using SmaI (Bens et al. 2006); (ii) they belong to clonal

complex (CC) 398 by multi-locus sequence typing (MLST) (Enright et al. 2000); (iii) the staphylococcal chromosomal cassette (SCC)*mec* types IV and V predominate but are structurally different from the corresponding types carried by MRSA genotypes endemic in community and healthcare settings (Li et al. 2011); and (iv) most isolates lacks toxins such as Panton–Valentine leukocidin (PVL) and other enterotoxins (Hallin et al. 2011). LA-MRSA seemed to be less virulent and less transmissible than community- and healthcare-associated MRSA (van Rijen et al. 2008; Wassenberg et al. 2011; Bootsma et al. 2011), although secondary transmission to household members, people, and animals in contact has been reported (Denis et al. 2009). In a Danish case–control study, a relatively high proportion of case patients reported skin and soft tissue infections (10/21), one patient developed sinusitis and another developed bacteremia after knee surgery (Lewis et al. 2008). In Europe, geographical variation in the number of human LA-MRSA cases (both asymptomatic carriage and infections) is directly linked to pig and veal calf densities (van Cleef et al. 2011). In areas where LA-MRSA is endemic in the agricultural setting, the carriage rate in pig farm workers is higher than in any other population, including patients with predisposing risk factors such as exposure to foreign healthcare facilities (van Rijen et al. 2008). In countries with a low incidence of MRSA, this epidemiological change threatens existing control policies developed to prevent spread of MRSA in the community and into hospitals. For example, a Dutch survey conducted during 2002–2006 found that 32 % of patients carried MRSA on admission to a hospital located in an area with a high pig density compared to a nationwide incidence of 0.03 % in 2000 (van Rijen et al. 2008).

In Belgium and Denmark, a study confirmed that veterinarians are at high risk of acquiring LA-MRSA through exposure to pigs, and particularly to live pigs, rather than other animal species. Moreover, the fact that carriage was not dependent on a recent exposure to pigs may suggest that veterinarians could become chronic carriers, and therefore, they could be implicated in the spread of LA-MRSA between farms (Garcia-Graells et al. 2012).

In most European countries, CC398 remains the most commonly identified type of LA-MRSA (Smith and Pearson 2011); however, while CC398 strains have been found in livestock across the globe, the epidemiology of livestock-associated *S. aureus* differs in other geographic areas. Asian studies have demonstrated that a different strain of MRSA, ST9, appears to be the prominent type of LA-MRSA in several Asian countries (Larsen et al. 2012; Patchanee et al. 2014). Poultry may harbor CC398 strains (Argudin et al. 2013; Wendlandt et al. 2013; Nemeghaire et al. 2013) but also other types unrelated to CC398, including CC5 (Argudin et al. 2013; Buyukcangaz et al. 2013). The epidemiology of CC398 and other strains, found in both animals and humans (Wagenaar et al. 2009), has led to abandon the idea of species specificity in *S. aureus*. Although CC398 appears to be frequently shared between animals and humans and it is capable of causing active symptomatic infections in both species (Smith et al. 2009; Graveland et al. 2011; Hanson et al. 2011), other studies also documented CC398 infections in populations with no obvious livestock contact (Uhlemann et al. 2012; David et al. 2013). Furthermore,

both CC398 and a poultry-adapted *S. aureus* strains of CCT5 have been phylogenetically analyzed and appear to have originated in humans, who transmitted strains to animals, in which the strains subsequently spread and evolved a variety of host adaptations (Lowder et al. 2009).

7 Conclusions

From all data reported in the present article, it is clear that genotypes of *S. aureus* commonly infect species that are not their native host. Between lineages, there is evidence of preferential association and adaptation which dispels any notion that animals (including humans) represent a uniform host landscape to the pathogen. However, there have been several occasions where a genotype has switched host and founded a novel lineage. The findings presented here suggest that major host switches are infrequent, but spillover events are common and represent frequent opportunities for a genotype to become established in an additional host. This supports the concept that human and veterinary medicine should be treated as a strongly linked fields, both clinically and epidemiologically, particularly with respect to practices such as the use of antibiotics.

Regarding livestock role in *S. aureus* epidemiology, as a consequence of studies carried out on swine farms in the USA, we have identified human strains within the noses of live animals (Osadebe et al. 2013; Gordoncillo et al. 2012) or as components of environmental samples of farm dust (Frana et al. 2013) documenting that CC398 can have LA as well as human versions and that human strains of *S. aureus* could also been found in livestock.Spoor et al. (2013) suggested that the bidirectional transmission of strains of *S. aureus* between humans and livestock is not a rare occurrence. Moreover, in addition to the movement of CC398 between animals and humans, studies have suggested that a human pandemic clone, CC97, had its origin in cattle (Spoor et al. 2013), and antibiotic resistance genes, including mecA (Wu et al. 1996) and mecC (García-Álvarez et al. 2011), have been suggested to have an animal origin.

S. aureus surveillance is most commonly carried out within a human clinical or hospital setting, with far fewer research dollars devoted to the analysis of carriage within communities, particularly in a rural setting, and very little research examining animal strains. As such, it is likely we are missing other spillover events of *S. aureus* from livestock to humans or vice versa. To track such events and facilitate both surveillance and source tracking of novel isolates, the buy-in of industry is needed. All too often, the relationship between public health and the agricultural and food industry is one of antagonism rather than assistance. Working together will mean both safer food products and well-protected workers. More attention to this type of research is needed, as we are rapidly approaching a “post-antibiotic era” (WHO 2014). The effectiveness of antimicrobial stewardship in the clinical setting may be reduced if pathogens and resistance genes from the agricultural

environment are repeatedly, but silently, being introduced into the human population (Smith et al. 2002).

8 Future Directions

A large mass of papers demonstrate that there is some evidence in the medical literature to support a potential role for pet animals in the transmission of MRSA in households. Although these data are clearly only hypothesis generating at this point, they do suggest that future work to elucidate the exact role of pet animals in MRSA household transmission would be very worthwhile. These studies should focus on identifying the prevalence of MRSA colonization among pet animals of humans infected or colonized with MRSA. In addition, longitudinal assessment of MRSA colonization in household members as well as their pets should be undertaken to determine the transmission dynamics in the households. Further, identifying potential high-risk behaviors for transmission (e.g., face licking) should be emphasized.

Future investigations could lead to important novel interventions, which could include enhanced surveillance for MRSA among pets as well as potential decolonization of pets with MRSA carriage. In addition, surveillance should be expanded to companion animals other than cats and dogs, particularly those with exposure to agricultural, veterinary, and healthcare settings.

References

Abdel-moein KA, Samir A (2011) Isolation of Enterotoxigenic *Staphylococcus aureus* from pet dogs and cats: a public health implication. Vector-borne Zoonotic Dis 11(6):627–629

Abdel-moein KA, El-Hariri M, Samir A (2012) Methicillin-resistant staphylococcus aureus: an emerging pathogen of pets in Egypt with a public health burden. Transbound Emerg Dis 59:331–335

Ameni G, Regassa A, Kassa T, Medhin G (2001) Survey on bovine tuberculosis in cattle and its public health implications to cattle raising families in Wolaita Soddo, Southern Ethiopia. Ethiopian J Anim Prod 1:55–62

Andersson GU, Wallensten A, Hæggman S, Greko C, Hedin G, Hökeberg I, Lindström F, Olsson-Liljequist B, Smedjegård J, Söderblom T, Windahl U, Struwe J (2014) Outbreaks of methicillin-resistant *Staphylococcus aureus* among staff and dogs in Swedish small animal hospitals. Scand J Infect Dis 46:310–314

Argudin MA, Cariou N, Salandre O, Le Guennec J, Nemeghaire S, Butaye P (2013) Genotyping and antimicrobial resistance of *Staphylococcus aureus* isolates from diseased turkeys. Avian Pathol 42:572–580

Armand-Lefevre L, Ruimy R, Andremont A (2005) Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11:711–714

Baptiste KE, Williams K, Willams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CA (2005) Methicillin resistant Staphylococci in companion animals. Emerg Infect Dis 11 (12):1942–1944

Bender JB, Minicucci L (2007) Diseases pets and people share. Minn Med 90(4):43–47
Benić M, Habrun B, Kompes G (2012) Clinical and epidemiological aspects of cow mastitis caused by *S. aureus* and its methicillin-resistant strains. Med Sci 37:113–122
Bens CC, Voss A, Klaassen CH (2006) Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. J Clin Microbiol 44:1875–1876
Bonelli F, Barsotti G, Attili AR, Mugnaini L, Cuteri V, Preziuso S, Corazza M, Preziuso G, Sgorbini M (2014) Conjunctival bacterial and fungal flora in clinically normal sheep. Vet Rec Open 1(1):e000017
Bootsma MC, Wassenberg MW, Trapman P, Bonten MJ (2011) The nosocomial transmission rate of animal-associated ST398 meticillin-resistant *Staphylococcus aureus*. J R Soc Interface 8:578–584
Burstiner LC, Faires M, Weese JS (2010) Methicillin-resistant *Staphylococcus aureus* colonization in personnel attending a veterinary surgery conference. Vet Surg 39:150–157
Butterworth A, Reeves NA, Harbour D, Werrett G, Kestin SC (2001) Molecular typing of strains of *Staphylococcus aureus* isolated from bone and joint lesions in lame broilers by random amplification of polymorphic DNA. Poult Sci 80:1339–1343
Buyukcangaz E, Velasco V, Sherwood JS, Stepan RM, Koslofsky RJ, Logue CM (2013) Molecular typing of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from animals and retail meat in North Dakota, United States. Foodborne Pathog Dis 10:608–617
Carolan MG (1986) Staphylococcosis in rabbits. Vet Rec 119(16):412
Catry BE, Van Duijkeren E, Pomba MC, Greko C, Moreno MA, Pyorala S, Ruzauskas M, Sanders P, Threlfall EJ, Ungemach F, Torneke K, Munoz-Madero C, Torren-Edo J (2010) Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. Epidemiol Infect 138:626–644
Cefai C, Ashurst S, Owens C (1991) Human carriage of methicillin-resistant *Staphylococcus aureus* linked with pet dog. The Lancet 344:539–540
Cinquepalmi V, Monno R, Fumarola L, Ventrella G, Calia C, Greco MF, de Vito D, Soleo L (2013) Environmental contamination by dog's faeces: a public health problem? Int J Environ Res Public Health 10:72–84
Coughlan K, Olsen KE, Boxrud D, Bender JB (2010) Methicillin-resistant *Staphylococcus aureus* in resident animals of a long-term care facility. Zoonoses Public Health 57:220–226
David MZ, Siegel J, Lowy FD, Zychowski D, Taylor A, Lee CJ, Boyle-Vavra S, Daum RS (2013) Asymptomatic carriage of sequence type 398, spa type t571 methicillin-susceptible *Staphylococcus aureus* in an urban jail: a newly emerging, transmissible pathogenic strain. J Clin Microbiol 51:2443–2447
de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, van Oosterom RA, Vila A, Heuvelink AE (2009) Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 134:52–56
Denis O, Suetens C, Hallin M, Catry B, Ramboer I, Dispas M, Willems G, Gordts B, Butaye P, Struelens MJ (2009) Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. Emerg Infect Dis 15:1098–1101
Deurenberg RH, Stobberingh EE (2008) The evolution of Staphylococcus aureus. Infect Genet Evol 8(6):747–763
Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE (2007) The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 13 (3):222–235

Devriese LA, Okerman L, Maertens L, Okerman F, Godard C (1987) Infecties met een special konijnpathogeen *Staphylococcus aureus* biotype op een konijnen proefbedrijf: resultaten van behandelingen en eradicatiepogingen. Landbouwtijdschrift 5:1243–1245

Didelot X, Lawson D, Darling A, Falush D (2010) Inference of homologous recombination in bacteria using whole-genome sequences. Genetics 186:1435–1449

Duquette RA, Nuttall TJ (2004) Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? J Small Anim Pract 45:591–597

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38:1008–1015

Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A 99 (11):7687–7692

European Food safety Authority (2008) Analysis of the baseline survey on the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs in the EU. EFSA J 7:1376

Faires M, Tater KC, Weese JS (2009) An investigation of methicillin-resistant *Staphylococcus aureus* colonization in people and pets in the same household with an infected person or infected pet. J Am Vet Med Assoc 235:540–543

Fluit AC (2012) Livestock-associated Staphylococcus aureus. Clin Microbiol Infect 18:735–744

Foti M, Fisichella V, Giacopello C (2013) Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in the microbial flora from the conjunctiva of healthy donkeys from Sicily (Italy). Vet Ophthalmol 16(2):89–92

Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC (2013) Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS ONE 8(1):e53738. doi:10.1371/journal.pone.0053738

García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA (2011) Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11:595–603

Garcia-Graells C, Antoine J, Larsen J, Catry B, Skov R, Denis O (2012) Livestock veterinarians at high risk of acquiring methicillin-resistant *Staphylococcus aureus* ST398. Epidemiol Infect 140:383–389

Goni P, Vergara Y, Ruiz J, Albizu I, Vila J, Gomez RL (2004) Antibiotic resistance and epidemiological typing of *Staphylococcus aureus* strains from ovine and rabbit mastitis. Int J Antimicrob Agents 23(3):268–272

Gordoncillo MJ, Abdujamilova N, Perri M, Donabedian S, Zervos M, Bartlett P (2012) Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in backyard pigs and their owners, Michigan, USA. Zoonoses Public Health 59:212–216

Gosbell IB (2011) Methicillin-resistant *Staphylococcus aureus* in veterinary practice. Aust Vet J 89(5):148–151

Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA (2011) Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. Int J Med Microbiol 301:630–634

Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, Cartwright RA, Smyth DS, Smyth CJ, Lindsay JA, Gould KA, Witney A, Hinds J, Bollback JP, Rambaut A, Penadés JR, Fitzgerald JR (2010) Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol Evol 2:454–466

Haenni M, Saras E, Châtre P, Médaille C, Bes M, Madec JY, Laurent F (2012) A USA300 variant and other human-related methicillin-resistant *Staphylococcus aureus* strains infecting cats and dogs in France. J Antimicrob Chemother 67:326–329

Hagen KW (1963) Disseminated staphylococcic infection in young domestic rabbits. J Am Vet Med Assoc 142:1421–1422

Hallin M, De Mendonça R, Denis O, Lefort A, El Garch F, Butaye P, Hermans K, Struelens MJ (2011) Diversity of accessory genome of human and livestock-associated ST398 methicillin resistant *Staphylococcus aureus* strains. Infect Genet Evol 11:290–299

Hanselman BA, Kruth SA, Rousseau J, Low DE, Willey BM, McGeer A, Weese JS (2006) Methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel. Emerg Infect Dis 12(12):1933–1938

Hanselman BA, Kruth SA, Rousseau J, Weese JS (2009) Coagulase positive staphylococcal colonization of humans and their household pets. Can Vet J 50:954–958

Hanson BM, Dressler AE, Harper AL, Scheibel RP, Wardyn SE, Roberts LK, Kroeger JS, Smith TC (2011) Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J Infect Public Health 4:169–174

Heller J, Armstrong SK, Girvan EK, Reid SWJ, Moodley A, Mellor DJ (2009) Prevalence and distribution of meticillin-resistant *Staphylococcus aureus* within the environment and staff of a university veterinary clinic. J Small Anim Pract 50:168–173

Heller J, Innocent GT, Denwood M, Reid SWJ, Kelly L, Mellor DJ (2011) Assessing the probability of acquisition of meticillin-resistant *Staphylococcus aureus* (MRSA) in a dog using a nested stochastic simulation model and logistic regression sensitivity analysis. Prev Vet Med 99:211–224

Hoet AE, van Balen J, Nava-Hoet RC, Bateman S, Hillier A, Dyce J, Wittum TE (2013) Epidemiological profiling of methicillin-resistant *Staphylococcus aureus*-positive dogs arriving at a veterinary teaching hospital. Vector-Borne Zoonotic Dis 13(6):385–393

Holliman A, Girvan GA (1986) Staphylococcosis in a commercial rabbitry. Vet Rec 119:187

Holmes MA, Zadocks RN (2011) Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia 16(4):373–382

Ishihara K, Shimokubo N, Sakagami A, Ueno H, Muramatsu Y, Kadosawa T, Yanagisawa C, Hanaki H, Nakajima C, Suzuki Y, Tamura Y (2010) Occurrence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in an academic veterinary hospital. Appl Environ Microbiol 76(15):5165–5174

Ishihara K, Saito M, Shimokubo N, Muramatsu Y, Maetani S, Tamura Y (2014) Methicillin-resistant *Staphylococcus aureus* carriage among veterinary staff and dogs in private veterinary clinics in Hokkaido. Japan Microbiol Immunol 58:149–154

Khanna T, Friendship R, Dewey C, Weese JS (2008) Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol 128:298–303

Klein E, Smith DL, Laxminarayan R (2007) Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg Infect Dis 13:1840–1846

Kluytmans J, Struelens M (2009) Meticillin resistant *Staphylococcus aureus* in the hospital. BMJ 338:b364

Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, Bae W, Lim JY, Kim JY, Kim JM, Hong SK, Park YH (2006) Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol 117:304–312

Lai CH, Chang LL, Lin JN, Liao MH, Liu SS, Lee HH, Chen Y-H (2015) Association of human Q fever with animal husbandry, Taiwan, 2004–2012. Emerg Infect Dis 21(12):2217–2220

Larsen J, Imanishi M, Hinjoy S, Tharavichitkul P, Duangsong K, Davis MF, Nelson KE, Larsen AR, Skov RL (2012) Methicillin-resistant *Staphylococcus aureus* ST9 in pigs in Thailand. PLoS ONE 7:e31245. doi:10.1371/journal.pone.0031245

Leonard FC, Abbott Y, Rossney A, Quinn PJ, O'Mahony R, Markey BK (2006) Methicillin-resistant *Staphylococcus aureus* isolated from a veterinary surgeon and five dogs in one practice. Vet Rec 158:155–159

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ,

Mevius DJ; National ESBL surveillance group (2011) Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17:873–880

Lewis HC, Mølbak K, Reese C, Aarestrup FM, Selchau M, Sørum M, Skov RL (2008) Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. Emerg Infect Dis 14:1383–1389

Li S, Skov RL, Han X, Larsen AR, Larsen J, Sørum M, Wulf M, Voss A, Hiramatsu K, Ito T (2011) Novel types of staphylococcal cassette chromosome mec elements identified in clonal complex 398 methicillin resistant *Staphylococcus aureus* strains. Antimicrob Agents Ch 55:3046–3050

Lloyd-Smith JO, George D, Pepin KM, Pitzer VE, Pulliam JR, Dobson AP, Hudson PJ, Grenfell BT (2009) Epidemic dynamics at the human-animal interface. Science 326:1362–1367

Loeffler A, Lloyd DH (2010) Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect 138:595–605

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH (2005) Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J Antimicrob Chemother 56:692–697

Loeffler A, Pfeiffer DU, Lloyd DH, Smith H, Soares-Magalhaes R, Lindsay JA (2010) Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups. J Hosp Infect 74:282–288

Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nübel U, Fitzgerald JR (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci USA 106:19545–19550

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532

Lowy FD (2003) Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest 111:1265–1273

Makovec JA, Ruegg PL (2003) Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci 86:3466–3472

Manian FA (2003) Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis 36:26–28

McLean CL, Ness MG (2008) Meticillin-resistant *Staphylococcus aureus* in a veterinary orthopaedic referral hospital: staff nasal colonisation and incidence of clinical cases. J Small Anim Pract 49:170–177

McNamee PT, Smyth JA (2000) Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avian Pathol 29:253–270

Middleton JR, Fales WH, Luby CD, Oaks JL, Sanchez S, Kinyon JM, Wu CC, Maddox CW, Welsh RD, Hartmann F (2005) Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. J Clin Microbiol 43(6):2916–2919

Mitchell BA, Brown MH, Skurray RA (1998) QacA multidrug efflux pump from *Staphylococcus aureus*: comparative analysis of resistance to diamidines, biguanidines, and guanylhydrazones, antimicrob. Agents Chemother 42(2):475–477

Moodley A, Stegger M, Bagcigil AF, Baptiste KE, Loeffler A, Lloyd DH, Williams NJ, Leonard N, Abbott Y, Skov R, Guardabassi L (2006) *spa* typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. J Antimicrob Chemother 58:1118–1123

Moodley A, Nightingale EC, Stegger M, Nielsen SS, Skov RL, Guardabassi L (2008) High risk for nasal carriage of methicillin-resistant *Staphylococcus aureus* among Danish veterinary practitioners. Scand J Work Environ Health 34(2):151–157

Morris DO, Lautenbach E, Zaoutis T, Leckerman K, Edelstein PH, Rankin SC (2012) Potential for pet animals to harbor methicillin-resistant *Staphylococcus aureus* (MRSA) when residing with human MRSA patients. Zoonoses Public Health 59(4):286–293

Muniz IM, Penna B, Lilenbaum W (2013) Treating animal bites: susceptibility of staphylococci from oral mucosa of cats. Zoonoses Public Health 60:504–509

Murphy CP, Reid-Smith RJ, Boerlin P, Weese JS, Prescott JF, Janecko N, Hassard L, McEwen SA (2010) *Escherichia coli* and selected veterinary and zoonotic pathogens isolated from environmental sites in companion animal veterinary hospitals in southern Ontario. Can Vet J 51:963–972

Nemeghaire S, Roelandt S, Argudin MA, Haesebrouck F, Butaye P (2013) Characterization of methicillin-resistant *Staphylococcus aureus* from healthy carrier chickens. Avian Pathol 42:342–346

Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerald-F. Gerlach, Schwarz S, Simon D, Nolte I (2009) Transmission of methicillin-resistant *Staphylococcus aureus* strains between humans and dogs: two case reports. J Antimicrob Chemother 64(3):660–662

O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S, Rossney AS (2005) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Vet Microbiol 109:285–296

Okerman L, Devriese LA, Maertens L, Okerman F, Godard C (1984) Cutaneous staphylococcosis in rabbits. Vet Rec 114:313–315

Okesola AO (2011) Community-acquired methicillin-resistant Staphylococcus aureus—A review of literature. Afr J Med Med Sci 40(2):97–107

Osadebe LU, Hanson B, Smith TC, Heimer R (2013) Prevalence and characteristics of *Staphylococcus aureus* in Connecticut swine and swine farmers. Zoonoses Public Health 60:234–243

Pal M (1997) Zoonoses. R.M. Publisher and Distributor Delhi, India

Paul NC, Moodley A, Ghibaudo G, Guardabassi L (2011) Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in small animal veterinarians: indirect evidence of zoonotic transmission. Zoonoses Public Health 58:533–539

Petinaki E, Spiliopoulou I (2012) Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. Clin Microbiol Infect 18:626–634

Piddock LJV (1996) Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic resistant bacteria that infect man and compromise antimicrobial chemotherapy? J Antimicrob Chemother 38:1–3

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods. J Food Prot 73(10):1919–1936

Patchanee P, Tadee P, Arjkumpa O, Love D, Chanachai K, Alter T, Hinjoy S, Tharavichitkul P (2014) Occurrence and characterization of livestock-associated methicillin-resistant *Staphylococcus aureus* in pig industries of northern Thailand. J Vet Sci 15(4):529–536. doi:10.4142/jvs.2014.15.4.529

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio 3(1): e00305–11. doi:10.1128/mBio.00305-11

Rasigade JP, Laurent F, Hubert P, Vandenesch F, Etienne J (2010) Lethal necrotizing pneumonia caused by an ST398 *Staphylococcus aureus* strain. Emerg Infect Dis 16:1330

Rossi G, Stanzel C, Witte W, (1995) *Staphylococcus aureus* infections in the rabbit and the transmission of the pathogens with the sperma. 9. Arbeitstagung u¨ber Haltung und Krankheiten der Kaninchen. Pelztiere und Heimtiere, Celle 251–257

Rutland BE, Weese JS, Bolin C, Au J, Malani AN (2009) Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 15(8):1328–1330

Sakwinska O, Giddey M, Moreillon M, Morisset D, Waldvogel A, Moreillon P (2011) *Staphylococcus aureus* host range and human-bovine host shift. Appl Environ Microbiol 77:5908–5915

Scott GM, Thomson R, Malone-Lee J, Ridgway GL (1988) Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans? J Hosp Infect 12:29–34

Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, Hollis RJ, Jones RJ, Pfaller MA (1999) Methicillin-resistant *Staphylococcus aureus* Outbreak in a veterinary teaching hospital: potential human-to-animal transmission. J Clin Microbiol 37(5):1459–1463

Shepheard MA, Fleming VM, Connor TR, Corander J, Feil EJ, Fraser C, Hanage WP (2013) Historical zoonoses and other changes in host tropism of Staphylococcus aureus, identified by phylogenetic analysis of a population dataset. PLoS ONE 8:e62369. doi:10.1371/journal.pone.0062369

Silbergeld EK, Graham J, Price LB (2008) Industrial food animal production, antimicrobial resistance, and human health. Annu Rev Public Health 29:151–169

Silva NC, Guimarães FF, Manzi MP, Júnior AF, Gómez-Sanz E, Gómez P, Langoni H, Rall VL, Torres C (2014) Methicillin-resistant *Staphylococcus aureus* of lineage ST398 as cause of mastitis in cows. Lett Appl Microbiol 59(6):665–669

Sing A, Tuschak C, Hörmansdorfer S (2008) Methicillin-resistant *Staphylococcus aureus* in a family and its pet cat. N Engl J Med 358(11):1200–1201

Smith TC (2015) Livestock-associated Staphylococcus aureus: the United States experience. PLoS One. doi:10.1371/journal.ppat.1004564

Smith TC, Pearson N (2011) The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis 11:327–339

Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG Jr (2002) Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci U S A 99:6434–6439

Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Herwaldt LA, Diekema DJ (2009) Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Midwestern U.S. swine and swine workers. PLoS ONE 4:e4258. doi:10.1371/journal.pone.0004258

Smyth DS, Feil EJ, Meaney WJ, Hartigan PJ, Tollersrud T et al (2009) Molecular genetic typing reveals further insights into the diversity of animal-associated *Staphylococcus aureus*. J Med Microbiol 58:1343–1353

Snyder GM, Thom KA, Furuno JP, Perencevich EN, Roghmann MC, Strauss SM, Netzer G, Harris AD (2008) Detection of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci by healthcare workers on infection control gown and gloves. Infect Control Hosp Epidemiol 29(7):583–589

Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, et al (2013) Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus. MBio 4:e00356-13. doi:10.1128/mBio.00356-13

Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, Witte W, Schwarz S (2006) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. J Antimicrob Chemother 57:461–465

Sung JM, Lindsay JA (2007) *Staphylococcus aureus* strains that are hypersusceptible to resistance gene transfer from Enterococci. Antimicrob Agents Chemother 51:2189–2191

Sung JM, Lloyd DH, Lindsay JA (2008) *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154:1949–1959

Trinidad P, Nickerson SC, Alley TK (1990) Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. J Dairy Sci 73:107–114

Türkyilmaz S, Tekbiyik S, Oryasin E, Bozdogan B (2010) Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. Zoonoses Public Health 57(3):197–203

Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, et al (2012) Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. mBio 3:e00027–12. doi:10.1128/mBio.00027-12

van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, Kluytmans JA (2011) Persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol 49:1030–1033

van Duijkeren E, Wolfhagen MJHM, Box ATA, Heck MEOC, Wannet WJB, Fluit AC (2004) Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 10(12):2235–2237

van Duijkeren E, Wolfhagen MJMH, Heck MEOC, Wannet WJB (2005) Transmission of a panton-valentine leucocidin-positive, methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. J Clin Microbiol 43(12):6209–6211

van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N et al (2007) Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis 13:1834–1839

van Rijen MM, Van Keulen PH, Kluytmans JA (2008) Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. Clin Infect Dis 46:261–263

Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P (2010a) Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. Vet Microbiol 144:166–171

Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P (2010b) Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol Infect 138:606–625

Varshney AK, Mediavilla JR, Robiou N, Guh A, Wang X, Gialanella P, Levi MH, Kreiswirth BN, Fries BC (2009) Diverse enterotoxin gene profiles among clonal complexes of *Staphylococcus aureus* isolates from the Bronx, New York. Appl Environ Microbiol 75:6839–6849

Vengust M, Anderson MEC, Rousseau J, Weese JS (2006) Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett Appl Microbiol 43:602–606

Viana D, Selva L, Segura P, Penades JR, Corpa JM (2007) Genotypic characterization of *Staphylococcus aureus* strains isolated from rabbit lesions. Vet Microbiol 121:288–298

Viana D, Blanco J, Tormo-Mas MA, Selva L, Guinane CM et al (2010) Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77:1583–1594

Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lübke-Becker A, Walther B (2014) Risk factors for MRSA infection in companion animals: Results from a case—Control study within Germany. Int J Med Microbiol 304:787–793

Virgin JE, Van Slyke TM, Lombard JE, Zadoks RN (2009) Short communication: methicillin-resistant *Staphylococcus aureus* detection in US bulk tank milk. J Dairy Sci 92:4988–4991

Vitale CB, Gross TL, Weese JS (2006) Methicillin-resistant *Staphylococcus aureus* in cat and owner. Emerg Infect Dis 12(12):1998–2000

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005) Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis 11:1965–1966

Waage S, Mørk T, Røros A, Aasland D, Hunshamar A, Odegaard SA (1999) Bacteria associated with clinical mastitis in dairy heifers. J Dairy Sci 82:712–719

Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X et al (2009) Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. Vet Microbiol 139:405–409

Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ (2011) Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus* (ST398) in Dutch hospitals. Clin Microbiol Infect 17:316–319

Wedley AL, Dawson S, Maddox TW, Coyne KP, Pinchbeck GL, Clegg P, Jamrozy D, Fielder MD, Donovan D, Nuttall T, Williams NJ (2014) Carriage of *Staphylococcus species* in the veterinary visiting dog population in mainland UK: molecular characterization of resistance and virulence. Vet Microbiol 170:81–88

Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE (2006) Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol 115:148–155

Weinert LA, Welch JJ, Suchard MA, Lemey P, Rambaut A et al (2012) Molecular dating of human-to-bovid host jumps by *Staphylococcus aureus* reveals an association with the spread of domestication. Biol Lett 8:829–832

Wendlandt S, Kadlec K, Fessler AT, Monecke S, Ehricht R et al (2013) Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers. J Antimicrob Chemother 68:2458–2463

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5:751–762

Wettstein Rosenkranz K, Rothenanger E, Brodard I, Collaud A, Overesch G, Bigler B, Marschall J, Perreten V (2014) Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among Swiss veterinary health care providers: detection of livestock- and healthcare-associated clones. Schweiz Arch Tierheilkd 156(7):317–325

WHO (1967) Joint WHO/FAO expert committee on zoonoses. 3th report. WHO technical report series no. 378. Word Health Organization, Geneva

WHO (1979) Parasitic zoonoses, WHO Technical Report Series No. 637. Word Health Organization, Geneva

WHO (1982) Bacterial and viral zoonoses, WHO Technical Report Series No. 682. Word Health Organization, Geneva

WHO (2014) Antimicrobial resistance: global report on surveillance, pp. 257. Word Health Organization, Geneva

Wiles S, Hanage WP, Frankel G, Robertson B (2006) Modelling infectious disease-time to think outside the box? Nat Rev Microbiol 4:307–312

Witte W, Strommenger B, Stanek C, Cuny C (2007) Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals Central Europe. Emerg Infect Dis 13(2):255–258

Wolfe ND, Dunavan CP, Diamond J (2007) Origins of major human infectious diseases. Nature 447:279–283

Wu S, Piscitelli C, de Lencastre H, Tomasz A (1996) Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of mecA from a methicillin susceptible strain of Staphylococcus sciuri. Microb Drug Resist 2:435–441

Yihan L, Barker E, Kislow J, Kaldhone P, Stemper ME, Pantrangi M, Moore FM, Hall M, Fritsche TR, Novicki T, Foley SL, Shukla SK (2011) Evidence of multiple virulence subtypes in nosocomial and community-associated MRSA genotypes in companion animals from the upper midwestern and Northeastern United States. Clin Med Res 9(1):7–16

Treatment of *Staphylococcus aureus* Infections

Michael Z. David and Robert S. Daum

Abstract *Staphylococcus aureus*, although generally identified as a commensal, is also a common cause of human bacterial infections, including of the skin and other soft tissues, bones, bloodstream, and respiratory tract. The history of *S. aureus* treatment is marked by the development of resistance to each new class of antistaphylococcal antimicrobial drugs, including the penicillins, sulfonamides, tetracyclines, glycopeptides, and others, complicating therapy. *S. aureus* isolates identified in the 1960s were sometimes resistant to methicillin, a ß-lactam antimicrobial active initially against a majority *S. aureus* strains. These MRSA isolates, resistant to nearly all ß-lactam antimicrobials, were first largely confined to the health care environment and the patients who attended it. However, in the mid-1990s, new strains, known as community-associated (CA-) MRSA strains, emerged. CA-MRSA organisms, compared with health care-associated (HA-) MRSA strain types, are more often susceptible to multiple classes of non ß-lactam antimicrobials. While infections caused by methicillin-susceptible *S. aureus* (MSSA) strains are usually treated with drugs in the ß-lactam class, such as cephalosporins, oxacillin or nafcillin, MRSA infections are treated with drugs in other antimicrobial classes. The glycopeptide drug vancomycin, and in some countries teicoplanin, is the most common drug used

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to treat severe MRSA infections. There are now other classes of antimicrobials available to treat staphylococcal infections, including several that have been approved after 2009. The antimicrobial management of invasive and noninvasive *S. aureus* infections in the ambulatory and in-patient settings is the topic of this review. Also discussed are common adverse effects of antistaphylococcal antimicrobial agents, advantages of one agent over another for specific clinical syndromes, and the use of adjunctive therapies such as surgery and intravenous immunoglobulin. We have detailed considerations in the therapy of noninvasive and invasive *S. aureus* infections. This is followed by sections on specific clinical infectious syndromes including skin and soft tissue infections, bacteremia, endocarditis and intravascular infections, pneumonia, osteomyelitis and vertebral discitis, epidural abscess, septic arthritis, pyomyositis, mastitis, necrotizing fasciitis, orbital infections, endophthalmitis, parotitis, staphylococcal toxinoses, urogenital infections, and central nervous system infections.

Abbreviations

ABSSSI	Acute bacterial skin and skin structure infection
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CAP	Community-acquired pneumonia
CDC	United States Centers for Disease Control and Prevention
CNS	Central nervous system
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
ETA; ETB	Exfoliative toxins A and B
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
HA-MRSA	Healthcare-associated methicillin-resistant *Staphylococcus aureus*
hVISA	Heterogeneous vancomycin-intermediate *Staphylococcus aureus*
IDSA	Infectious Diseases Society of America
IV	Intravenous
IVIG	Intravenous immune globulin
MAO	Monoamine oxidase
MIC	Minimum inhibitory concentration
MRI	Magnetic resonance imaging
MSSA	Methicillin-susceptible *Staphylococcus aureus*
PBP	Penicillin binding protein
PVL	Panton-Valentine leukocidin
RCT	Randomized, controlled trial
SCC*mec*	Staphylococcal cassette chromosome *mec*
SIRS	Systemic inflammatory response syndrome
SSRI	Selective serotonin reuptake inhibitor
SSSS	Staphylococcal scalded skin syndrome
SSTI	Skin and soft tissue infection

TEE	Transesophageal echocardiogram
TMP-SMX	Trimethoprim-sulfamethoxazole
TOA	Tubo-ovarian abscess
TSS	Toxic shock syndrome
tsst	Toxic shock syndrome toxin
TTE	Transthoracic echocardiogram
UK	United Kingdom
USA	United States of America
UTI	Urinary tract infection
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*

Contents

1 Introduction

Staphylococcus aureus is a common cause of human bacterial infections (Table 1). Despite its pathogenicity, it is generally identified as a commensal; it is estimated that 25–40% of the population is nasally colonized at any time (Lowy 1998; Tong et al. 2015). Colonization at other sites, e.g., the throat, in some studies, has been found to be more frequent. *S. aureus* colonization is asymptomatic, but colonized

Table 1 *Staphylococcus aureus* disease syndromes described in this review

Syndrome	Features	Therapeutic considerations
Skin and soft tissue infections (SSTI)	Broad range of severity; most are uncomplicated	Drainage is indicated if there is a drainable focus; antibacterial therapy increases likelihood of cure and decreases recurrence. Oral therapy is appropriate for uncomplicated SSTI. For complicated SSTI, parenteral therapy may be necessary for cure and to prevent complications
Bacteremia	Mortality 15–50%; source should be sought aggressively; bacteremia may be complicated by secondary infections of heart, bone, or abscess in an internal organ	If source can be removed or debrided (such as a foreign body or infected tissue) or drained (such as an abscess), this increases likelihood of cure; if source is addressed then IV therapy for 2 weeks is usually adequate; if there is secondary seeding of an organ, vascular site, bone or other tissue, a more prolonged duration is necessary
Endocarditis, native valve	May be a complication of bacteremia from any source; the diagnosis often suggested by persistently positive blood cultures, onset of heart failure, arrhythmias, aortic root abscess, septic emboli to any organ or tissue (usually to the lung in the setting of right-sided endocarditis), or CVA; an echocardiogram is often an important means of supporting the diagnosis as part of the modified Duke criteria for endocarditis	If uncomplicated, IV antimicrobial therapy alone is adequate and monotherapy is usually appropriate; however, in certain cases surgical valve replacement is indicated (see text)
Endocarditis, prosthetic valve	May be a complication of bacteremia from any source; often suggested by persistently positive blood cultures, onset of heart failure; complications include those noted above; as well as dehiscence of the prosthetic valve; an echocardiogram is often an important means of supporting the diagnosis as part of the modified Duke criteria for endocarditis	Combination IV therapy is indicated for prosthetic valve endocarditis with a primary agent for *S. aureus* in combination with rifampin, both for 6 weeks plus gentamicin for the first 2 weeks. Valve replacement is often indicated and a team approach with early involvement of cardiothoracic surgery is essential

(continued)

Table 1 (continued)

Syndrome	Features	Therapeutic considerations
Intravascular infection	A complication of bacteremia, these may result from direct extension of an infection from a foreign body into the wall of a blood vessel or they may result from an infected embolus, as sometimes is the case in a septic pulmonary embolus	Require prolonged IV therapy, usually 4–6 weeks
Pneumonia	*S. aureus* is a relatively rare cause of CAP and is more common as a cause of nosocomial pneumonia and particularly ventilator-associated pneumonia; PVL-positive *S. aureus* strains may be more likely to cause a severe, necrotizing infection; *S. aureus* pneumonia has been found to be more common when pneumonia is a complication of influenza or other respiratory viral infection	While an uncomplicated pneumonia may be treated for only 7 days, in cases of necrotizing pneumonia, the duration may be extended to 4 or more weeks. Importantly, daptomycin is not used for pneumonia
Osteomyelitis	*S. aureus* may infect any bone in the body either by direct extension from another tissue or by hematogenous spread	Surgery is not usually required for cure unless a foreign body must be removed. Antimicrobial therapy is generally required for a minimum of 4–6 weeks although the duration is determined by the clinical course and sometimes requires several months. In the event that there is a foreign body that cannot be removed, long-term or indefinite suppressive therapy may be required
Intervertebral discitis	May arise as a complication of bacteremia and less often from direct spread; often accompanies vertebral osteomyelitis, but it also may occur in the absence of any bone infection	May require surgical debridement
Epidural abscess	Can be a sequela of bacteremia or direct spread from vertebral osteomyelitis, discitis, or a parameningeal focus of a central nervous system infection	Often require drainage by interventional radiologists or neurosurgeons for cure
Septic arthritis	Usually a complication of bacteremia. Monoarticular arthritis is most common with a neutrophilic predominance of the often purulent fluid aspirated from the joint space	Prompt drainage, followed by a washout or serial needle taps are often required to preserve the full function of the joint

(continued)

Table 1 (continued)

Syndrome	Features	Therapeutic considerations
Pyomyositis	More common in the tropical regions of the world; *S. aureus* is the predominant cause; most commonly affected muscles are in the pelvis; imaging with ultrasound, CT or MRI is often necessary for diagnosis	Drainage is necessary by interventional radiology or surgery for larger abscesses; for multiple abscesses surgery may be superior. Treatment for a minimum of 21 days is recommended
Necrotizing fasciitis	Severe, rapidly progressive infection of the fascial plane deep to the subcutaneous tissues; usually diagnosed by clinical examination; there is often crepitus, pain beyond the margin of erythema, a grayish color to the skin affected, and there is no bleeding when the affected, necrotic tissue is cut; the bacterial cause may be recovered from tissue culture, and blood cultures are often positive as well	A surgical emergency, and surgery should not be delayed for imaging studies; immediate debridement of necrotic tissue is indicated; broad-spectrum antibacterial drugs are essential as the etiology is not usually known immediately; *S. aureus* is a possible etiology and vancomycin should be used; as a toxin-mediated process may be contributing to the pathogenesis of the infection, clindamycin or linezolid is recommended empirically as well
Impetigo	A superficial infection of the skin with distinctive crusting	May be treated expectantly or with a topical agent (see text)
Mastitis and breast abscess	Inflammation of the breast that often occurs in the setting of lactation; should be distinguished from granulomatous mastitis that is thought to be noninfectious; if draining or drainable focus visible on ultrasound, *S. aureus* may be recovered from needle drainage specimen, and this suggests breast abscess	Mastitis may improve with no systemic therapy; breast abscess requires drainage, and subsequent oral therapy is usually adequate
Conjunctivitis	Infection of the conjunctivae	Usually treated topically
Orbital infections	Preseptal, or facial cellulitis involves the anterior of the orbit, while retro-orbital cellulitis involves deeper tissues within the orbit; either infection may arise from lacrimal duct infection, blepharitis, conjunctivitis, sinus infection or from hematogenous spread; more complicated orbital infections include endophthalmitis, orbital abscess and subperiosteal abscess, the last 2 of which can be distinguished by MRI;	If limited to cellulitis, antimicrobial therapy is adequate, but an orbital abscess usually requires surgical drainage by an ophthalmologist or neurosurgeon; a subperiosteal abscess generally does not require drainage

(continued)

Table 1 (continued)

Syndrome	Features	Therapeutic considerations
	concerning symptoms are photophobia, limited range of motion of the eye, and pain with movement of the eye	
Endophthalmitis	A surgical emergency, this is an infection of the globe of the eye, distinguished often by the presence of a hypopyon visible on physical examination	Emergent vitrectomy may be necessary to prevent blindness; immediate systemic antimicrobial therapy is essential
Parotitis	An infection of the parotid gland, often caused by *S. aureus*; complications are rare and usually readily responds to therapy; can be diagnosed by culture of drainage from Stensen's duct or by needle drainage	Treatment with antimicrobial therapy alone is often adequate.
Staphylococcal toxic shock syndrome (TSS)	A potentially fatal syndrome caused by the toxic shock syndrome toxins (tsst-1 and -2), sometimes in the absence of a primary, clinically apparent *S. aureus* infection; has been associated with super-absorbent tampon use as well as surgical dressings; results from nonspecific stimulation of T cells by the toxin which acts as a superantigen	Treatment with antibacterials is usually required; debridement even in the absence of a clinically obvious infection may be necessary in the case of an infected wound; removal of the responsible tampon or dressing should be immediate; some advocate the use of IVIG therapy as an adjunct
Staphylococcal scalded skin syndrome (SSSS)	Syndrome of skin sloughing caused by the ETA and ETB toxins. Desquamation is often on the face and skin folds; blood cultures may be positive, especially in adults	Burn unit treatment is optimal; antimicrobial therapy is indicated; fresh frozen plasma is often administered, as is IVIG for 5 days
Urogenital infections	*S. aureus* is a rare cause of urogenital infection but has been reported to cause infections in all urinary and genital organs; there is a risk of abscess formation in the case of *S. aureus* infections	Treatment may require drainage for cure (see text for considerations in specific organ involvement)
Lemierre's syndrome	Infection extending from a soft tissue source in the neck, leading to thrombophlebitis of the internal jugular vein; may extend into the mediastinum; septic emboli may occur from infected vasculature; *S. aureus* is not a common cause of this syndrome	Usually treated with antimicrobial therapy alone; drainage of secondary focus of infection may be needed; rarely drainage or debridement of foci in soft tissues of the neck is required as well

(continued)

Table 1 (continued)

Syndrome	Features	Therapeutic considerations
Meningitis	*S. aureus* is a rare cause of meningitis in the absence of prior surgery or placement of a foreign body in the CNS; may occur as a complication of epidural injections	Usually treated with 2 weeks of IV therapy after removal of any focus or foreign body (see text for considerations related to antimicrobial drug choice)
Other central nervous system infections	Most common as a complication of foreign bodies, but can develop brain abscesses and other CNS infections as a complication of bacteremia and hematogenous seeding	Abscesses often require drainage if they are large or causing neurologic deficit. Foreign bodies should be removed if possible (see text for considerations related to antimicrobial drug choice)

CAP community-acquired pneumonia; *CNS* central nervous system; *CT* computed tomography; *CVA* cerebrovascular accident; *IV* intravenous; *IVIG* intravenous immune globulin; *MRI* magnetic resonance imaging; *PVL* Panton-Valentine leucocidin

individuals may develop an infection if the colonizing organism is pathogenic. Colonization may be short or long term, and may clear spontaneously. Transmission is believed to be largely via skin-to-skin contact from a colonized or infected individual. Acquisition of the organism from a fomite source is a possibility but has not been proven.

New *S. aureus* isolates were identified in the 1960s that were resistant to methicillin, a ß-lactam antimicrobial active at that time against nearly all *S. aureus* strains. Although methicillin is not used any more (other members of the family that are used are oxacillin and nafcillin), the name "methicillin-resistant *S. aureus* (MRSA)" persists. Such MRSA isolates were initially largely confined to the health care environment, and they became major causes of nosocomial infections, particularly among patients after procedures or with devices that necessitate piercing the skin. These MRSA strains were believed to be acquired in health care environments and have been termed health care-associated or HA-MRSA. They are often resistant to multiple classes of non ß-lactam antibiotics (David and Daum 2010; Peacock and Paterson 2015).

In the mid-1990s, there was a major shift in the clinical and molecular epidemiology of MRSA infections in the United States (USA) and other countries. HA-MRSA strains continued to infect patients, usually originating from the health care environment. However, the incidence of MRSA among children and adults lacking previous exposure to the health care system increased significantly. Infection caused by these new strains could be severe. A report documented rapidly fatal infections caused by MRSA in North Dakota and Minnesota in 1997–1999 (CDC 1999). Because the genetically distinct MRSA strain types causing infections were often acquired by patients who lacked contact with the healthcare environment, they were termed community-associated MRSA (CA-MRSA) infections (David and Daum 2010).

CA-MRSA strains have been studied extensively. Since 2000, the vast majority of the circulating strains in the USA and Canada have belonged to the clonotype

designated USA300 as defined by pulsed field gel electrophoresis. For several years before 2000, they were caused most often by a different MRSA clonotype (USA400) which has disappeared from people in the USA. Both clonotypes carry the *mecA* gene which is necessary for methicillin resistance, and a novel staphylococcal cassette chromosome termed SCC*mec* type IV. SCC*mec* is the chromosomal genetic element that carries *mecA*. The two CA-MRSA strain types also usually carry the Panton-Valentine Leukocidin (PVL) genes, present in <10% of unselected *S. aureus* strains, and >90% of CA-MRSA strains, although the role of PVL in pathogenesis and fitness is still unclear (David and Daum 2010). On other continents, the predominant CA-MRSA strain types, many of which carry the PVL genes like USA300, differ (Vandenesch et al. 2003; David and Daum 2010).

Paradoxically, these CA-MRSA organisms, compared with HA-MRSA strain types, are more often susceptible to multiple classes of non ß-lactam antimicrobials.

Vancomycin, a licensed glycopeptide antimicrobial introduced in the 1950s, was termed the 'antimicrobial of last resort' in the US because it was reliably bactericidal for all *S. aureus* strains, but resistant isolates have been identified both at low level (so-called vancomycin-intermediate resistant, or VISA isolates) and at high level (so-called vancomycin resistant or VRSA isolates). Additional antimicrobials have received US Food and Drug Administration (FDA) approval for the management of MRSA infections as well as infections with VISA and VRSA isolates. These will be detailed in this article.

The CA-MRSA era has seen a substantial increase in the use of clindamycin and trimethoprim-sulfamethoxazole (TMP-SMX) for the treatment of infections caused by CA-MRSA strain types. Two recent studies have documented that one of these antimicrobials should be used in addition to incision and drainage for a skin infection, the commonest clinical syndrome caused by CA-MRSA (Daum et al. 2017; Talan et al. 2016), and another recent randomized, controlled trial (RCT) demonstrated that for uncomplicated skin and soft tissue infections (SSTIs), clindamycin or TMP-SMX were effective (Miller et al. 2015).

The antimicrobial management of *S. aureus* infections in the ambulatory and in-patient settings is the topic of this review. Table 1 lists the major clinical syndromes that are examined.

2 Guidelines for Therapy of *S. aureus* Infections

There are a number of guidelines that have been published by professional organizations and governmental institutions around the world that bear upon the therapy of all or specific *S. aureus* infections. These include the Infectious Diseases Society of America (IDSA) guidelines for the therapy of MRSA infections (Liu et al. 2011) and for SSTIs (Stevens et al. 2014). A Guideline for UK Practice for the Diagnosis and Management of MRSA Infections Presenting in the Community (Nathwani et al. 2008), another for MRSA management in the UK (Gould et al. 2009) and a Canadian Guideline for CA-MRSA prevention and management (Barton et al.

2006) have also been published. While there are differences in these guidelines, the general principles are similar, and many differing recommendations reflect variations in local antimicrobial susceptibility patterns in different countries. Other distinct recommendations are dictated by differences in antimicrobials approved by national agencies as well as approved indications for their use.

3 General Considerations for Therapy of *S. aureus* Infections

3.1 Noninvasive Skin and Soft Tissue Infections

SSTIs, infections of the skin and the subcutaneous tissues, are the most common type of human infections caused by *S. aureus*. Many SSTIs are termed *uncomplicated* when they are not severe, while severe or deep SSTIs, especially with systemic signs and symptoms of infection, are classified as being *complicated*. Uncomplicated SSTIs are those in which there are no systemic symptoms, such as fever, chills, or hypotension; in the absence of the Systemic Inflammatory Response Syndrome (SIRS); and when there is not rapid progression, evidence of fasciitis or signs or symptoms of another deeper tissue infection.

S. aureus can cause a variety of specific noninvasive infections of the skin and subcutaneous tissues, including most commonly folliculitis, impetigo, cellulitis, paronychia, and abscess. *Furuncle* is a term that is sometimes used to describe an abscess that arises from an infection of a hair follicle, while *carbuncle* is a term used to describe a cluster of furuncles or larger abscesses. In this review, we will use the term *abscess* in place of *carbuncle* and *furuncle*. We consider impetigo separately below. Uncomplicated sinus infections can also be caused by *S. aureus* (Whitby et al. 2011).

The primary treatment of noninvasive *S. aureus* infections is incision and drainage, when appropriate, followed by the use of antimicrobial therapy that is directed at *S. aureus*. If drainage is performed, it is appropriate to culture the fluid for bacterial pathogens and obtain susceptibility testing on bacteria grown from culture to ensure that the antimicrobial therapy is optimal and effective (Stevens et al. 2014).

Recommended choices for empiric antimicrobial therapy of uncomplicated SSTIs are summarized in Table 2 (Stevens et al. 2014). For uncomplicated SSTIs, in addition to incision and drainage when indicated, oral antibiotic therapy increases the likelihood of cure and may decrease the risk of recurrence (Daum et al. 2017; Talan et al. 2016).

For empiric therapy of an uncomplicated SSTI, if MRSA comprises <10% of *S. aureus* locally causing uncomplicated SSTIs, some authors recommend only drugs active against MSSA (Liu et al. 2011). If MRSA accounts for >10% of *S. aureus* SSTIs in a region, antimicrobial agents should be chosen to target MRSA and MSSA according to local antibiogram data.

The majority of *S. aureus*, both MSSA and MRSA, isolated from human infections are resistant to penicillin (Chambers 2001). For empiric or targeted

Table 2 Antimicrobial drugs for therapy of *S. aureus* invasive and noninvasive infections [in part adapted from (Baddour et al. 2015; Liu et al. 2011; Drew 2007)]

Drug	Typical adult dose*	Typical pediatric dose*	Comment
Oral agents			
Oral antistaphylococcal β-lactam	Varies by drug	Varies by drug	Optimal choice for MSSA infections; MRSA is resistant to this class
Clindamycin	300–450 mg TID	10–13 mg/kg/dose every 6–8 h (total <40 mg/kg/day)	Concern for nausea; increases risk of *Clostridium difficile* disease; may decrease risk of recurrent infection; no adjustment needed for renal or liver dysfunction; useful for inhibiting ribosomal production of toxins when there is suspicion for severe, toxin-mediated infection
Trimethoprim (TMP)-sulfamethoxazole (SMX)	1–2 DS tabs BID	TMP 4–6 mg/kg/dose; SMX 20–30 mg/kg/dose every 12 h	Must adjust for renal dysfunction; may cause myelosuppression, hyperkalemia, or acute kidney injury; cannot be used with G6PD deficiency; hypersensitivity reaction occurs; avoid use in 3rd trimester of pregnancy; rare photosensitivity; rare Stevens-Johnson syndrome; avoid in children <2 months old; few data support use as monotherapy in invasive *S. aureus* infections; use with rifampin in osteomyelitis; often used for long-term suppression of *S. aureus* foreign body infections
Doxycycline	100 mg BID	≤45 kg: 2 mg/kg/dose every 12 h; >45 kg: adult dosing	Risk of rash with sun exposure; few adverse effects; avoid in children <8 years old; gastrointestinal intolerance possible; poorly studied for therapy of invasive infections; often used for long-term suppression of *S. aureus* foreign body infections

(continued)

Table 2 (continued)

Drug	Typical adult dose*	Typical pediatric dose*	Comment
Minocycline	200 mg once and then 100 mg BID	4 mg/kg once and then 2 mg/kg/dose thereafter every 12 h	Avoid in children <8 years old; gastrointestinal intolerance possible; poorly studied for therapy of invasive infections
Linezolid	600 mg po BID	10 mg/kg every 8 h (total $\leq$ 600 mg/dose)	Concern for myelosuppression, particularly thrombocytopenia, with >14 days of use; rare neuropathy; gastrointestinal intolerance occurs; serotonin syndrome may result if used with SSRIs, MAO inhibitors, and sympathomimetic drugs; no adjustment needed for renal or liver dysfunction; coverage for many anaerobes; bacteriostatic and therefore not recommended as monotherapy for bacteremia; in children >11 years old some would add linezolid 600 mg BID to primary therapy for MRSA meningitis; generic formulations likely to be available; useful for inhibiting ribosomal production of toxins
Tedizolid	200 mg po daily	Undetermined	Approved in U.S. for SSTIs; less likely to cause myelosuppression and fewer drug interactions than linezolid; no adjustment needed for renal or liver dysfunction; *S. aureus* not resistant when it carries the *cfr* gene

(continued)

Table 2 (continued)

Drug	Typical adult dose*	Typical pediatric dose*	Comment
Rifampin	300 mg po (or IV) every 12 h	5 mg/kg/dose every 8 h	Not for monotherapy; valuable in combination in the setting of a foreign body infection; many drug interactions; no oral suspension available; avoid in liver disease if possible or monitor liver tests; hypersensitivity reaction occurs; indicated in *S. aureus* prosthetic valve endocarditis (6 weeks) in addition to vancomycin (6 weeks) plus gentamicin (2 weeks) for MRSA, and in addition to an antistaphylococcal β-lactam (6 weeks) plus gentamicin (2 weeks) for MSSA; some add rifampin (300-450 mg po BID) to vancomycin for MRSA meningitis in adult patients
*Intravenous agents***			
Vancomycin	15–20 mg/kg/dose IV every 8–12 h	15 mg/kg/dose IV every 6 h	Renal insufficiency is leading adverse effect; much less common marrow suppression; must monitor serum trough level; rare Redman syndrome can be addressed with slowed infusion; VISA strains may be more common over time, so must monitor MIC and change to alterative therapy if MIC rises; first-line therapy for most invasive MRSA infections
Daptomycin	6–10 mg/kg/dose IV every 24 h	1 to <2 years: 10 mg/kg IV; 2–6 years: 9 mg/kg; 7–11 years: 7 mg/kg; 12–17 years: 5 mg/kg IV, and all regimens are dosed every 24 h	Concern for rhabdomyolysis so must monitor CK weekly; rare eosinophilic pneumonia; dosage used varies; can be used as monotherapy for

(continued)

Table 2 (continued)

Drug	Typical adult dose*	Typical pediatric dose*	Comment
			bacteremia and other invasive infections although resistance may emerge on therapy; not appropriate sole salvage therapy for vancomycin failure
Linezolid	600 mg IV BID	10 mg/kg IV every 8 h (total $\leq$600 mg/dose)	Concern for myelosuppression, particularly thrombocytopenia with >14 days of use; serotonin syndrome may result if used with SSRIs or MAO inhibitors; no adjustment needed for renal or liver dysfunction; coverage for many anaerobes; bacteriostatic and therefore not recommended as monotherapy for bacteremia; in children >11 years old some would add linezolid 600 mg BID to primary therapy for MRSA meningitis; generic formulations likely to be available; useful for inhibiting ribosomal production of toxins
Tedizolid	200 mg IV daily	Undetermined	Less likely to cause myelosuppression and fewer drug interactions than linezolid; no adjustment needed for renal or liver dysfunction; high cost; approved in the U.S. for skin infections; not studied in controlled trials for invasive infections and thus its efficacy is not known
Tigecycline	100 mg IV once and then 50 mg every 12 h thereafter	8–11 years: 1.2 mg/kg every 12 h; 12–17 years: 50 mg every 12 h	Approved in U.S. for complicated intra-abdominal infections, CAP, and skin and soft tissue infections; Black box warning

(continued)

Table 2 (continued)

Drug	Typical adult dose*	Typical pediatric dose*	Comment
			because death rate was increased relative to comparator drug groups in clinical trials; nausea, vomiting, and diarrhea are common adverse effects; no adjustment needed for renal dysfunction or for mild hepatic impairment; should not be used in pregnancy; avoid in children <8 years of age as for all tetracycline derivatives
Ceftaroline	600 mg IV every 12 h	2 months to <2 years old: 8 mg/kg IV every 8 h 2–18 years old: $\leq$ 33 kg: 12 mg/kg IV every 8 h; >33 kg: 400 mg IV every 8 h OR 600 mg every 12 h	Well tolerated; approved for complicated skin infections (ABSSSI) and Gram-positive CAP; no controlled data for use in other invasive infections
Dalbavancin	1500 mg IV once	Undetermined	Single dose for complicated skin infections (ABSSSI); long half-life (346 h) is a benefit but concern for removal if adverse reaction occurs; few adverse effects; no controlled data available to support its use in invasive infections
Oritavancin	1200 mg IV once	Undetermined	Single dose for complicated skin infections (ABSSSI); long half-life (393 h) is a benefit but concern for removal if adverse reaction occurs; few adverse effects; no controlled data available to support its use in invasive infections
Telavancin	10 mg/kg IV daily	Undetermined	Avoid in renal failure; black box warning for higher mortality in patients with renal insufficiency; approved in U.S. for skin and soft

(continued)

Table 2 (continued)

Drug	Typical adult dose*	Typical pediatric dose*	Comment
			tissue infection therapy and hospital-acquired pneumonia; few adverse effects
Quinupristin/dalfopristin	7.5 mg/kg IV every 8–12 h	Undetermined	Severe myalgias; must be administered through a central venous catheter; may cause hyperbilirubinemia; monitor liver tests in blood; many drug interactions; reserved for use in infections that are very resistant or in which few other options exist

Abbreviations: *ABSSSI* acute bacterial skin and skin structure infection; *BID* twice daily; *CAP* community-acquired pneumonia; *CK* creatine kinase; *IV* intravenous; *kg* kilogram; *mg* milligrams; *MAO* monoamine oxidase; *MSSA* methicillin-susceptible *Staphylococcus aureus*; *MRSA* methicillin-resistant *S. aureus*; *po* oral; *SSRI* selective serotonin reuptake inhibitor; *TID* three times per day; *VISA* vancomycin-intermediate *S. aureus*

*May require adjustment for renal function, liver function, or other factors

**Not included here are intravenous β-lactam antibiotics, which are often used for the treatment of MSSA infections

therapy for MSSA, an antistaphylococcal β-lactam drug is the most appropriate choice, usually administered for 10 days. The optimal duration is not certain because different durations of therapy have been used in different studies. These drugs include oral agents such as cloxacillin, dicloxacillin, or cephalexin or another oral cephalosporin.

For an uncomplicated SSTI in which MRSA is suspected or proven, in a locale where MRSA is frequent, or an allergy to β-lactam drugs is documented or suspected, recommended enteral therapy includes clindamycin (Miller et al. 2015), TMP-SMX (Cenizal et al. 2007; Miller et al. 2015; Szumowski et al. 2007; Talan et al. 2016), doxycycline (Ruhe and Menon 2007; Ruhe et al. 2007), or, less often, linezolid (Itani et al. 2010), an oxazolidinone that targets the bacterial ribosome. Resistance to TMP-SMX among clinical *S. aureus* isolates has remained rare, even with increased use of the drug during the CA-MRSA era (McDougal et al. 2010; Wood et al. 2012). Minocycline, another tetracycline antibiotic, has been used for empiric SSTI therapy by some (Bishburg and Bishburg 2009; Carris et al. 2015; Colton et al. 2016). However, minocycline has not been well studied, has been associated with skin and nail hyperpigmentation (Ali et al. 2015), and should not be used in children younger than 9 years of age. Tedizolid is a new orally available oxazolidnone that is noninferior to linezolid in the therapy for acute bacterial skin and skin structure infections (ABSSSIs) (Durkin and Corey 2015; Moran et al. 2014; Prokocimer et al. 2013). It has several advantages over linezolid, including

fewer drug interactions, possibly a shorter duration of therapy, activity in the presence of the *cfr* gene (Chen et al. 2014), and a lower incidence of thrombocytopenia, but its use is limited by lack of experience and high cost (Rybak et al. 2014; Rybak and Roberts 2015; Zhanel et al. 2015). Susceptibility testing for tedizolid is often not available, but linezolid susceptibility is generally a reliable surrogate (Zurenko et al. 2014). The specific choice of antimicrobial agent should be dictated by local antibiogram data. Usually one or more of the oral drugs discussed here will be effective against *S. aureus* isolated from uncomplicated SSTIs diagnosed in the community or in the healthcare setting.

For patients with an SSTI who cannot tolerate or absorb enteral therapies, when multiple allergies are present, when adverse effects of orally available drugs occur, when a deep soft tissue infection is suspected, or when an unusually resistant isolate of *S. aureus* is cultured from an SSTI, intravenous antistaphylococcal agents may be indicated. These alternatives are discussed below, in relation to complicated infections.

Signs of failure of an antibiotic regimen for a *S. aureus* skin infection include lack of improvement in or worsening of erythema, tenderness, edema, drainage and warmth at the site of SSTI while on therapy after approximately 72 h. Also persistence of fever and an elevated peripheral white blood cell count after this period of therapy may suggest failure. In such cases, the clinician should reassess the need for additional drainage of a protected focus of infection, removal of foreign bodies if relevant, or the possibility of an occult site of infection, including bacteremia. The clinician may also consider a change in or an addition to the antimicrobial agent chosen as initial therapy.

3.2 Invasive Infections

S. aureus can cause a host of different invasive infection syndromes. These may occur in the previously healthy host although they are more common in patients who have comorbid conditions. *S. aureus* infections can occur in any organ. The most common pathologic lesion of human tissues caused by *S. aureus* infections is an abscess, a collection of purulent fluid surrounded by a fibrous capsule that walls off the infection. Abscesses can form, for example, in the skin and subcutaneous tissues, muscles, bones, intra-abdominal organs, the peritoneum, the walls of blood vessels, the lung, or the central nervous system. Bacteremia, or bloodstream infection, occurs frequently as a complication of an initial focus of infection in the body or as a complication of an indwelling vascular catheter. Bloodstream infections can then be complicated by seeding of organs or bones. One of the most serious complications of a bloodstream infection is endocarditis, as discussed in detail below. Invasive *S. aureus* infections may manifest as sepsis, severe sepsis, or septic shock. Septic shock caused by *S. aureus* has a fatality rate of >20% (van Hal et al. 2012).

The pathogenesis of invasive infections may in some cases be driven by the elaboration of staphylococcal toxins. For example, certain superantigen toxins (e.g.,

toxic shock syndrome toxin [tsst]-1 and tsst-2) produced by some strains of *S. aureus* cause nonspecific activation of T cells and can lead to toxic shock syndrome. Other toxins, known as leukocidins, bind and kill human white blood cells. Among these are PVL, leukocidin AB/GH (LukAB/GH), and leukocidin ED (LukED) (Alonzo and Torres 2014). PVL, specifically associated with a number of CA-MRSA strain types (including USA300) as noted above, may be responsible for dermonecrosis in skin lesions and for necrotizing pneumonia (Lina et al. 1999). A third class of toxin genes is the enterotoxins. These are associated with food poisoning leading to gastroenteritis when a toxin is ingested, but when inoculated, these toxins may lead to shock. Staphylococcal scalded skin syndrome (sometimes called Ritter von Ritterschein disease or Ritter disease) is caused by exfoliative toxins (ETA or ETB). In this syndrome, there is blistering of the skin leading to desquamation varying in severity. This syndrome is distinguished from pemphigus vulgaris by the presence of Nikolsky's sign, which is considered to be positive when a shearing force applied to skin results in sloughing of the superficial epidermis.

Treatment of invasive *S. aureus* infections must be tailored to the specific circumstances of each patient, but the primary treatment has features in common with those of noninvasive infections: drainage of relevant foci, removal of foreign bodies, if possible, and therapy with an effective antimicrobial agent. In general, intravenous (IV) therapy is indicated for invasive *S. aureus* infections although if long-term therapy exceeds many weeks, if the patient can adequately absorb an oral drug, and if an oral drug is available to which the infecting *S. aureus* isolate is susceptible, many experts agree that transition to oral therapy is appropriate.

For most invasive infections, an IV β-lactam for MSSA and IV vancomycin for MRSA are usually the first-line therapy in the USA as Guidelines suggest (Liu et al. 2011). In many countries, teicoplanin can be used in place of vancomycin. Alternative parenteral agents may be appropriate in specific situations, and they are listed in Table 2. Alternatives are required when the minimum inhibitory concentration (MIC) of vancomycin is >2 mcg/mL, as in the case of certain VISA or heterogeneous VISA (hVISA) isolates (Casapao et al. 2013), or when serial isolates obtained from a source of infection during vancomycin therapy in a patient show a rising trend in the MIC of vancomycin. hVISA isolates are *S. aureus* that are susceptible to vancomycin by broth microdilution testing, but additional testing reveals subpopulations that are resistant (Hiramatsu et al. 1997). Some patients cannot tolerate vancomycin because of severe Redman syndrome, a reaction caused by histamine release with flushing of the skin, or acute kidney injury during vancomycin administration. Vancomycin is inferior to antistaphylococcal β-lactam drugs in the therapy of MSSA infections (Liu et al. 2011).

There are a number of alternatives to vancomycin for the treatment of invasive infections (Table 2). Data supporting their use in treating specific *S. aureus* syndromes will be discussed below in detail. Linezolid is bacteriostatic and yet has been used successfully to treat many invasive infections, including pneumonia and osteomyelitis, since it was approved for use in 2000 (Chastre et al. 2014; Stevens et al. 2002). Daptomycin has a track record in the treatment of bacteremia and right-sided endocarditis. Both daptomycin and linezolid are active against MRSA as

well as MSSA. Tedizolid, like linezolid, is available in IV and enteral formulations, and it is likely that tedizolid is also effective in invasive infections although experience with this agent is limited (Rybak et al. 2014). Another newer agent is the cephalosporin ceftaroline, which was the first cephalosporin antibiotic that binds to PBP2a, resulting in effective killing of many strains of MRSA (Saravolatz et al. 2011; Stryjewski et al. 2015). Oritavancin (Corey et al. 2014, 2015) and dalbavancin (Boucher et al. 2014; Dunne et al. 2016) are new lipoglycopeptide antibiotics with very long half-lives of >10 days that have been approved only to treat complicated skin infections. These long-acting agents have a very good safety profile, but their role in the therapy of invasive infections has not yet been defined (Kaasch and Seifert 2016; Smith et al. 2015). Telavancin is another recently approved lipoglycopeptide that requires daily doing and is approved for hospital-acquired pneumonia as well as skin infections (Rubinstein et al. 2011; Sandrock and Shorr 2015; Stryjewski et al. 2008). Tigecycline, an analog of minocycline available only in a parenteral preparation, is often effective against MRSA and MSSA and has been approved for the treatment of skin infections, community-acquired pneumonia (CAP), and complicated intra-abdominal infections (Rose and Rybak 2006).

Novel compounds are being developed in a number of different antibiotic classes that may be effective in the treatment of MRSA infections (reviewed in Vuong et al. 2016), but these will not be discussed further in the present review.

4 Considerations in the Therapy of Specific Clinical Syndromes

4.1 Bacteremia

S. aureus is the second most common cause of bacteremia after *Escherichia coli*. In a study of Olmstead County, Minnesota in 1999–2005, the incidence of *S. aureus* bacteremia was 28.3/100,000 person-years in females, and 53.5/100,000 in males (El Atrouni et al. 2009). It is estimated that in Western countries, the incidence of community onset *S. aureus* bacteremia is 15/100,000 population (Laupland and Church 2014). Patients with bacteremia often present with fever, malaise, and fatigue, and the infection can quickly progress to life-threatening sepsis. Mortality from *S. aureus* bacteremia overall remains high (approximately 15–50%) despite optimal therapy (Allard et al. 2008; Tong et al. 2015).

In many countries, the incidence of nosocomial MRSA bacteremia decreased after the year 2000 (Dantes et al. 2013; Duerden et al. 2015; Newitt et al. 2015). The proportion of *S. aureus* bacteremia cases that is caused by MRSA varies greatly in different countries (Borg et al. 2012). In the USA, with the emergence of CA-MRSA strains, trends in MRSA epidemiology in bacteremia have varied geographically (David et al. 2014), and so knowledge of the local epidemiology of *S. aureus* is essential in choosing empiric therapy. For example, one study from an

urban public hospital in 2007–2013 showed that 36% of *S. aureus* bacteremia cases were caused by MRSA, and 70% of the isolates were USA300 (Rhee et al. 2015), suggesting that in cases of *S. aureus* bacteremia in that setting, empiric therapy for MRSA should be considered.

S. aureus bacteremia may result from direct inoculation of the blood although this is rare. In some cases, bacteremia with no identified source is diagnosed. More often, *S. aureus* bacteremia arises from a prior SSTI, surgical site infection (SSI), pneumonia, or a foreign body infection, including indwelling intravenous catheters.

The fundamental principles of therapy for bacteremia include identification of a primary site of infection, removal of an infected foreign body, if present, drainage or evacuation of a source of infection that cannot be sterilized with antimicrobial therapy alone, documentation that blood has become sterile, and therapy for the bacteremia with an effective antibacterial drug (Holland et al. 2014; Thwaites et al. 2011).

In the event that bacteremia is uncomplicated, that is, the source of bacteria is known and controlled, parenteral therapy for 14 days beginning on the first day with a negative blood culture is usually recommended (Liu et al. 2011). The choice of antibiotic for definitive therapy is determined by the antimicrobial susceptibility of the *S. aureus* isolate recovered in the blood culture. Agents of choice for empiric therapy are shown in Table 2. For MSSA bacteremia, the primary agent has traditionally been oxacillin, nafcillin or another semisynthetic, antistaphylococcal penicillin. Alternatives include cephalosporins; a recent retrospective cohort study demonstrated that there was no significant difference in mortality comparing cefazolin and nafcillin used for MSSA bacteremia, with less common renal injury in those treated with cefazolin (Flynt et al. 2017).

For MRSA bacteremia or in patients with penicillin allergy, vancomycin is the most common choice. Alternative agents for MRSA or suspected MRSA are used empirically in the event that there is high suspicion that the MIC of vancomycin is >2 or is rising rapidly on therapy, or if the patient cannot tolerate vancomycin. As noted above, if vancomycin is started empirically, it should be changed to a β-lactam drug in the event that MSSA is identified in a blood culture because vancomycin has been shown to be inferior to β-lactams in the therapy of MSSA infections (Liu et al. 2011).

The duration of therapy for bacteremia varies depending on the presence of a foreign body or seeding of internal organs as a complication of bacteremia (e.g., the lungs, heart, liver, spleen, kidney, or brain), often with the formation of one or more abscesses in these tissues (Thwaites et al. 2011). Source control is essential in the treatment process. It is essential to aggressively seek a source of infection to ensure that it is drained or otherwise treated in order to avoid recrudescence. This includes a recommendation to perform a transthoracic echocardiogram (TTE) in adult patients with *S. aureus* bacteremia to assess for a valvular vegetation, followed by a transesophageal echocardiogram (TEE) if the TTE is negative (Holland et al. 2014). The presence of a valvular vegetation would suggest infectious endocarditis (see below). TTE, however, is not recommended routinely in young children when blood cultures clear rapidly.

In the event that there is an infected foreign body, such as a central venous catheter or orthopedic, neurosurgical, cardiac, or other hardware, this foreign body should be removed if possible. If the foreign body cannot be removed, as is often true for pacemaker or defibrillator wires or a left- or right ventricular assist device (Athan et al. 2012), the risk of antibiotic failure exists. In other cases, the source may be infected tissue in the body, a septic joint, infected bone, soft tissue abscess (including infected deep skin ulcers), or an abscess in an internal organ. For osteomyelitis, surgical drainage and debridement is not usually required for cure unless there is a large sequestrum that fails to resolve with antimicrobial therapy. For other sites of infection, debridement, drainage, and in extreme cases, amputation may be required for cure. These surgical interventions should be performed as soon as possible if required.

Alternatives to vancomycin may be indicated in certain cases of bacteremia for optimal tissue penetration, if there is an adverse reaction to vancomycin, or if there is an increased MIC of vancomycin (Table 2). Daptomycin has been found to be noninferior to IV vancomycin in bacteremia associated with right-sided endocarditis (Fowler et al. 2006), and daptomycin was superior in clinical success in the therapy of *S. aureus* bacteremia in the absence of pneumonia in one retrospective study (failure 45% with vancomycin vs. 29% with daptomycin, $p = 0.007$) (Claeys et al. 2016). Daptomycin (recently reviewed in Holubar et al. 2016) thus remains an alternative to vancomycin although due to cross resistance, it is not an optimal single agent for salvage therapy in the event that vancomycin fails due to high MIC (Cui et al. 2006). Also of concern, resistance frequently emerges on daptomycin therapy (Fowler et al. 2006). The most common serious adverse reaction to daptomycin is rhabdomyolysis (Gonzalez-Ruiz et al. 2016), and it has also been associated with rare cases of eosinophilic pneumonia (Hirai et al. 2017; Kakish et al. 2008; Kalogeropoulos et al. 2011; Lal and Assimacopoulos 2010; Miller et al. 2010; Patel et al. 2014; Nickerson et al. 2017).

Tigecycline is an alternative to vancomycin for patients older than 9 years of age (Cai et al. 2011; Stein and Babinchak 2013). Tigecycline has efficacy against many Gram-negative as well as Gram-positive pathogens, which is an important advantage. However, a black box warning on tigecycline was announced by the US FDA in 2013 because it was found that patients treated with this drug in registration trials had an increased mortality risk relative to comparator regimens used in clinical trials (Tsoulas and Nathwani 2015). This warning has dampened the initial enthusiasm for this drug. While tigecycline is not licensed for use in the USA to treat bacteremia, it has been used successfully in the treatment of bacteremia secondary to SSTIs, intra-abdominal infections and CAP (Gardiner et al. 2010). It is particularly useful in settings of a concomitant intra-abdominal infection, when a broad-spectrum antibiotic is beneficial. However, it has not been studied in controlled trials to treat bacteremia and therefore is not a recommended agent.

Quinupristin/dalfopristin is an antibiotic fixed combination that has been used as salvage therapy for resistant Gram-positive bacteremia (Eliopoulos 2003). However, its use is infrequent because it causes severe arthralgias and myalgias that often require narcotic therapy for pain control (Rubinstein et al. 1999).

A newer cephalosporin, ceftaroline, the first cephalosporin licensed in the USA that has efficacy against most circulating MRSA strains, has been approved for the treatment of acute bacterial skin and skin structure infections (ABSSSI) (Corey et al. 2010; Wilcox et al. 2010) and CAP (File TM Jr et al. 2011; Low et al. 2011). Ceftaroline has been used as salvage therapy in the setting of vancomycin or daptomycin failure. It has excellent penetration into the cerebrospinal fluid. While effective against some Gram-negative pathogens, it is not a broad-spectrum drug that is appropriate empiric therapy for suspected Gram-negative infections because the risk of failure is high. In combination with avibactam, a β-lactamase inhibitor, its spectrum of Gram-negative activity is much broader (Karlowsky et al. 2013). Resistance to ceftaroline in *S. aureus* may be related to mutations in the *mecA* gene, which codes for PBP2a (Alm et al. 2014; Harrison et al. 2016; Lahiri et al. 2015; Schaumburg et al. 2016) and resistance has been found to be common in ST239 and ST228 MRSA isolates (Abbott et al. 2015; Mubarak et al. 2015; Strommenger et al. 2015). Successful therapy for MSSA or MRSA bacteremia with ceftaroline, often in combination with another antimicrobial agent, has ranged from 31 to 83.3% (Arshad et al. 2017; Burnett et al. 2016; Paladino et al. 2014; Sakoulas et al. 2014; Vazquez et al. 2015; White et al. 2017). For example, in 2011–2013 in Detroit a retrospective study was performed with 30 MRSA bacteremia patients treated with ceftaroline compared with 102 control patients treated with daptomycin or vancomycin; a composite failure outcome did not differ significantly in the 30 ceftaroline-treated patients compared with the controls (23.3% vs. 21.6%) (Arshad et al. 2017).

Ceftobiprole, another cephalosporin that binds to PBP2a, is not licensed in the USA but is licensed in Europe. It has shown efficacy against *S. aureus* in vitro (Chung et al. 2008; Farrell et al. 2014; Yun et al. 2007), and also in *S. aureus* pneumonia (Liapikou et al. 2015; Scheeren 2015) and bacteremia (Dauner et al. 2010). It will not be discussed further in this article.

Linezolid is a bacteriostatic drug that is not usually recommended for the treatment of bacteremia. Similarly, the newer, related agent tedizolid, which has been approved by the US FDA for skin infections, does not yet have a role in the therapy of bacteremia.

The three recently licensed parenteral lipoglycopeptides, oritavancin, dalbavancin, and telavancin have been approved by the US FDA for the treatment of skin infections, as noted above. Only a small case series has been published on the use of oritavancin in invasive infections (Stewart et al. 2017). Telavancin, which has been shown in vitro to be effective against MRSA (Karlowsky et al. 2015; Rodvold 2015), in addition to skin infections (Stryjewski et al. 2008), has been approved to treat hospital-acquired pneumonia (Rubinstein et al. 2011; Sandrock and Shorr 2015). A case series of refractory MRSA bacteremia or endocarditis treated with telavancin showed 57% survival (Ruggero et al. 2015), while in another series of uncomplicated *S. aureus* bacteremia, cure was achieved in 88% (Stryjewski et al. 2014). Telavancin, however, has a black box warning that states that the drug should not be used in patients with renal insufficiency as there was an elevated risk of death in patients treated with telavancin in this population (Tsoulas and Nathwani 2015). The role of these the novel lipoglycopeptide agents in the

treatment of bacteremia is still undefined as there are few available data for this indication. Therefore, they are not recommended for the therapy of *S. aureus* bacteremia at this time.

Indications of failure of a drug regimen for *S. aureus* bacteremia include persistence of positive blood cultures, persistence of fever, fatigue, and other presenting symptoms, and also a rising MIC of the antibacterial drug being used to treat the bacteremia in sequentially recovered *S. aureus* isolates. Persistence of sepsis is, of course, possible and is a particularly concerning sign of treatment failure. Often in cases of failure, the problem is not the choice of drug, but rather a secondary focus that must be drained or removed. The clinician in these circumstances therefore should pursue an aggressive search for a secondary focus of infection, particularly endocarditis, septic arthritis, osteomyelitis, septic pulmonary emboli, foreign body infections, intervertebral disc infections, or epidural abscesses. The clinician should certainly consider changing therapy, particularly if there is no secondary focus found and/or if there is a rising MIC of the antibacterial drug being used.

4.2 Endocarditis and Intravascular Infection

When *S. aureus* bacteremia is diagnosed in an adult, as noted above, a TTE followed by a TEE, if the TTE is negative, is indicated in order to assess for echocardiographic evidence of a cardiac valvular vegetation. In children TTE may be an adequate study to assess for evidence of a vegetation because the chest wall is thinner. When a vegetation is present, *S. aureus* endocarditis should be suspected. Endocarditis, however, is diagnosed when the Duke criteria for infectious endocarditis are met (Li et al. 2000). The classical physical findings of endocarditis, such as subungual or conjunctival splinter hemorrhages, Janeway lesions, and Osler nodes, may be present although they are now rarely seen (Hoen and Duval 2013). However, fever, malaise, and sepsis are common at presentation. *S. aureus* endocarditis, including MRSA strains, may arise as a complication of other anatomic sites of infection, such as skin abscesses (Bahrain et al. 2006). Overall mortality from *S. aureus* endocarditis varies in different published studies, and ranges from 26 to 40% have been reported (Abdallah et al. 2016; Bassetti et al. 2017). Mortality from infective endocarditis among people who used IV drugs tends to be much lower (Asgeirsson et al. 2016). Embolic phenomena occur as complications of infective endocarditis, such as septic pulmonary, splenic, renal, or brain emboli, which themselves can cause local abscesses or a myriad of additional sequelae (Hoen and Duval 2013).

Septic pulmonary embolism is a vascular complication of hematogenous spread of bacteria from any site of acute infection. In a systematic review of 76 publications, a blood culture was obtained in 151 of 168 reported cases of septic pulmonary emboli. In the review, *S. aureus* was obtained in 55% (75/137) of cases with a positive blood culture and was the leading cause of septic pulmonary emboli (Ye et al. 2014).

For endocarditis, it is recommended that antibiotic therapy be started as soon as possible and that source control be obtained if there is an identified focus of infection to drain or remove aside from the infected site in the heart. Right-sided endocarditis generally responds to antimicrobial treatment more readily and is associated with fewer complications than is left-sided endocarditis.

In most cases of uncomplicated native valve *S. aureus* endocarditis, antimicrobial therapy alone is recommended for 6 weeks after the blood culture becomes sterile. For MSSA, the first-line choice of therapy is oxacillin, nafcillin, or another antistaphylococcal penicillin. Alternatives include first-generation cephalosporins such as cefazolin, vancomycin, and daptomycin. For MRSA native valve endocarditis, vancomycin remains an effective therapy and is recommended as the first-line choice, while daptomycin is also a recommended option (Baddour et al. 2015; Liu et al. 2011). For prosthetic valve endocarditis with MSSA, nafcillin or oxacillin plus rifampin for 6–8 weeks plus gentamicin for the first 2 weeks is recommended. Rifampin is included because it is effective in treating biofilm-associated infections in the setting of a foreign body (Jørgensen et al. 2016). For MRSA prosthetic valve endocarditis or for MSSA prosthetic valve endocarditis in the setting of penicillin allergy, vancomycin plus rifampin for 6–8 weeks plus gentamicin for the first 2 weeks is recommended (Baddour et al. 2015; Liu et al. 2011). Evidence for the use of newer agents for the treatment of endocarditis is limited to case reports and case series for regimens including ceftaroline in combination with daptomycin (Baxi et al. 2015; Cunha and Gran 2015; Fabre et al. 2014; Ho et al. 2012; Jongsma et al. 2013; Lin et al. 2013b; Nigo et al. 2017; Pagani et al. 2014; Tattevin et al. 2014).

The decision to perform a valve replacement surgery in *S. aureus* endocarditis must be individualized, taking into account the risks of the surgery and the anticipated complications of medical therapy alone (Cahill et al. 2017). Surgical intervention in native valve *S. aureus* endocarditis for valve replacement or repair is often necessary. For prosthetic valve endocarditis caused by *S. aureus*, this surgery should be strongly considered in all patients to replace the prosthetic valve. Valve replacement evaluation is indicated in any *S. aureus* endocarditis for moderate to severe congestive heart failure, persistent bacteremia or fever on optimal therapy, presence of an unstable prosthetic valve, extension of the infection beyond the valve into the endomyocardium, a vegetation >10 mm in size, new heart block, one or more embolic event occurring in the first 14 days of treatment, and relapse of endocarditis after completion of antibiotic therapy (Liu et al. 2011).

4.3 Pneumonia

S. aureus pneumonia can be severe and can result in necrosis of lung tissue, respiratory failure, empyema, bacteremia, and death. While *S. aureus* is a well-described cause of CAP, it is relatively uncommon (Aliberti et al. 2016). Certain comorbid conditions, including cystic fibrosis, are associated with a high risk of *S. aureus* pneumonia (Wong et al. 2013) while pneumonia in patients

without underlying lung disease is rarely caused by *S. aureus*. Nosocomial pneumonia is more commonly caused by *S. aureus*, both MRSA and MSSA, often as a complication of intubation or preexisting respiratory disease. Severe necrotizing *S. aureus* pneumonia has been reported by many authors often, but not always, complicating influenza and other respiratory viruses (Al-Tawfiq and Aldaabil 2005; Chickering and Park 1919; Shah et al. 2015; Spencer and Thomas 2014; Valentini et al. 2008), and some have found an association of necrotizing pneumonia and PVL-positive *S. aureus* strains (Lina et al. 1999; Rájová et al. 2016; Schwartz and Nourse 2012). Mortality in *S. aureus* pneumonia is high. For example, in one case series from Spain in 2000–2014, 30-day mortality among 98 patients with *S. aureus* pneumonia with bacteremia was 46.9% (De la Calle et al. 2016).

Treatment of *S. aureus* pneumonia can often be accomplished with an effective antibiotic for 7 days, although longer courses may be required for more severe infections. For MSSA, recommended antimicrobials include antistaphylococcal penicillins, such as nafcillin or oxacillin, and if the pathogen is susceptible, clindamycin or linezolid. For MRSA, vancomycin remains the first-line therapy. Alternatives would be considered when vancomycin is contraindicated or if there is an elevated MIC of vancomycin. They include linezolid and telavancin. The former should be avoided in patients with thrombocytopenia or leukopenia (Zahedi Bialvaei et al. 2017) and in those taking selective serotonin reuptake inhibitors (SSRIs) or monoamine oxidase (MAO) inhibitors (Huang and Gortney 2006), and the latter should be avoided in patients with renal insufficiency (Tsoulas and Nathwani 2015). There are no data to support the use of tedizolid for pneumonia, but it may be useful for this indication. Daptomycin, which is inactivated by pulmonary surfactant, is not recommended (Silverman et al. 2005). Tigecycline is approved for the treatment of community-acquired bacterial pneumonia (Townsend et al. 2011) although there are limitations to its use, noted above. Ceftaroline is also approved for the therapy of community-acquired bacterial pneumonia, having proved noninferior to comparator drugs in 2 adult Phase III trials and in a recent RCT in children as well (Blumer et al. 2016; File TM Jr et al. 2011; Low et al. 2011).

For less severe cases of pneumonia with no evidence of lung necrosis, treatment is often started with an intravenous agent and transitioned to an enteral agent to complete therapy after the patient's condition has improved.

For severe pneumonia, especially with necrosis of pulmonary tissue, the complete course of therapy should often be administered intravenously, and a course of 3 weeks or more is generally indicated, particularly if there is suspicion of extensive vascular involvement of the infection. If the infection is complicated by empyema, drainage of pleural fluid is often necessary using a chest tube, and decortication is sometimes indicated by video-assisted thoracoscopic surgery or an open procedure. A recent review of 8 RCTs did not find a mortality difference in empyema patients with a variety of pathogens who had surgical intervention compared with those who did not (Redden et al. 2017), but it is possible that drainage is more important for cure in cases of *S. aureus* empyema than in empyema with other bacterial species.

4.4 Osteomyelitis, Including Discitis

Osteomyelitis caused by *S. aureus* can occur as a complication of bacteremia, with seeding of a bone by hematogenous spread, or by direct extension from a site of infection in an adjacent tissue. *S. aureus* is the most common cause of bacterial osteomyelitis (Lew and Waldvogel 2004; Russell et al. 2015; Tong et al. 2015). Some have found that acute osteomyelitis was more severe when caused by a MRSA strain that carries the PVL toxin than when caused by a PVL-negative *S. aureus* strain (Kaplan 2014). Discitis (i.e., infection of the intervertebral discs) is sometimes diagnosed in the absence of osteomyelitis, including in children (Tyagi 2016).

Acute hematogenous osteomyelitis is more common in children, with an incidence of 1 in 4000, estimated at 2 pediatric hospitals in New Zealand in 1997–2007 and 1998–2008, respectively. Among 813 cases of acute hematogenous osteomyelitis in New Zealand, 50% had a cultured bacterial cause, and *S. aureus* was identified in 81% (339/417) (Street et al. 2015). In children, bacterial osteomyelitis was estimated to have an incidence of 1.2 per 100,000 population in Germany, and *S. aureus* was the cause of 83% of cases (Grote et al. 2017). In a study from Edinburgh in 2007–2013, *S. aureus* was responsible for 20/26 cases of pediatric osteomyelitis with or without septic arthritis in which a pathogen was recovered (Russell et al. 2015). In a hospital in Florence, Italy, *S. aureus* was the most common cause of acute hematogenous osteomyelitis in a study of 121 children in 2010–2015, and just 10% of *S. aureus* cases were MRSA strains (Chiappini et al. 2017). In the USA, in contrast, an increasing proportion of pediatric osteomyelitis was caused by MRSA strains after 2001 and in some studies have accounted for >50% of *S. aureus* (Arnold et al. 2006; Bocchini et al. 2006; Saavedra-Lozano et al. 2008).

Vertebral osteomyelitis is most often caused by *S. aureus* (Corrah et al. 2011; Pigrau et al. 2005) and is more common in adults than in children. When it occurs, it is often a complication of bacterial endocarditis although it is estimated that the condition occurs in <2% of cases of infective endocarditis (Mansur et al. 1992). For example, one study from Spain showed that of 91 cases of pyogenic vertebral osteomyelitis excluding patients after trauma or surgery, 30.8% were complications of endocarditis. *S. aureus* was the etiology in 42.9% of patients with endocarditis and in 39.7% in patients without endocarditis (Pigrau et al. 2005). Other studies have shown that vertebral osteomyelitis was only rarely a complication of endocarditis, perhaps because of differences in study inclusion criteria.

Vertebral osteomyelitis is often accompanied by infection of the intervertebral discs (i.e., discitis) in adults although this is less common in children (Tyagi 2016). Neurologic complications of vertebral osteomyelitis may result from local spread to adjacent tissues, including the development of an epidural abscess (McHenry et al. 2002; Pigrau et al. 2005). Among 253 patients with 255 episodes of vertebral osteomyelitis in 1950–1994 followed up for a median of more than 6.5 years at the Cleveland Clinic, the most common cause was *S. aureus*. In assessing outcomes,

the study found that 11.3% died within 1 year, 14% had a recurrence, and 31% recovered with some residual pain, weakness, impaired mobility or other debility (McHenry et al. 2002). It is therefore important that vertebral osteomyelitis be diagnosed and treated promptly in order to reduce the likelihood of such sequelae.

Diagnosis of osteomyelitis at any body site in both children and adults requires imaging. A plain X-ray or a radionuclide bone scan may be diagnostic, but MRI is more sensitive (Browne et al. 2008; Simpfendorfer 2017). The ESR and CRP are usually higher than normal.

S. aureus is not an uncommon pathogen in polymicrobial diabetic foot soft tissue infections that can extend to bone, ligaments, and tendons, and thus it is important that empiric therapy for these infections, including those with community onset, includes coverage for MRSA in high-burden countries such as the USA.

Treatment of *S. aureus* adult osteomyelitis may rarely require surgical drainage of infected bone abscesses or debridement of necrotic bone followed by a minimum of 6 weeks of antimicrobial therapy although the IDSA Guidelines for MRSA recommend a minimum of 8 weeks (Liu et al. 2011). For acute hematogenous osteomyelitis in children, surgical drainage may be needed for cure, in addition to antimicrobial therapy (Kaplan 2014). Acute hematogenous osteomyelitis in children may be treated for 4–6 weeks (Liu et al. 2011). For children and adults surgical intervention may be required for cure in the presence of other comorbidities such as diabetes or peripheral arterial disease, prior exposure to radiation at the site of infection, presence of an open ulcer exposing bone, and involved foreign bodies (Liu et al. 2011).

Evidence is lacking to support the choice of intravenous or oral route of administration of antimicrobial therapy for osteomyelitis. Many practitioners treat with an intravenous agent for the initial period of treatment and then use oral therapy for the latter part of the course. Our general practice is treat with intravenous therapy for the first 2 weeks and then change to oral therapy, although this pattern differs depending on the antibiogram of the infecting pathogen, tolerability of the optimal antimicrobial agents, concerns about adherence with therapy, and the severity and complications of the osteomyelitis being treated. The total duration is generally determined by the resolution of bone inflammation as suggested by improvement in pain and normalization of inflammatory markers in the blood, such as the ESR or the CRP; some rely on normalization the CRP alone as a marker of resolution. However, these tests of systemic inflammation are not as useful in patients with chronic inflammatory conditions, and their normalization cannot be regarded as a definitive test of cure (Liu et al. 2011).

Specific considerations in choosing an antimicrobial agent to treat *S. aureus* osteomyelitis include the ability of the agent to penetrate bone and to reach appropriate concentration there (Liu et al. 2011). For MSSA, a β-lactam drug is first-line therapy. For MRSA or for MSSA osteomyelitis in patients with a β-lactam allergy, vancomycin or daptomycin is recommended. Three alternatives are TMP-SMX plus oral rifampin; linezolid; or clindamycin. Some recommend the addition of oral rifampin to the latter two regimens (Liu et al. 2011) (Table 2).

While there are no randomized studies for the value of surgical intervention in the treatment of vertebral osteomyelitis in addition to antibiotic therapy, in cohort studies and case series, surgical intervention has been required for cure in up to half of cases (Chelsom and Solberg 1998; McHenry et al. 2002; Ozuna and Delamarter 1996). Some experts recommend 4–6 weeks of intravenous therapy followed by 3–6 months of oral therapy (Ozuna and Delamarter 1996). As for therapy of other sites of osteomyelitis, the duration for vertebral osteomyelitis is usually guided by improvement or resolution of symptoms and normalization of CRP and/or ESR. The choice of antimicrobial agent is the same as for other sites of osteomyelitis unless a concomitant central nervous system (CNS) infection is suspected (see below) (Table 2).

4.5 Epidural Abscess

The epidural abscess caused by *S. aureus* is a complication of bacteremia or direct spread from infected tissues, sometimes after spinal surgery. It is a rare condition in children although there are case reports in both neonates (Stewart et al. 2015) and older children (Vallejo et al. 2017). Epidural abscesses can lead to meningitis, vertebral osteomyelitis, spread to cause adjacent soft tissue infections, cord compression, or persistence of bacteremia with distant metastatic infection (Shioya et al. 2012). It is often diagnosed by CT scan or MRI. *S. aureus* is the most common cultured etiology (Tong et al. 2015). Epidural abscesses may complicate intrathecal injections and should be considered in the differential diagnosis of back pain in patients who have recently undergone such procedures. An epidural abscess can also complicate the placement of a neurosurgical catheter, for example from intrathecal pumps or neurosurgically placed wires from stimulator devices. If an epidural abscess is diagnosed and there is no alternative source of infection, infective endocarditis should be considered.

Drainage of an epidural abscess can sometimes be accomplished by an interventional radiology procedure (when there is no indication for debridement of adjacent soft tissues) or it may require surgical management. The benefit of the former is that a surgery is avoided with all of its potential complications, while the latter may offer more definitive therapy in the case of complex or multiple abscesses or when soft tissues are involved that require debridement. Epidural abscesses should be evaluated by a neurosurgeon as they may require drainage (Liu et al. 2011; Suppiah et al. 2016). Drained fluid, if obtained, should be cultured so that optimal therapy can be chosen. This should be followed by intravenous antimicrobial therapy, as for other invasive *S. aureus* infections. A minimum duration of therapy of 4–6 weeks is often required for cure (Liu et al. 2011). For MSSA abscess, a β-lactam drug should be chosen as first-line therapy. For MRSA or for MSSA abscesses in patients with a β-lactam allergy intravenous vancomycin is recommended. Alternatives are intravenous TMP-SMX or oral or intravenous linezolid. Some experts recommend the addition of oral rifampin with these

regimens (Liu et al. 2011) (Table 2). A case report has shown the successful use of ceftaroline to treat a MRSA epidural abscess (Bucheit et al. 2014), but more data are required before this can be considered a first-line therapy.

If a foreign body is present at the site of an epidural abscess, cure is more likely if the foreign body is removed. If a foreign body at the site of infection cannot be removed, rifampin should be used in conjunction with the primary antistaphylococcal antimicrobial agent. Associated infection of bone or an intervertebral disc is not uncommon and is addressed in the previous section.

It is appropriate to repeat an imaging study to document resolution of an epidural abscess, particularly if no drainage procedure is performed. In 21 patients with an epidural abscess treated without surgical intervention in Greece between 2012 and 2015, 7 had culture-proven *S. aureus* infections. Failure of conservative therapy, i.e., antibiotic therapy alone, was associated with MRSA infection and presence of neurological complications (Spernovasilis et al. 2017).

4.6 Septic Arthritis

S. aureus is a common cause of septic arthritis of both large and small joints. It was estimated that 16,382 patients were treated for septic arthritis in US emergency departments in 2012 (Singh and Yu 2017a). This infection can be a complication of an adjacent SSTI (such as bursitis) or bone infection, it can be postoperative or a complication of an intra-articular injection, it can result from direct inoculation by an animal bite, or, perhaps most commonly, it can follow seeding by hematogenous spread (Singh and Vogelgesang 2017b). *S. aureus* usually results in a grossly purulent, tender monoarticular arthritis that can be readily diagnosed by arthrocentesis and culture. Imaging such as by CT or MRI may be useful in diagnosis although in most cases imaging is not necessary (Kaplan 2014). Septic arthritis can be difficult to distinguish on physical examination alone from other diagnoses, such as gout or pseudogout (Singh and Vogelgesang 2017b). Typical symptoms of septic arthritis include pain, swelling, erythema, subjective fever and inability to use the joint while suggestive signs include fever, tenderness to palpation, edema, tenderness elicited with both passive and active range of motion, and ability to palpate fluid in the joint cavity. Children may present with "fever and a limp" in cases of lower extremity septic arthritis, and such patients should be evaluated carefully. Importantly, pain can be referred from an infected joint to another anatomic site, which may confuse the clinician. Previously damaged native joints and particularly prosthetic joints are at increased risk for *S. aureus* septic arthritis (Singh and Vogelgesang 2017b). Concomitant osteomyelitis should always be considered as an additional diagnosis in the evaluation of a septic joint.

In regions of the world with a high incidence of CA-MRSA, empiric coverage for MRSA in septic arthritis has become more important. For example, a study in children from Texas in 2001–8 demonstrated that USA300 MRSA was the leading *S. aureus* strain type causing septic arthritis (Carillo-Marquez 2009).

The treatment of native joint septic arthritis caused by *S. aureus* may differ for small and large joints. In small joints with effusions, such as the toes, antimicrobial therapy alone may be adequate. However, septic joints of the hand in particular, due to the risk of debility from permanent damage, should undergo surgical evaluation for possible drainage (Singh and Vogelgesang 2017b). In large joints, optimal therapy is either an open or arthroscopic washout procedure, or alternatively serial (usually daily) needle arthrocentesis can be performed until the joint space is dry, in addition to appropriate antimicrobial therapy (see Table 2). If these aggressive interventions are not performed, infected large joints, such as the knee, hip, shoulder and ankle, are at risk of intra-articular scarring that may lead to a frozen joint (Singh and Vogelgesang 2017b).

Antimicrobial therapy for septic native joints should be given for a minimum of 21 days, although many experts recommend a minimum of 28 days (Liu et al. 2011). If there is associated deep soft tissue infection or osteomyelitis, these conditions may dictate a longer duration of therapy. Imaging studies are not usually necessary to document clearance of the infection.

Infections of prosthetic joints raise special concerns for therapy. For treatment of prosthetic joints infected by *S. aureus*, there is a distinction between early- and late-onset infection after arthroplasty. If an infection is early, i.e., diagnosed in a prosthetic joint within approximately 30 days of implantation, it can most often be cured with a washout procedure for drainage of the infection, exchange of the polyethylene components of the prosthesis, and 6 weeks of intravenous antimicrobial therapy. If it is late, i.e., more than about 30 days after implantation, while there have been many reports of successful cure with conservative, medical therapy alone, there is a significant risk of failure of therapy, and the prosthetic joint itself may loosen (Liu et al. 2011; Osmon et al. 2013). A prosthetic joint infection may sometimes be considered "early" for purposes of this classification scheme if the surgical wound has not completely healed after surgery and appears infected, even though more than 30 days has elapsed from the surgery. Many patients require a two-step procedure for cure of a late prosthetic joint infection. This involves removal of prosthetic joint components and placement of a temporary cement spacer in the joint space, followed by treatment of the septic arthritis (which is often accompanied by osteomyelitis) for at least 6 weeks of intravenous therapy, and finally, replacement of the prosthetic joint (Osmon et al. 2013).

4.7 Pyomyositis

Pyomyositis, sometimes called "tropical pyomyositis," "bacterial pyomyositis," or "primary pyomyositis," is an infection of a muscle by a pyogenic bacterial species. This condition is most often caused by *S. aureus* although it has also been reported as a complication of bacteremia caused by a host of other species (Bickels et al. 2002; Chiedozi 1979; Small and Ross 2005). The authors of one study of 60 patients from two centers in Ohio in 1990–2010 found that *S. aureus* was more

commonly culture-proven as the etiology of pyomyositis in patients with previous trauma and in younger patients (Burdette et al. 2012). The disease is more common in Africa and the South Pacific than in other regions of the world, but some observers have suggested that the condition has become more common in temperate climates during the past twenty years (Unnikrishnan et al. 2010). MRSA as a cause of pyomyositis has been more frequently recognized since 2005 (Burdette et al. 2012; Fowler and Mackay 2006; Pannaraj 2006; Ruiz et al. 2005). Pyomyositis is rare in the USA and Europe, as are reliable data on population-based incidence, and some suggest that it is more common in children than in adults (Gubbay and Isaacs 2000).

The source of bacterial infection of muscle can also be by direct spread, for example, from an intra-abdominal infection or from an infected tendon, as opposed to hematogenous spread, which is the presumed mechanism of infection in primary pyomyositis (Small and Ross 2005). In the U.S, with the emergence of CA-MRSA strain types, several reports have been published of so-called "pelvic syndrome," in which extensive infection of muscles with thrombophlebitis of pelvic vasculature and septic arthritis of the hip occurs (David and Daum 2010).

Although any muscle in body can be infected by *S. aureus* in primary pyomyositis, the most common sites include muscles of the lower extremities, iliopsoas, obturator internus, piriformis, gluteus maximus or minimus, and other pelvic muscles (Chiedozi 1979; Unnikrishnan et al. 2010). *S. aureus* often causes an abscess in muscle after an infection begins, and these can grow to be large, resulting in severe pain and inability to use the affected muscle(s) and progressing to septic shock, at times even with prompt treatment.

Although the condition may be suspected based on findings from physical examination, such as local tenderness, pain with use of the affected muscle, and warmth at the site of infection, it is often confirmed by imaging, including ultrasound, CT scan, or MRI. MRI is likely better than other modalities at defining muscular architecture. On MRI, the abscess of pyomyositis is suggested by a fluid collection in a muscle with rim enhancement. Imaging is important because the size and location of lesions may dictate the approach to therapy. The creatine kinase, white blood cell count, CRP and the ESR are almost universally elevated above normal (Burdette et al. 2012; Unnikrishnan et al. 2010). From the history and physical examination, iliopsoas pyomyositis may be mistaken for pathologic processes of the urologic or reproductive systems, septic arthritis or bursitis of the hip, appendicitis, osteomyelitis, or vertebral discitis as pyomyositis frequently results in no lesion visible on the skin. The condition may also present as an occult source of pain and/or fever. The pathogen in pyomyositis is often identified by blood culture or culture of a lesion elsewhere in the body. In the case of *S. aureus* pyomyositis, the bacterium is usually easily recovered from culture of a drainage specimen from the infected muscle.

Therapy of *S. aureus* pyomyositis can be limited to antimicrobials alone if the lesions are small and involve only a single muscle. In more extensive lesions, antimicrobial therapy combined with needle drainage by interventional radiologists or open surgical drainage is needed for cure. Antimicrobial therapy is often given

for 21 days although the duration is determined by the clinical course. Parenteral agents are recommended for initial therapy of bacterial pyomyositis although oral agents may be used to complete the course if the duration of therapy is prolonged. For the choice of antibiotic some recommend clindamycin and a penicillinase-resistant penicillin (Zimbelman et al. 1999). In areas with a high incidence of MRSA, vancomycin should be included in the initial empiric regimen. If *S. aureus* is cultured, the choices for definitive therapy are similar to those used for other invasive infections (see Table 2).

4.8 Necrotizing Fasciitis

Necrotizing fasciitis is a severe SSTI, with crude mortality in the USA estimated for 2003–2013 of 4.8 deaths per 1,000,000 person-years (Arif et al. 2016). Although *Streptococcus* is the leading bacterial genus implicated in this syndrome, necrotizing fasciitis caused by *S. aureus* has been recorded in many case reports and case series as the sole pathogen (Chhetry et al. 2016; de Carvalho et al. 2012; Miller et al. 2005; Perbet et al. 2010; Thapaliya et al. 2015; Tsitsilonis et al. 2013; Wood 2015; Young and Price 2008) after the emergence of CA-MRSA. This is an infection of the fascial tissue deep to the skin and subcutaneous fat, where bacterial infections can spread rapidly along tissue planes and result in necrosis of more superficial tissues. Necrotizing fasciitis is a surgical and infectious diseases emergency. Management of the condition requires a multidisciplinary approach, often including surgeons, infectious diseases specialists, critical care experts, wound care specialists, and specialty burn units. The PVL toxin or other specific virulence factors in cases caused by USA300 MRSA may play an important role in the pathogenesis of necrotizing fasciitis (Miller et al. 2005). Risk factors for necrotizing fasciitis include the presence of a foreign body, IV drug use, prior hematologic cancer, a deep skin laceration, and diabetes mellitus (Sultan et al. 2012; Waldron et al. 2015).

Whether necrotizing fasciitis is caused by *S. aureus* or another bacterial species, patients generally present with painful, rapidly progressing skin lesions that often demonstrate pain beyond the margin of erythema and that do not bleed when cut due to the lack of blood flow to necrotic tissue. Crepitus may be palpable. The skin may have a gray discoloration. Diagnosis is clinical and may be supported by ultrasound, CT scan, or MRI showing gas or fluid collections in the affected tissues as well as evidence of necrotic tissue adjacent to the fascia on biopsy. Diagnosis of the pathogen is often made from surgical tissue culture, and surgical intervention should not be delayed for imaging studies (Sultan et al. 2012).

Cases of monomicrobial Fournier's gangrene, i.e., necrotizing fasciitis in the perineal and genital region (Shyam and Rapsang 2013), caused by MRSA have been reported (Bjurlin et al. 2013; Lin et al. 2015), including cases caused by CA-MRSA strain types (Burton et al. 2008; Kalorin and Tobin 2007).

Surgical intervention for necrotizing fasciitis must not be delayed. Aggressive debridement of necrotic tissue is essential to prevent the need for limb amputation

or loss of other tissues. Tissue cultures should be sent to identify the pathogen responsible and to obtain antimicrobial susceptibilities to tailor therapy. Empiric antimicrobial therapy should be broad, in order to cover the most common causes of necrotizing fasciitis, such as anaerobes (with clindamycin, for example), Group A Streptococcus (with penicillin or another β-lactam), Gram-negatives, and MRSA (using vancomycin as first-line therapy) (Hussein and Anaya 2013). Antimicrobial drugs should be initiated immediately on suspicion of this diagnosis; they should not be delayed until after a procedure is performed. In order to decrease protein production by the infecting bacteria as quickly as possible, many experts recommend that in necrotizing fasciitis, clindamycin or linezolid be included in the treatment regimen initially for at least several days. These agents, which act upon the ribosome to curtail protein synthesis, also provide coverage of anaerobic bacteria, which may complicate a necrotic wound (Sultan et al. 2012). Some recommend the addition of intravenous immunoglobulin (IVIG) therapy although evidence for its use is very limited (Koch et al. 2015).

4.9 Impetigo

Impetigo is an often self-limited infection of the superficial skin that is most commonly caused by *S. aureus* or by *Streptococcus pyogenes*. The therapy is either expectant treatment, or topical mupirocin or retapamulin. Outside of the USA, topical fusidic acid is an effective therapy as well (Koning et al. 2012).

4.10 Mastitis and Breast Abscess

A distinctive type of SSTI is mastitis, an infection of the breast tissue. It is a common complication of lactation (i.e., puerperal mastitis) and is often caused by *S. aureus* (Lee et al. 2010). CA-MRSA mastitis has been reported in previously healthy women (Holmes and Zadoks 2011; Lee et al. 2010; Pérez et al. 2013; Schoenfeld and McKay 2010), suggesting that if MRSA is prevalent locally in the community and if antibiotics are used to treat empirically, MRSA should be considered in the choice of empiric therapy. However, a systematic review has shown that it is not clear if antimicrobial therapy is necessary in the treatment of mastitis in a breastfeeding woman (Jahanfar et al. 2013).

Mastitis can lead to a breast abscess, which can often be managed by ultrasound-guided needle drainage with culture of the aspirated fluid followed by appropriate oral antimicrobial therapy, preferably using antimicrobials safe for lactation if the women is breastfeeding (Lam et al. 2014). In a series of 33 post-partum breast abscesses diagnosed in Texas in 2000–2006, *S. aureus* was the only bacterial pathogen recovered from cultures, and MRSA was common (Berens

et al. 2010), suggesting that empiric therapy for a breast abscess should include coverage for MRSA according to local susceptibility patterns.

4.11 Conjunctivitis

S. aureus conjunctivitis, an infection of the conjunctivae, is a condition that is usually easily treated with topical antimicrobial therapy, but it may advance to the deeper tissues of the eye and surrounding structures (Alfonso et al. 2015; Freidlin et al. 2007; Sato 2015). One study of 20,180 bacterial cultures of the conjunctivae in New York state in 1997–2008 showed that *S. aureus* was the most common pathogen isolated and the percentage of *S. aureus* that was MRSA increased from 7.2% in 1997–1998 to 41.6% in 2007–2008 (Adebayo et al. 2011). Diagnosis is clinical, with redness of the conjunctivae and frequently accompanied by purulent exudate. Treatment is usually with topical antibiotic preparations including erythromycin ophthalmic ointment, an aminoglycoside, azithromycin or sulfacetamide (Azari and Barney 2013), although alternatives may be necessary in the event that *S. aureus* is resistant. Besifloxacin or moxifloxacin, among other fluoroquinolones, are available as ophthalmic suspensions and are alternatives (Deschênes and Blondeau 2015; Mah and Sanfilippo 2016). Ointments may be preferred for children and in adult patients with poor compliance (Tarff and Behrens 2017). Many cases of *S. aureus* conjunctivitis are self-limited within 14 days, but topical treatments shorten the duration of symptoms and decrease the transmissibility. There are limited data from controlled studies to guide the choice of therapy. Systemic therapy and ophthalmologic evaluation may be needed if the disease progresses despite topical therapy.

4.12 Orbital Infections

Orbital cellulitis is an infection of the soft tissues surrounding the orbit. It is not nearly as common as conjunctivitis, the most common ophthalmologic infection caused by *S. aureus* (Amato et al. 2013; Fukuda et al. 2002; Ross and Abate 1990). However, *S. aureus* is also a common cause of orbital cellulitis, which can arise from *S. aureus* conjunctivitis, blepharitis, eyelid abscess, infection of a lacrimal duct, facial (prefrontal) cellulitis, a laceration, sinusitis, or hematogenous spread from another site in the body. Orbital cellulitis varies in severity and risk to the eye. Prefrontal and retro-orbital cellulitis have been identified among patients with CA-MRSA infection (Blomquist 2006; Chung et al. 2011; Kobayashi et al. 2011; Lei et al. 2013).

If an infection is not limited to the preseptal tissues, complications include orbital abscess; superior ophthalmic vein thrombosis or infection; and abscess of the skull, optic nerve, extraocular muscles, or posterior or anterior chamber of the

eye (endophthalmitis, see below). In the case of orbital cellulitis, an MRI is necessary to assess for complications. MRI often can distinguish between a subperiosteal abscess and an orbital abscess. A subperiosteal abscess may arise from acute sinusitis caused by *S. aureus* (Sharma and Josephson 2014). CA-MRSA orbital abscesses have been reported (Lin et al. 2013a), and should be considered in the diagnostic process if MRSA is a common pathogen locally. Occasionally, orbital cellulitis can progress to meningitis and other intracranial infections including cavernous sinus thrombosis.

Preseptal cellulitis typically presents with pain, erythema and edema of the face, sometimes leading to swelling shut of the eye. It may arise from a bacterial infection of a sinus, and thus sinus imaging may be useful to establish the origin of the infection. Imaging is indicated if there are ominous symptoms of an invasive infection, such as pain with eye movements, photophobia, and diplopia. Signs such as proptosis or ophthalmoplegia should also prompt imaging of the orbit, usually with a CT scan initially, to assess for deeper tissue involvement of the infection that may require urgent surgical exploration and drainage. MRI of the orbit is necessary to assess the smaller structures of the eye.

Orbital cellulitis, if uncomplicated, requires only antimicrobial therapy. However, if there is evidence of a drainable focus in the orbit or in the structures surrounding the orbit, including the sinuses, complicating the cellulitis, surgical drainage or debridement may be necessary by an otolaryngologist, ophthalmologist, or a neurosurgeon. If such a procedure is performed, a culture is essential of any removed tissue or drained fluid to guide choice of antimicrobial therapy. Orbital abscesses usually require drainage for cure while subperiosteal abscesses are often curable with antimicrobial therapy alone. The choice of therapy is similar to other invasive *S. aureus* infections (Table 2).

4.13 Endophthalmitis and Panophthalmitis

Endophthalmitis is the infection of the globe of the eye, often identified after the observation in the eye examination of a hypopyon. Two distinct forms of endophthalmitis are recognized, endogenous endophthalmitis (Bhavsar et al. 2017) and panophthalmitis. Panophthalmitis is endophthalmitis with inflammation of all structures in the globe, while endogenous (metastatic) endophthalmitis is the inflammation of the interior structures of the globe (i.e., uvea and retina with pus in the vitreous and aqueous humors). Both entities have been associated with MRSA infections (Larson and Carrillo-Marquez 2015), including the USA300 MRSA strain in previously healthy patients (Rutar et al. 2006).

Treatment of endophthalmitis involves immediate ophthalmologic evaluation for vitrectomy, debridement of infected tissue, and systemic antimicrobial therapy. Often culture data are lacking when the infection is initially treated. Systemic therapy should therefore empirically include IV vancomycin and a β-lactam for *S. aureus* coverage if there is no contraindication and if local epidemiology suggests a

risk for MRSA (Rutar et al. 2006). Cultures from ophthalmologic tissue or fluid is essential to guide definitive therapy. Therapy can be tailored to antibiotic susceptibilities when they are available.

4.14 Parotitis

Parotitis is an infection of the parotid gland (Bradley 2002). Although the condition can be caused by viruses, especially mumps, bacterial infections, sometimes causing a condition called suppurative parotitis are most commonly caused by *S. aureus*, *Streptococcus pneumoniae*, or oral flora (Brook 2009). MRSA, including CA-MRSA, has been reported as a cause of this syndrome in adults and children (Donovan et al. 2013; Enoch et al. 2006; Mohammed and Hofstetter 2004; Nicolasora et al. 2009). *S. aureus* parotitis is marked by local tenderness over the gland, erythema and marked edema of the gland, and it is usually unilateral. Purulent material can sometimes be milked from the gland and cultured as it exits from salivary ducts. Rarely, *S. aureus* parotitis can result in bacteremia in adults (Enoch et al. 2006) and children (Donovan et al. 2013). The infection can usually be treated with antimicrobials, but occasionally an abscess in the gland requires surgical or needle drainage (Bradley 2002; Donovan et al. 2013). Choice of antibiotic is similar to that for other invasive *S. aureus* infections (see Table 2).

4.15 Toxinoses

4.15.1 Staphylococcal Toxic Shock Syndrome (TSS)

Staphylococcal TSS is a clinical syndrome caused by toxic shock syndrome toxin-1 or -2 (tsst-1 or tsst-2), which are superantigens produced by some strains of *S. aureus* (Kulhankova et al. 2014). TSS results in a life-threatening condition marked by a severe rash that can give rise to skin sloughing as well as hypotension. It may also affect the kidneys, gastrointestinal tract, liver, and central nervous system, and cause thrombocytopenia. Specific defining criteria have been determined by the CDC (1997). Mortality in TSS has been estimated at 4.1% (Hajjeh et al. 1999). The syndrome can be caused by exposure of mucous membranes to *S. aureus*, and inoculation of toxin can occur in the absence of a local, clinically apparent infection syndrome. For example, attention was drawn to TSS in the 1970s when there were a number of cases associated with the use of super-absorbent tampons (Berkley et al. 1987). Cases have also been reported with surgical packing of wounds, including after a burn (Garland et al. 2016), and packing of the nasopharynx to control bleeding (Allen et al. 1990). In some cases of surgical wound-associated TSS, the wound does not appear infected, but debridement may nevertheless be necessary for cure (Tong et al. 2015).

Other staphylococcal toxins, including the staphylococcal enterotoxins, can be responsible for similar shock syndromes by acting as nonspecific T cell superantigens (Krakauer et al. 2016).

Evidence for the optimal management of staphylococcal TSS is limited. Commonly, the therapeutic approach includes removal or debridement of the source of the infection, an effective antibiotic and also administration of intravenous immune globulin (IVIG). If the condition is associated with a foreign body, the foreign body should be removed immediately. Antibiotics are of secondary importance in the treatment of TSS although antiribosomal drugs, such as clindamycin and linezolid are often used in order to decrease toxin production (Lappin and Ferguson 2009).

4.15.2 Staphylococcal Scalded Skin Syndrome (SSSS)

SSSS is a potentially fatal disease caused by certain strains of *S. aureus* that carry the exfoliating toxin A or B (ETA or ETB). The condition has a low incidence of 0.09–0.56 per million and is most common in young children. It may diagnosed histologically although it is often a clinical diagnosis. The pathologic process is toxin-mediated, and the bacterium is often culturable in the nares but not in the skin lesions. The infection may present with malaise, fever, and Nikolsky's sign. The disease results in the development of patches of erythematous skin that coalesce and in many cases bullae that can slough, leading to life-threatening lesions that mimic a severe burn, hence the name of the disease. There is often desquamation on the face and skin folds in SSSS. The mucous membranes are spared, and the desquamation is limited to the superficial epidermis, unlike toxic epidermal necrolysis. Therefore, on recovery from SSSS, there is no scarring. Adults with the condition may have positive blood cultures for *S. aureus*, which is less common in children (Handler and Schwartz 2014).

Treatment of SSSS is usually in a burn unit, if one is available, with antibiotic therapy with the same medications recommended for invasive *S. aureus* infections, and the use of penicillinase-resistant penicillin is strongly recommended in the empiric regimen if there is no concern for allergy (Table 2). Fresh frozen plasma is sometimes used in children to help neutralize the toxin. Some also advocate a 5-day course of IVIG (Handler and Schwartz 2014; Ladhani and Joannou 2000). Studies have suggested that 91% of adults >40 years of age carry antibodies to ETA (Schmidt et al. 2002); the utility of IVIG in adults is controversial. The site of primary infection may or may not be evident. If it is, this site should be treated as appropriate. In children, the site of infection may be a minor upper respiratory infection, while in adults there is more variability in the anatomic site. Fluid and skin dressing management is critical, and the approach is similar to that used in burn patients (Handler and Schwartz 2014).

4.16 Urogenital Infections

Urogenital infections caused by *S. aureus* occur at a broad variety of specific sites, including cystitis; pyelonephritis; prostatitis, epididymitis, and orchitis in men; and abscesses in the female genital tract. Uncomplicated lower urinary tract infections (UTIs) are not commonly caused by *S. aureus* (Wada et al. 2016), and complicated *S. aureus* UTIs are often related to the presence of a catheter (Baraboutis et al. 2010). When a urine culture grows *S. aureus*, particularly in the absence of pyuria, this should raise concern for *S. aureus* bacteremia, and this may not indicate a UTI (Asgeirsson et al. 2012; Choi et al. 2009). *S. aureus* is an occasional cause of pyelonephritis (Baraboutis et al. 2009; Czaja et al. 2007; Kempker et al. 2009; Kim et al. 2016; Yock and Boyce 2015), and intrarenal and perinephric abscesses (Dembry and Andriole 1997; Linder and Granberg 2016), which can usually be identified by ultrasound (Vourganti et al. 2006). An abscess of the kidney caused by Gram-negative bacilli (Dembry and Andriole 1997) or *Candida species* (Kauffman et al. 2011) should be considered in the differential diagnosis if an abscess is visualized on imaging and culture data are not available.

One study estimated that 14,294 women who are pregnant or post-partum have an invasive MRSA infection each year in the USA (Beigi et al. 2009), with mastitis being the leading syndrome, but such infections may occur in any anatomic site. In women, chorioamnionitis or endometritis is usually caused by vaginal flora (Martens et al. 1991); *S. aureus* is an unusual pathogen in these infections (Geisler et al. 1998; Maher et al. 1993). Urogenital abscesses are often diagnosed by ultrasound, CT scan, or MRI. Abscesses of the ovaries or Fallopian tubes (tubo-ovarian abscesses [TOA]) may complicate pelvic inflammatory disease and may be diagnosed by transvaginal ultrasound. TOA are usually caused by vaginal flora and are often polymicrobial (Gjelland et al. 2005; Granberg et al. 2009). However, they are occasionally caused by *S. aureus*. They may require drainage, which can often be accomplished using transvaginal aspiration guided by ultrasound (Gjelland et al. 2005; Granberg et al. 2009). TOA and endometritis are treated for at least 14 days.

The choice of antibiotic in the cases of *S. aureus* infection of the reproductive and urinary system organs is similar to other invasive infections (see Table 2). Treatment of a renal abscess requires a minimum of 4 weeks of antimicrobial therapy and does not usually require surgical drainage (Dembry and Andriole 1997). Acute or subacute cases of bacterial prostatitis often require a prolonged period of therapy, for many weeks (Coker and Dierfeldt 2016; Gill and Shoskes 2016), whatever the bacterial cause, which is rarely *S. aureus* (Baker et al. 2004; Beckman and Edson 2007; Brede and Shoskes 2011). Chronic prostatitis is usually caused by Gram-negative bacilli and is treated with fluoroquinolones or TMP-SMX (Le and Schaeffer 2011; Perletti et al. 2013). Orchitis and epididymitis can be evaluated by ultrasound and may be caused by *S. aureus* (Raychaudhuri and Chew 2012). In our experience, these infections require at least 14 days of antibiotic

therapy after drainage, if it is indicated, but the duration of therapy should be determined by the clinical course of the infection.

Prostatic abscess is an unusual diagnosis that is more common in people with diabetes and in immunosuppressed patients. It is increasingly commonly caused by *S. aureus* (Baker et al. 2004; Deshpande et al. 2013). Prostatic abscesses can be diagnosed with transrectal ultrasound, may require drainage transrectally, and should be treated with antibiotics for at least 4 weeks (Susaníbar Napurí et al. 2011).

For all urogenital *S. aureus* infections, the clinician should remain alert for the possibility of an abscess developing or enlarging during therapy that requires drainage for cure and should not hesitate to reimage the area if symptoms persist.

4.17 Lemierre's Syndrome

Lemierre's syndrome, also called postanginal sepsis, is thrombophlebitis of the internal jugular vein often resulting from extension of an initial focus of infection, sometimes an abscess, in the oropharynx. There have been numerous reports of *S. aureus*, including MRSA, as a cause of retropharyngeal abscesses (Brown et al. 2015; Fleisch et al. 2007), for example, which may progress to deeper neck infections. Symptoms of Lemierre's syndrome usually include severe neck pain and swelling around the jugular vein, and there may be septic emboli that spread to the lungs, liver, kidneys, joints, or other organs and perhaps leading to local abscess formation. A recent study suggests that the mortality rate for Lemierre's syndrome is approximately 2% (Johannesen and Bodtger 2016). Although this syndrome is classically caused by *Fusobacterium necrophorum*, an increasing number of case reports of this syndrome caused by *S. aureus*, including MRSA, have been published (Abhishek et al. 2013; Bentley and Brennan 2009; Bilal et al. 2009; Chanin et al. 2011; Gokçe Ceylan et al. 2009; Gunatilake et al. 2014; Herek et al. 2010; Jariwala et al. 2017; Kizhner et al. 2013; Molloy et al. 2012). Therapy for this condition is primarily antimicrobial agents targeting the pathogen. The neck or other organs affected by septic emboli sometimes require drainage or local debridement. Rarely is ligation of the infected jugular vein necessary for cure (Johannesen and Bodtger 2016).

4.18 Meningitis and Other CNS Infections

Meningitis, subdural empyema, and other CNS infections caused by *S. aureus* are rare in the absence of previous surgery or foreign bodies. It has been estimated that *S. aureus* is the cause of 0.3–8.8% of cases of meningitis (Aguilar et al. 2010; Durand et al. 1993), and the mortality was reported to be 36% in a series of 33 cases in Detroit in 1999–2008 (Aguilar et al. 2010) and 35.3% in 34 patients in Taiwan in 2000–2008 (Huang et al. 2010). In preterm infants, MRSA meningitis had a

mortality of 26% and MSSA had a mortality of 24% in 2006–2008 at 20 US medical centers (Shane et al. 2012). There are a number of recent case reports of *S. aureus* meningitis from around the world in children and adults (Aguilar et al. 2010; Al Kandari et al. 2010; De Schryver et al. 2011; Dylewski and Martel 2004; Gattuso et al. 2009; Huang et al. 2010; Lee et al. 2008; Pereira et al. 2015; Pintado et al. 2012; Shane et al. 2012; Smetana et al. 2013; Udassi et al. 2015; Vallejo et al. 2017) including CA-MRSA meningitis in the absence of other known meningitis risk factors (Laurens et al. 2008; Munckhof et al. 2008; Naesens et al. 2009; Valentini et al. 2008; Yonezawa et al. 2015). Typical symptoms of bacterial meningitis are fever, stiff neck, photophobia and headache. Focal neurologic symptoms may or may not be present.

As for other causes of meningitis, *S. aureus* may be cultured from the cerebrospinal fluid (CSF) obtained by a lumbar puncture or a sample of CSF from another site. As for other causes of meningitis, a neutrophilic pleocytosis, elevated protein, and decreased glucose level is usually expected in the CSF in cases of *S. aureus* meningitis. *S. aureus* meningitis may be complicated by a parameningeal focus of infection, including cavernous sinus thrombosis (Munckhof et al. 2008; Naesens et al. 2009) that can require drainage and/or prolonged antimicrobial therapy for cure. Treatment of meningitis is generally parenteral and for 14 days (Liu et al. 2011) after removal of relevant foreign bodies and drainage of any large foci.

Ventriculo-peritoneal, ventriculo-pleural, and ventricular shunts to other organs can become infected during the immediate postoperative period or from later seeding from the bloodstream or by direct extension from another site of infection. Staphylococci and other skin flora are often responsible for infections in the postoperative period. *S. aureus* may cause a shunt infection leading to abscess formation in the CNS or at a site at the distal end of the shunt that requires drainage. Similarly, implanted intrathecal pumps may be infected by skin flora, including *S. aureus*, with abscess development in the pump pocket or along the path of the catheter. For these foreign body-associated *S. aureus* infections, generally removal of the foreign body is required for cure. In patients who require a permanent ventricular shunt to treat hydrocephalus, a temporary lumbar or other external CSF drain should be placed after removal of the infected shunt, with regular sampling of the CSF for cell counts and culture. Replacement of the permanent shunt should be delayed until the CSF cell counts are normal and the CSF culture is negative. Therapy for such infections is usually parenteral, and therapy is recommended for at least 2 weeks after normalization of the cell counts in the CSF (Liu et al. 2011).

Brain abscesses may arise from direct spread from a contiguous site, trauma, hematogenous spread or from an unknown source (Brook 2017). They have many possible bacterial etiologies, but brain abscesses caused by *S. aureus* have been reported (Arora et al. 2012; El-Khashab et al. 2012; Kao et al. 2008; Leotta et al. 2005; Narayanan et al. 2013; Park et al. 2015; Ramos et al. 2009), including some caused by CA-MRSA strains (Enany et al. 2007; Lo and Erwin 2008; Mutale et al. 2014; Naesens et al. 2009; Sifri et al. 2007), and often require drainage for cure. Linezolid has been reported as a possible therapy for MRSA brain abscesses in case series (Ntziora and Falagas 2007; Saito et al. 2010).

The choice of antimicrobial agent for therapy for CNS infections should be determined by the susceptibility profile of *S. aureus* causing the infection and also take into consideration penetration of the drug across the blood–brain barrier. For MSSA, a parenteral β-lactam drug is optimal, such as oxacillin or nafcillin. Vancomycin is the leading choice for therapy of MRSA infections, which has been shown to cross-inflamed meninges (Aguilar et al. 2010; Albanèse et al. 2000; Liu et al. 2011). There is some clinical and experimental evidence of efficacy of linezolid in *S. aureus* meningitis (Aguilar et al. 2010; Cabellos et al. 2014; Calik et al. 2012; Gattuso et al. 2009; Kessler and Kourtis 2007; Ntziora and Falagas 2007; Sipahi et al. 2013), some evidence for linezolid in combination with rifampicin (Al Kandari et al. 2010), and one case report with both daptomycin and rifampin (Kalesidis 2011). TMP-SMX is an alternative recommended in the IDSA MRSA treatment guidelines (Liu et al. 2011). Ceftaroline, which showed good CNS penetration in a rabbit model (Saravolatz et al. 2011), has been used to treat MRSA meningitis (Balouch et al. 2015), but evidence for its efficacy is limited to case reports. Daptomycin has poor CNS penetration; there are few case reports of success (Lee et al. 2008) as well as failure (Wahby and Alangaden 2012) with this agent as monotherapy in *S. aureus* meningitis. Some advise the addition of rifampin for combination therapy with vancomycin in MRSA meningitis (Liu et al. 2011).

5 Conclusions

Treatment of *S. aureus* infections is complex. *S. aureus* is most often a commensal, and thus the first challenge is to ensure that a culture of this species indicates the presence of a clinically significant infection. *S. aureus* is capable of causing infection in any human tissue, and it often causes purulent infections with abscess formation.

Penicillin is no longer useful to treat *S. aureus* infections in the majority of cases, but for MSSA strains, there are often a great number of alternative semisynthetic antistaphylococcal penicillins and other β-lactam drugs available, such as the cephalosporins. For MRSA there are also many drug classes available, including the glycopeptides vancomycin and teicoplanin (in Europe, Asia and South America), which are first-line for invasive infections. Daptomycin is also a useful choice. For uncomplicated MRSA skin infections, doxycycline, minocycline, clindamycin, TMP-SMX, and linezolid are all well-established agents with limited toxicities. Tigecycline is generally reserved for infections in which other drugs are not adequate or several drugs would be needed to replace it. Tedizolid is a promising new oxazolidinone with oral and parenteral preparations. The newer agents active against MRSA include cephalosporins (ceftobiprole in Europe and ceftaroline in the USA), and the lipoglycopeptides (oritavancin, dalbavancin, and telavancin). The role of these newer agents is not yet defined, but as they are used more widely, they may be useful in the treatment of invasive infections.

S. aureus, which has been a constant companion of humanity for millennia, has developed resistance to every new class of antibiotics developed. It will remain a common pathogen and a persistent challenge for surgeons and internists unless a vaccine is developed to prevent staphylococcal disease. The discovery and development of antimicrobials with new antibiotic targets are thus essential.

References

Abbott IJ, Jenney AW, Jeremiah CJ, Mirčeta M, Kandiah JP, Holt DC, Tong SY, Spelman DW (2015) Reduced in vitro activity of ceftaroline by Etest among clonal complex 239 methicillin-resistant *Staphylococcus aureus* clinical strains from Australia. Antimicrob Agents Chemother 59(12):7837–7841

Abdallah L, Remadi JP, Habib G, Salaun E, Casalta JP, Tribouilloy C (2016) Long-term prognosis of left-sided native-valve *Staphylococcus aureus* endocarditis. Arch Cardiovasc Dis 109 (4):260–267

Abhishek A, Sandeep S, Tarun P (2013) Lemierre syndrome from a neck abscess due to methicillin-resistant *Staphylococcus aureus*. Braz J Infect Dis 17(4):507–509

Adebayo A, Parikh JG, McCormick SA, Shah MK, Huerto RS, Yu G, Milman T (2011) Shifting trends in in vitro antibiotic susceptibilities for common bacterial conjunctival isolates in the last decade at the New York Eye and Ear Infirmary. Graefes Arch Clin Exp Ophthalmol 249 (1):111–119

Aguilar J, Urday-Cornejo V, Donabedian S, Perri M, Tibbetts R, Zervos M (2010) *Staphylococcus aureus* meningitis: case series and literature review. Medicine (Baltimore) 89(2):117–125

Albanèse J, Léone M, Bruguerolle B, Ayem ML, Lacarelle B, Martin C (2000) Cerebrospinal fluid penetration and pharmacokinetics of vancomycin administered by continuous infusion to mechanically ventilated patients in an intensive care unit. Antimicrob Agents Chemother 44 (5):1356–1358

Alfonso SA, Fawley JD, Alexa LuX (2015) Conjunctivitis. Prim Care 42(3):325–345

Ali FR, Yiu ZZ, Ogden S (2015) Minocycline-induced pigmentation of the skin and nails. Postgrad Med J 91(1081):662

Aliberti S, Reyes LF, Faverio P, Sotgiu G, Dore S, Rodriguez AH, Soni NJ, Restrepo MI; GLIMP investigators (2016) Global initiative for meticillin-resistant *Staphylococcus aureus* pneumonia (GLIMP): an international, observational cohort study. Lancet Infect Dis 16(12):1364–1376

Al Kandari M, Jamal W, Udo EE, El Sayed A, Al Shammri S, Rotimi VO (2010) A case of community-onset meningitis caused by hospital methicillin-resistant *Staphylococcus aureus* successfully treated with linezolid and rifampicin. Med Princ Pract 19(3):235–239

Allard C, Carignan A, Bergevin M, Boulais I, Tremblay V, Robichaud P, Duperval R, Pepin J (2008) Secular changes in incidence and mortality associated with *Staphylococcus aureus* bacteraemia in Quebec, Canada, 1991–2005. Clin Microbiol Infect 14(5):421–428

Allen ST, Liland JB, Nichols CG, Glew RH (1990) Toxic shock syndrome associated with use of latex nasal packing. Arch Intern Med 150(12):2587–2588

Alm RA, McLaughlin RE, Kos VN, Sader HS, Iaconis JP, Lahiri SD (2014) Analysis of *Staphylococcus aureus* clinical isolates with reduced susceptibility to ceftaroline: an epidemiological and structural perspective. J Antimicrob Chemother 69(8):2065–2075

Alonzo F 3rd, Torres VJ (2014) The bicomponent pore-forming leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78(2):199–230

Al-Tawfiq JA, Aldaabil RA (2005) Community-acquired MRSA bacteremic necrotizing pneumonia in a patient with scrotal ulceration. J Infect 51(4):e241–e243

Amato M, Pershing S, Walvick M, Tanaka S (2013) Trends in ophthalmic manifestations of methicillin-resistant *Staphylococcus aureus* (MRSA) in a northern California pediatric population. J AAPOS 17(3):243–247

Arif N, Yousfi S, Vinnard C (2016) Deaths from necrotizing fasciitis in the United States, 2003–2013. Epidemiol Infect 144(6):1338–1344

Arnold SR, D. Elias D, Buckingham SC, Thomas ED, Novais E, Arkader A, Howard C (2006) Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant *Staphylococcus aureus*. J Pediatr Orthop 26:703–708

Arora P, Kalra VK, Pappas A (2012) Multiple brain abscesses in a neonate after blood stream infection with methicillin-resistant *Staphylococcus aureus*. J Pediatr 161(3):563–563.e1

Arshad S, Huang V, Hartman P, Perri MB, Moreno D, Zervos MJ (2017) Ceftaroline fosamil monotherapy for methicillin-resistant *Staphylococcus aureus* bacteremia: a comparative clinical outcomes study. Int J Infect Dis 57:27–31

Asgeirsson H, Kristjansson M, Kristinsson KG, Gudlaugsson O (2012) Clinical significance of *Staphylococcus aureus* bacteriuria in a nationwide study of adults with *S. aureus* bacteraemia. J Infect 64(1):41–46

Asgeirsson H, Thalme A, Weiland O (2016) Low mortality but increasing incidence of *Staphylococcus aureus* endocarditis in people who inject drugs: experience from a Swedish referral hospital. Medicine (Baltimore) 95(49):e5617

Athan E, Chu VH, Tattevin P, Selton-Suty C, Jones P, Naber C, Miró JM, Ninot S, Fernández-Hidalgo N, Durante-Mangoni E, Spelman D, Hoen B, Lejko-Zupanc T, Cecchi E, Thuny F, Hannan MM, Pappas P, Henry M, Fowler VG Jr, Crowley AL, Wang A, Investigators ICE-PCS (2012) Clinical characteristics and outcome of infective endocarditis involving implantable cardiac devices. JAMA 307(16):1727–1735

Azari AA, Barney NP (2013) Conjunctivitis: a systematic review of diagnosis and treatment. JAMA 310(16):1721–1729

Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA; American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Stroke Council (2015) Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation 132(15):1435–1486

Bahrain M, Vasiliades M, Wolff M, Younus F (2006) Five cases of bacterial endocarditis after furunculosis and the ongoing saga of community-acquired methicillin-resistant *Staphylococcus aureus* infections. Scand J Infect Dis 38(8):702–707

Baker SD, Horger DC, Keane TE (2004) Community-acquired methicillin-resistant *Staphylococcus aureus* prostatic abscess. Urology 64(4):808–810

Balouch MA, Bajwa RJ, Hassoun A (2015) Successful use of ceftaroline for the treatment of MRSA meningitis secondary to an infectious complication of lumbar spine surgery. J Antimicrob Chemother 70(2):624–625

Baraboutis IG, Koukoulaki M, Belesiotou H, Platsouka E, Papastamopoulos V, Kontothanasis D, Kontogianni-Katsarou K, Tsagalou EP, Paniara O, Skoutelis AT (2009) Community-acquired methicillin-resistant *Staphylococcus aureus* as a cause of rapidly progressing pyelonephritis with pyonephrosis, necessitating emergent nephrectomy. Am J Med Sci 338(3):233–235

Baraboutis IG, Tsagalou EP, Lepinski JL, Papakonstantinou I, Papastamopoulos V, Skoutelis AT, Johnson S (2010) Primary *Staphylococcus aureus* urinary tract infection: the role of undetected hematogenous seeding of the urinary tract. Eur J Clin Microbiol Infect Dis 29(9):1095–1101

Barton M, Hawkes M, Moore D, Conly J, Nicolle L, Allen U, Boyd N, Embree J, Van Horne L, Le Saux N, Richardson S, Moore A, Tran D, Waters V, Vearncombe M, Katz K, Weese JS, Embil J, Ofner-Agostini M, Ford-Jones EL; Writing Group of the Expert Panel of Canadian Infectious Disease, Infection Prevention and Control, and Public Health Specialists (2006)

Guidelines for the prevention and management of community-associated methicillin-resistant *Staphylococcus aureus*: a perspective for Canadian health care practitioners. Can J Infect Dis Med Microbiol 17(Suppl C):4C–24C

Bassetti M, Peghin M, Trecarichi EM, Carnelutti A, Righi E, Del Giacomo P, Ansaldi F, Trucchi C, Alicino C, Cauda R, Sartor A, Spanu T, Scarparo C, Tumbarello M (2017) Characteristics of *Staphylococcus aureus* bacteraemia and predictors of early and late mortality. PLoS ONE 12(2):e0170236

Baxi SM, Chan D, Jain V (2015) Daptomycin non-susceptible, vancomycin-intermediate *Staphylococcus aureus* endocarditis treated with ceftaroline and daptomycin: case report and brief review of the literature. Infection 43(6):751–754

Beckman TJ, Edson RS (2007) Methicillin-resistant *Staphylococcus aureus* prostatitis. Urology 69 (4):779.e1–e3

Beigi RH, Bunge K, Song Y, Lee BY (2009) Epidemiologic and economic effect of methicillin-resistant *Staphylococcus aureus* in obstetrics. Obstet Gynecol 113(5):983–991

Bentley TP, Brennan DF (2009) Lemierre's syndrome: methicillin-resistant *Staphylococcus aureus* (MRSA) finds a new home. J Emerg Med 37(2):131–134

Berens P, Swaim L, Peterson B (2010) Incidence of methicillin-resistant *Staphylococcus aureus* in postpartum breast abscesses. Breastfeed Med 5(3):113–115

Berkley SF, Hightower AW, Broome CV, Reingold AL (1987) The relationship of tampon characteristics to menstrual toxic shock syndrome. JAMA 21;258(7):917–920

Bhavsar MM, Devarajan TV, Nembi PS, Ramakrishnan N, Mani AK (2017) Metastatic endogenous endophthalmitis: a rare presentation with methicillin-resistant *Staphylococcus aureus* prostatic abscess. Indian J Crit Care Med 21(3):172–175

Bickels J, Ben-Sira L, Kessler A, Wientroub S (2002) Primary pyomyositis. J Bone Joint Surg Am 84-A(12):2277–2286

Bilal M, Cleveland KO, Gelfand MS (2009) Community-acquired methicillin-resistant *Staphylococcus aureus* and Lemierre syndrome. Am J Med Sci 338(4):326–327

Bishburg E, Bishburg K (2009) Minocycline–an old drug for a new century: emphasis on methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. Int J Antimicrob Agents 34(5):395–401

Bjurlin MA, O'Grady T, Kim DY, Divakaruni N, Drago A, Blumetti J, Hollowell CM (2013) Causative pathogens, antibiotic sensitivity, resistance patterns, and severity in a contemporary series of Fournier's gangrene. Urology 81(4):752–758

Blomquist PH (2006) Methicillin-resistant *Staphylococcus aureus* infections of the eye and orbit (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc 104:322–345

Blumer JL, Ghonghadze T, Cannavino C, O'Neal T, Jandourek A, Friedland HD, Bradley JS (2016) A multicenter, randomized, observer-blinded, active-controlled study evaluating the safety and effectiveness of ceftaroline compared with ceftriaxone plus vancomycin in pediatric patients with complicated community-acquired bacterial pneumonia. Pediatr Infect Dis J 35 (7):760–766

Bocchini CE, Hulten KG, Mason EO Jr, Gonzalez BE, Hammerman WA, Kaplan SL (2006) Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. Pediatrics 117(2):433–440

Borg MA, Camilleri L, Waisfisz B (2012) Understanding the epidemiology of MRSA in Europe: do we need to think outside the box? J Hosp Infect 81(4):251–256

Boucher HW, Wilcox M, Talbot GH, Puttagunta S, Das AF, Dunne MW (2014) Once-weekly dalbavancin versus daily conventional therapy for skin infection. N Engl J Med 370(23):2169–2179

Bradley PJ (2002) Microbiology and management of sialadenitis. Curr Infect Dis Rep 4(3):217–224

Brede CM, Shoskes DA (2011) The etiology and management of acute prostatitis. Nat Rev Urol 8 (4):207–212

Brook I (2009) The bacteriology of salivary gland infections. Oral Maxillofac Surg Clin North Am 21(3):269–274

Brook I (2017) Microbiology and treatment of brain abscess. J Clin Neurosci 38:8–12

Brown NK, Hulten KG, Mason EO, Kaplan SL (2015) *Staphylococcus aureus* retropharyngeal abscess in children. Pediatr Infect Dis J 34(4):454–456

Browne LP, Mason EO, Kaplan SL, Cassady CI, Krishnamurthy R, Guillerman RP (2008) Optimal imaging strategy for community-acquired *Staphylococcus aureus* musculoskeletal infections in children. Pediatr Radiol 38(8):841–847

Bucheit J, Collins R, Joshi P (2014) Methicillin-resistant *Staphylococcus aureus* epidural abscess treated with ceftaroline fosamil salvage therapy. Am J Health Syst Pharm 71(2):110–113

Burdette SD, Watkins RR, Wong KK, Mathew SD, Martin DJ, Markert RJ (2012) *Staphylococcus aureus* pyomyositis compared with non-*Staphylococcus aureus* pyomyositis. J Infect 64 (5):507–512

Burnett YJ, Echevarria K, Traugott KA (2016) Ceftaroline as salvage monotherapy for persistent MRSA bacteremia. Ann Pharmacother 50(12):1051–1059

Burton MJ, Shah P, Swiatlo E (2008) Community-acquired methicillin-resistant *Staphylococcus aureus* as a cause of Fournier's gangrene. Amer J Med Sci 335:327–328

Cabellos C, Garrigós C, Taberner F, Force E, Pachón-Ibañez ME (2014) Experimental study of the efficacy of linezolid alone and in combinations against experimental meningitis due to *Staphylococcus aureus* strains with decreased susceptibility to beta-lactams and glycopeptides. J Infect Chemother 20(9):563–568

Cahill TJ, Baddour LM, Habib G, Hoen B, Salaun E, Pettersson GB, Schäfers HJ, Prendergast BD (2017) Challenges in infective endocarditis. J Am Coll Cardiol 69(3):325–344

Cai Y, Wang R, Liang B, Bai N, Liu Y (2011) Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease. Antimicrob Agents Chemother 55(3):1162–1172

Calik S, Turhan T, Yurtseven T, Sipahi OR, Buke C (2012) Vancomycin versus linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis in an experimental rabbit model. Med Sci Monit 18(11):SC5–SC8

Carrillo-Marquez MA, Hulten KG, Hammerman W, Mason EO, Kaplan SL (2009) USA300 is the predominant genotype causing *Staphylococcus aureus* septic arthritis in children. Pediatr Infect Dis J 28(12):1076–1080

Carris NW, Pardo J, Montero J, Shaeer KM (2015) Minocycline as a substitute for doxycycline in targeted scenarios: a systematic review. Open Forum Infect Dis 2(4):ofv178

Casapao AM, Leonard SN, Davis SL, Lodise TP, Patel N, Goff DA, LaPlante KL, Potoski BA, Rybak MJ (2013) Clinical outcomes in patients with heterogeneous vancomycin-intermediate *Staphylococcus aureus* bloodstream infection. Antimicrob Agents Chemother 57(9):4252–4259

Cenizal MJ, Skiest D, Luber S, Bedimo R, Davis P, Fox P, Delaney K, Hardy RD (2007) Prospective randomized trial of empiric therapy with trimethoprim-sulfamethoxazole or doxycycline for outpatient skin and soft tissue infections in an area of high prevalence of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 51(7):2628–2630

Centers for Disease Control and Prevention (1997) Case definitions for infectious conditions under public health surveillance. Centers for Disease Control and Prevention. MMWR Recomm Rep 46(RR-10):1–55

Centers for Disease Control and Prevention (1999) Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. MMWR Morb Mortal Wkly Rep 48:707–710

Chambers HF (2001) The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis 7:178–182

Chanin JM, Marcos LA, Thompson BM, Yusen RD, Dunne WM Jr, Warren DK, Santos CA (2011) Methicillin-resistant *Staphylococcus aureus* USA300 clone as a cause of Lemierre's syndrome. J Clin Microbiol 49(5):2063–2066

Chastre J, Blasi F, Masterton RG, Rello J, Torres A, Welte T (2014) European perspective and update on the management of nosocomial pneumonia due to methicillin-resistant *Staphylococcus aureus* after more than 10 years of experience with linezolid. Clin Microbiol Infect 20(Suppl 4):19–36

Chelsom J, Solberg CO (1998) Vertebral osteomyelitis at a Norwegian university hospital 1987–97: clinical features, laboratory findings and outcome. Scand J Infect Dis 30(2):147–151

Chen H, Yang Q, Zhang R, He W, Ma X, Zhang J, Xia F, Zhao F, Cao J, Liu Y, Wu W, Hu D, Wang Q, Zhao C, Zhang F, Wang X, Wang Z, Li H, Wang H (2014) In vitro antimicrobial activity of the novel oxazolidinone tedizolid and comparator agents against *Staphylococcus aureus* and linezolid-resistant Gram-positive pathogens: a multicentre study in China. Int J Antimicrob Agents 44(3):276–277

Chhetry M, Banerjee B, Subedi S, Koirala A (2016) Necrotizing fasciitis of anterior abdominal wall following cesarean section in a low-risk patient. J Surg Case Rep 2016(7)

Chiappini E, Camposampiero C, Lazzeri S, Indolfi G, De Martino M, Galli L (2017) Epidemiology and management of acute haematogenous osteomyelitis in a tertiary paediatric center. Int J Environ Res Public Health 14(5)

Chickering HT, Park JH (1919) *Staphylococcus aureus* pneumonia. JAMA 72:617–626

Chiedozi LC (1979) Pyomyositis: review of 205 cases in 112 patients. Am J Surg 137(2):255–259

Choi SH, Lee SO, Choi JP, Lim SK, Chung JW, Choi SH, Jeong JY, Woo JH, Kim YS (2009) The clinical significance of concurrent *Staphylococcus aureus* bacteriuria in patients with *S. aureus* bacteremia. J Infect 59(1):37–41

Chung M, Antignac A, Kim C, Tomasz A (2008) Comparative study of the susceptibilities of major epidemic clones of methicillin-resistant *Staphylococcus aureus* to oxacillin and to the new broad-spectrum cephalosporin ceftobiprole. Antimicrob Agents Chemother 52(8):2709–2717

Chung WC, Lin HJ, Foo NP, Chen KT (2011) Infantile orbital abscess caused by community-acquired methicillin-resistant *Staphylococcus aureus*. J Ophthalmic Inflamm Infect 1(4):181–183

Claeys KC, Zasowski EJ, Casapao AM, Lagnf AM, Nagel JL, Nguyen CT, Hallesy JA, Compton MT, Kaye KS, Levine DP, Davis SL, Rybak MJ (2016) Daptomycin improves outcomes regardless of vancomycin MIC in a propensity-matched analysis of methicillin-resistant *Staphylococcus aureus* bloodstream infections. Antimicrob Agents Chemother 60(10):5841–5848

Coker TJ, Dierfeldt DM (2016) Acute bacterial prostatitis: diagnosis and management. Am Fam Physician 93(2):114–120

Colton B, McConeghy KW, Schreckenberger PC, Danziger LH (2016) I.V. minocycline revisited for infections caused by multidrug-resistant organisms. Am J Health Syst Pharm 73(5):279–285

Corey GR, Good S, Jiang H, Moeck G, Wikler M, Green S, Manos P, Keech R, Singh R, Heller B, Bubnova N, O'Riordan W, Investigators SOLOII (2015) Single-dose oritavancin versus 7-10 days of vancomycin in the treatment of Gram-positive acute bacterial skin and skin structure infections: the SOLO II noninferiority study. Clin Infect Dis 60(2):254–262

Corey GR, Kabler H, Mehra P, Gupta S, Overcash JS, Porwal A, Giordano P, Lucasti C, Perez A, Good S, Jiang H, Moeck G, O'Riordan W, Investigators SOLOI (2014) Single-dose oritavancin in the treatment of acute bacterial skin infections. N Engl J Med 370(23):2180–2190

Corey GR, Wilcox MH, Talbot GH, Thye D, Friedland D, Baculik T; CANVAS 1 investigators (2010) CANVAS 1: the first Phase III, randomized, double-blind study evaluating ceftaroline fosamil for the treatment of patients with complicated skin and skin structure infections. J Antimicrob Chemother 65(Suppl 4):iv41–iv51

Corrah TW, Enoch DA, Aliyu SH, Lever AM (2011) Bacteraemia and subsequent vertebral osteomyelitis: a retrospective review of 125 patients. QJM 104(3):201–207

Cui L, Tominaga E, Neoh HM, Hiramatsu K (2006) Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. Antimicrob Agents Chemother 50(3):1079–1082

Cunha BA, Gran A (2015) Successful treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) aortic prosthetic valve endocarditis with prolonged high-dose daptomycin plus ceftaroline therapy. Int J Antimicrob Agents 46(2):225–226

Czaja CA, Scholes D, Hooton TM, Stamm WE (2007) Population-based epidemiologic analysis of acute pyelonephritis. Clin Infect Dis 45(3):273–280

Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC, Lynfield R, Nadle J, Petit S, Ray SM, Schaffner W, Townes J, Fridkin S, Emerging Infections Program–Active Bacterial Core Surveillance MRSA Surveillance Investigators (2013) National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. JAMA Intern Med 173(21):1970–1978

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23 (3):616–687

David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG Jr, Miller LG, Ostrowsky B, Baesa A, Boyle-Vavra S, Eells SJ, Garcia-Houchins S, Gialanella P, Macias-Gil R, Rude TH, Ruffin F, Sieth JJ, Volinski J, Spellberg B (2014) *Staphylococcus aureus* bacteremia at 5 US academic medical centers, 2008–2011: significant geographic variation in community-onset infections. Clin Infect Dis 59(6):798–807

Daum RS, Miller LG, Immergluck L, Fritz S, Young D, Kumar N, Downing M, Pettibone S, Hoagland R, Eels S, Boyle MG, Parker TC, Chambers HF, and the DMID 07-0051 team (2017) A placebo controlled trial of antibiotics for smaller skin abscesses. N Engl J Med 376 (26):2545–2555

Dauner DG, Nelson RE, Taketa DC (2010) Ceftobiprole: a novel, broad-spectrum cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. Am J Health Syst Pharm 67 (12):983–993

de Carvalho Ferreira D, Cisne Frota AC, Cavalcante FS, Abad ED, Netto Dos Santos KR (2012) Necrotizing fasciitis secondary to community pneumonia by Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*. Am J Respir Crit Care Med 186(2):202–203

De la Calle C, Morata L, Cobos-Trigueros N, Martinez JA, Cardozo C, Mensa J, Soriano A (2016) *Staphylococcus aureus* bacteremic pneumonia. Eur J Clin Microbiol Infect Dis 35(3):497–502

Dembry LM, Andriole VT (1997) Renal and perirenal abscesses. Infect Dis Clin North Am 11 (3):663–680

Deschênes J, Blondeau J (2015) Besifloxacin in the management of bacterial infections of the ocular surface. Can J Ophthalmol 50(3):184–191

De Schryver N, Cosnard G, van Pesch V, Godfraind C, Hantson P (2011) Extensive spinal cord injury following *Staphylococcus aureus* septicemia and meningitis. Case Rep Neurol 3(2):147–153

Deshpande A, Haleblian G, Rapose A (2013) Prostate abscess: MRSA spreading its influence into Gram-negative territory: case report and literature review. BMJ Case Rep

Donovan ST, Rohman GT, Selph JP, Rajan R, Stocks RM, Thompson JW (2013) Methicillin-resistant *Staphylococcus aureus* as a cause of neonatal suppurative parotitis: a report of two cases and review of the literature. Ear Nose Throat J 92(6):269–271

Drew RH (2007) Emerging options for treatment of invasive, multidrug-resistant *Staphylococcus aureus* infections. Pharmacotherapy 27(2):227–249

Duerden B, Fry C, Johnson AP, Wilcox MH (2015) The control of methicillin-resistant *Staphylococcus aureus* blood stream infections in England. Open Forum Infect Dis 12;2(2): ofv035

Dunne MW, Puttagunta S, Giordano P, Krievins D, Zelasky M, Baldassarre J (2016) A randomized clinical trial of single-dose versus weekly dalbavancin for treatment of acute bacterial skin and skin structure infection. Clin Infect Dis 62(5):545–551

Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS Jr, Swartz MN (1993) Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med 328 (1):21–28

Durkin MJ, Corey GR (2015) New developments in the management of severe skin and deep skin structure infections—focus on tedizolid. Ther Clin Risk Manag 11:857–862

Dylewski J, Martel G (2004) A case of spontaneous methicillin-resistant *Staphylococcus aureus* meningitis in a health care worker. Can J Infect Dis Med Microbiol 15(6):336–338

El Atrouni WI, Knoll BM, Lahr BD, Eckel-Passow JE, Sia IG, Baddour LM (2009) Temporal trends in the incidence of *Staphylococcus aureus* bacteremia in Olmsted County, Minnesota, 1998 to 2005: a population-based study. Clin Infect Dis 49(12):e130–e138

Eliopoulos GM (2003) Quinupristin-dalfopristin and linezolid: evidence and opinion. Clin Infect Dis 36(4):473–481

El-Khashab M, Zonouzi TH, Naghani IM, Nejat F (2012) Cerebellar staphylococcal abscess accompanied with high alfa-fetoprotein in a young infant. Iran J Pediatr 22(4):539–542

Enany S, Higuchi W, Okubo T, Takano T, Enany M, Yamamoto T (2007) Brain abscess caused by Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* in Egypt, April 2007. Euro Surveill 12(9):E070927.2

Enoch DA, Karas JA, Emery MM, Borland C (2006) Two cases of parotid gland infection with bacteraemia due to meticillin-resistant *Staphylococcus aureus*. J Med Microbiol 55(Pt 4):463–465

Fabre V, Ferrada M, Buckel WR, Avdic E, Cosgrove SE (2014) Ceftaroline in combination with trimethoprim-sulfamethoxazole for salvage therapy of methicillin-resistant *Staphylococcus aureus* bacteremia and endocarditis. Open Forum Infect Dis 1(2):ofu046

Farrell DJ, Flamm RK, Sader HS, Jones RN (2014) Ceftobiprole activity against over 60,000 clinical bacterial pathogens isolated in Europe, Turkey, and Israel from 2005 to 2010. Antimicrob Agents Chemother 58(7):3882–3888

File TM Jr, Low DE, Eckburg PB, Talbot GH, Friedland HD, Lee J, Llorens L, Critchley IA, Thye DA, FOCUS 1 investigators (2011) FOCUS 1: a randomized, double-blinded, multicentre, Phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. J Antimicrob Chemother 66(Suppl 3):iii19–iii32

Fleisch AF, Nolan S, Gerber J, Coffin SE (2007) Methicillin-resistant *Staphylococcus aureus* as a cause of extensive retropharyngeal abscess in two infants. Pediatr Infect Dis J 26(12):1161–1163

Flynt LK, Kenney RM, Zervos MJ, Davis SL (2017) The safety and economic impact of cefazolin versus nafcillin for the treatment of methicillin-susceptible *Staphylococcus aureus* bloodstream infections. Infect Dis Ther 6(2):225–231

Fowler A, Mackay A (2006) Community-acquired methicillin-resistant *Staphylococcus aureus* pyomyositis in an intravenous drug user. J Med Microbiol 55:123–125

Fowler VG, Boucher HW, Corey GR, Abrutyn E, Karchmer AW, Rupp ME, Levine DP, Chambers HF, Tally FP, Vigliani GA, Cabell CH, Link AS, DeMeyer I, Filler SG, Zervos M, Cook P, Parsonnet J, Bernstein JM, Price CS, Forrest GN, Fätkenheuer G, Gareca M, Rehm SJ, Brodt HR, Tice A, Cosgrove SE, *S. aureus* Endocarditis and Bacteremia Study Group (2006) Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. N Eng J Med 355:653–665

Freidlin J, Acharya N, Lietman TM, Cevallos V, Whitcher JP, Margolis TP (2007) Spectrum of eye disease caused by methicillin-resistant *Staphylococcus aureus*. Am J Ophthalmol 144 (2):313–315

Fukuda M, Ohashi H, Matsumoto C, Mishima S, Shimomura Y (2002) Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* ocular surface infection efficacy of chloramphenicol eye drops. Cornea 21(7 Suppl):S86–S89

Gardiner D, Dukart G, Cooper A, Babinchak T (2010) Safety and efficacy of intravenous tigecycline in subjects with secondary bacteremia: pooled results from 8 phase III clinical trials. Clin Infect Dis 50(2):229–238

Garland M, Zeller KA, Shetty AK (2016) Toxic shock syndrome due to methicillin-resistant *Staphylococcus aureus* infection after a pediatric scald burn. Am J Emerg Med 34(8):1735.e1–e2

Gattuso G, Palvarini L, Tomasoni D, Ferri F, Scalzini A (2009) Un caso di sepsi da MRSA comunitario (CA-MRSA) complicata da meningoencefalite ed ascesso cerebrale trattato con successo con linezolid [A case of community-acquired MRSA (CA-MRSA) sepsis complicated by meningoencephalitis and cerebral abscess, successfully treated with linezolid]. Infez Med 17 (4):244–248 (in Italian)

Geisler JP, Horlander KM, Hiett AK (1998) Methicillin resistant *Staphylococcus aureus* as a cause of chorioamnionitis. Clin Exp Obstet Gynecol 25(4):119–120

Gill BC, Shoskes DA (2016) Bacterial prostatitis. Curr Opin Infect Dis 29(1):86–91

Gjelland K, Ekerhovd E, Granberg S (2005) Transvaginal ultrasound-guided aspiration for treatment of tubo-ovarian abscess: a study of 302 cases. Am J Obstet Gynecol 193(4):1323–1330

Gokçe Ceylan B, Yavuz L, Baydar CL, Tuz M, Eroglu F, Kiris I, Akcam FZ, Erdem B (2009) Lemierre syndrome: a case of a rarely isolated microorganism, *Staphylococcus auerus*. Med Sci Monit 15(3):CS58–CS61

Gonzalez-Ruiz A, Seaton RA, Hamed K (2016) Daptomycin: an evidence-based review of its role in the treatment of Gram-positive infections. Infect Drug Resist 9:47–58

Gould FK, Brindle R, Chadwick PR, Fraise AP, Hill S, Nathwani D, Ridgway GL, Spry MJ, Warren RE, MRSA Working Party of the British Society for Antimicrobial Chemotherapy (2009) Guidelines (2008) for the prophylaxis and treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United Kingdom. J Antimicrob Chemother 63(5):849–861

Granberg S, Gjelland K, Ekerhovd E (2009) The management of pelvic abscess. Best Pract Res Clin Obstet Gynaecol 23(5):667–678

Grote V, Silier CC, Voit AM, Jansson AF (2017) Bacterial osteomyelitis or nonbacterial osteitis in children: a study involving the German Surveillance Unit for Rare Diseases in Childhood. Pediatr Infect Dis J 36(5):451–456

Gubbay AJ, Isaacs D (2000) Pyomyositis in children. Pediatr Infect Dis J 19(10):1009–1012

Gunatilake SS, Yapa LG, Gallala M, Gamlath R, Rodrigo C, Wimalaratna H (2014) Lemierre's syndrome secondary to community-acquired methicillin-resistant *Staphylococcus aureus* infection presenting with cardiac tamponade, a rare disease with a life-threatening presentation: a case report. Int J Emerg Med 7:39

Hajjeh RA, Reingold A, Weil A, Shutt K, Schuchat A, Perkins BA (1999) Toxic shock syndrome in the United States: surveillance update, 1979–1996. Emerg Infect Dis 5(6):807–810

Handler MZ, Schwartz RA (2014) Staphylococcal scalded skin syndrome: diagnosis and management in children and adults. J Eur Acad Dermatol Venereol 28(11):1418–1423

Harrison EM, Ba X, Blane B, Ellington MJ, Loeffler A, Hill RL, Holmes MA, Peacock SJ (2016) PBP2a substitutions linked to ceftaroline resistance in MRSA isolates from the UK. J Antimicrob Chemother 71(1):268–269

Herek PA, Lewis T, Bailitz JM (2010) An unusual case of Lemierre's syndrome due to methicillin-resistant *Staphylococcus aureus*. J Emerg Med 39(5):644–646

Hirai J, Hagihara M, Haranaga S, Kinjo T, Hashioka H, Kato H, Sakanashi D, Yamagishi Y, Mikamo H, Fujita J (2017) Eosinophilic pneumonia caused by daptomycin: six cases from two institutions and a review of the literature. J Infect Chemother 23(4):245–249

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40(1):135–136

Ho TT, Cadena J, Childs LM, Gonzalez-Velez M, Lewis JS 2nd (2012) Methicillin-resistant *Staphylococcus aureus* bacteraemia and endocarditis treated with ceftaroline salvage therapy. J Antimicrob Chemother 67(5):1267–1270

Hoen B, Duval X (2013) Clinical practice. Infective endocarditis. N Engl J Med 368(15):1425–1433

Holland TL, Arnold C, Fowler VG Jr (2014) Clinical management of *Staphylococcus aureus* bacteremia: a review. JAMA 312(13):1330–1341

Holmes MA, Zadoks RN (2011) Methicillin resistant *S. aureus* in human and bovine mastitis. J Mammary Gland Biol Neoplasia 16(4):373–382

Holubar M, Meng L, Deresinski S (2016) Bacteremia due to methicillin-resistant *Staphylococcus aureus*: new therapeutic approaches. Infect Dis Clin North Am 30(2):491–507

Huang V, Gortney JS (2006) Risk of serotonin syndrome with concomitant administration of linezolid and serotonin agonists. Pharmacotherapy 26(12):1784–1793

Huang WC, Lee CH, Liu JW (2010) Clinical characteristics and risk factors for mortality in patients with meningitis caused by *Staphylococcus aureus* and vancomycin minimal inhibitory concentrations against these isolates. J Microbiol Immunol Infect 43(6):470–477

Hussein QA, Anaya DA (2013) Necrotizing soft tissue infections. Crit Care Clin 29(4):795–806

Itani KM, Dryden MS, Bhattacharyya H, Kunkel MJ, Baruch AM, Weigelt JA (2010) Efficacy and safety of linezolid versus vancomycin for the treatment of complicated skin and soft-tissue infections proven to be caused by methicillin-resistant *Staphylococcus aureus*. Am J Surg 199 (6):804–816

Jahanfar S, Ng CJ, Teng CL (2013) Antibiotics for mastitis in breastfeeding women. Cochrane Database Syst Rev (2):CD005458

Jariwala RH, Srialluri S, Huang MZ, Boppana SB (2017) Methicillin-resistant *Staphylococcus aureus* as a cause of Lemierre's syndrome. Pediatr Infect Dis J 36(4):429–431

Johannesen KM, Bodtger U (2016) Lemierre's syndrome: current perspectives on diagnosis and management. Infect Drug Resist 9:221–227

Jongsma K, Joson J, Heidari A (2013) Ceftaroline in the treatment of concomitant methicillin-resistant and daptomycin-non-susceptible *Staphylococcus aureus* infective endocarditis and osteomyelitis: case report. J Antimicrob Chemother 68(6):1444–1445

Jørgensen NP, Skovdal SM, Meyer RL, Dagnæs-Hansen F, Fuursted K, Petersen E (2016) Rifampicin-containing combinations are superior to combinations of vancomycin, linezolid and daptomycin against *Staphylococcus aureus* biofilm infection in vivo and in vitro. Pathog Dis 74(4):ftw019

Kaasch AJ, Seifert H (2016) Oritavancin: a long-acting antibacterial lipoglycopeptide. Future Microbiol 11:843–855

Kakish E, Wiesner AM, Winstead PS, Bensadoun ES (2008) Acute respiratory failure due to daptomycin induced eosinophilic pneumonia. Respir Med 1:235–237

Kalogeropoulos AS, Tsiodras S, Loverdos D, Fanourgiakis P, Skoutelis A (2011) Eosinophilic pneumonia associated with daptomycin: a case report and a review of the literature. J Med Case Rep 5:13

Kalorin CM, Tobin EH (2007) Community-associated methicillin-resistant *Staphylococcus aureus* causing Fournier's gangrene and genital infections. J Urology 177:967–971

Kao KL, Wu KG, Chen CJ, Wu JJ, Tang RB, Chang KP, Wong TT (2008) Brain abscesses in children: analysis of 20 cases presenting at a medical center. J Microbiol Immunol Infect 41 (5):403–407

Kaplan SL (2014) Recent lessons for the management of bone and joint infections. J Infect 68 (Suppl 1):S51–S56

Karlowsky JA, Adam HJ, Baxter MR, Lagacé-Wiens PR, Walkty AJ, Hoban DJ, Zhanel GG (2013) In vitro activity of ceftaroline-avibactam against Gram-negative and Gram-positive pathogens isolated from patients in Canadian hospitals from 2010 to 2012: results from the CANWARD surveillance study. Antimicrob Agents Chemother 57(11):5600–5611

Karlowsky JA, Nichol K, Zhanel GG (2015) Telavancin: mechanisms of action, in vitro activity, and mechanisms of resistance. Clin Infect Dis 61(Suppl 2):S58–S68

Kauffman CA, Fisher JF, Sobel JD, Newman CA (2011) *Candida* urinary tract infections–diagnosis. Clin Infect Dis 52(Suppl 6):S452–S456

Kelesidis T, Humphries R, Ward K, Lewinski MA, Yang OO (2011) Combination therapy with daptomycin, linezolid, and rifampin as treatment option for MRSA meningitis and bacteremia. Diagn Microbiol Infect Dis 71(3):286–290

Kempker R, Difrancesco L, Martin-Gorgojo A, Franco-Paredes C (2009) Expanding spectrum of illness due to community-associated methicillin-resistant *Staphylococcus aureus*: a case report. Cases J 2:7437

Kessler AT, Kourtis AP (2007) Treatment of meningitis caused by methicillin-resistant *Staphylococcus aureus* with linezolid. Infection 35(4):271–274

Kim MJ, Koo HM, Lee WJ, Choi JH, Choi MN, Park SY, Kim WJ, Son SY (2016) Development of epidural and paraspinal abscesses after insufficient evaluation and treatment of acute pyelonephritis caused by *Staphylococcus aureus*. Korean J Fam Med 37(5):299–302

Kizhner V, Samara G, Panesar R, Krespi YP (2013) Methicillin-resistant *Staphylococcus aureus* bacteraemia associated with Lemierre's syndrome: case report and literature review. J Laryngol Otol 127(7):721–723

Koch C, Hecker A, Grau V, Padberg W, Wolff M, Henrich M (2015) Intravenous immunoglobulin in necrotizing fasciitis—a case report and review of recent literature. Ann Med Surg (Lond) 4 (3):260–263

Kobayashi D, Givner LB, Yeatts RP, Anthony EY, Shetty AK (2011) Infantile orbital cellulitis secondary to community-associated methicillin-resistant *Staphylococcus aureus*. J AAPOS 15 (2):208–210

Koning S, van der Sande R, Verhagen AP, van Suijlekom-Smit LW, Morris AD, Butler CC, Berger M, van der Wouden JC (2012) Interventions for impetigo. Cochrane Database Syst Rev 1:CD003261

Krakauer T, Pradhan K, Stiles BG (2016) Staphylococcal superantigens spark host-mediated danger signals. Front Immunol 7:23

Kulhankova K, King J, Salgado-Pabón W (2014) Staphylococcal toxic shock syndrome: superantigen-mediated enhancement of endotoxin shock and adaptive immune suppression. Immunol Res 59(1–3):182–187

Ladhani S, Joannou CL (2000) Difficulties in diagnosis and management of the staphylococcal scalded skin syndrome. Pediatr Infect Dis J 19(9):819–821

Lahiri SD, McLaughlin RE, Whiteaker JD, Ambler JE, Alm RA (2015) Molecular characterization of MRSA isolates bracketing the current EUCAST ceftaroline-susceptible breakpoint for *Staphylococcus aureus*: the role of PBP2a in the activity of ceftaroline. J Antimicrob Chemother 70(9):2488–2498

Lal Y, Assimacopoulos AP (2010) Two cases of daptomycin-induced eosinophilic pneumonia and chronic pneumonitis. Clin Infect Dis 50(5):737–740

Lam E, Chan T, Wiseman SM (2014) Breast abscess: evidence based management recommendations. Expert Rev Anti Infect Ther 12(7):753–762

Lappin E, Ferguson AJ (2009) Gram-positive toxic shock syndromes. Lancet Infect Dis 9(5):281–290

Larson KE, Carrillo-Marquez M (2015) Endogenous methicillin-resistant *Staphylococcus aureus* endophthalmitis after leg trauma. J AAPOS 19(4):387–389

Laupland KB, Church DL (2014) Population-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev 27(4):647–664

Laurens MB, Becker RM, Johnson JK, Wolf JS, Kotloff KL (2008) MRSA with progression from otitis media and sphenoid sinusitis to clival osteomyelitis, pachymeningitis and abducens nerve palsy in an immunocompetent 10-year-old patient. Int J Pediatr Otorhinolaryngol 72(7):945–951

Le B, Schaeffer AJ (2011) Chronic prostatitis. BMJ Clin Evid 2011. pii: 1802

Lee DH, Palermo B, Chowdhury M (2008) Successful treatment of methicillin-resistant *Staphylococcus aureus* meningitis with daptomycin. Clin Infect Dis 47(4):588–590

Lee IW, Kang L, Hsu HP, Kuo PL, Chang CM (2010) Puerperal mastitis requiring hospitalization during a nine-year period. Am J Obstet Gynecol 203(4):332.e1–e6

Lei TH, Huang YC, Chu YC, Lee CY, Lien R (2013) Orbital cellulitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in a previously healthy neonate. J Microbiol Immunol Infect 46(2):136–138

Leotta N, Chaseling R, Duncan G, Isaacs D (2005) Intracranial suppuration. J Paediatr Child Health 41(9–10):508–512

Lew DP, Waldvogel FA (2004) Osteomyelitis. Lancet 364(9431):369–379

Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, Bashore T, Corey GR (2000) Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 30(4):633–638

Liapikou A, Cillóniz C, Torres A (2015) Ceftobiprole for the treatment of pneumonia: a European perspective. Drug Des Devel Ther 9:4565–4572

Lin CY, Chiu NC, Lee KS, Huang FY, Hsu CH (2013a) Neonatal orbital abscess. Pediatr Int 55 (3):e63–e66

Lin JC, Aung G, Thomas A, Jahng M, Johns S, Fierer J (2013b) The use of ceftaroline fosamil in methicillin-resistant *Staphylococcus aureus* endocarditis and deep-seated MRSA infections: a retrospective case series of 10 patients. J Infect Chemother 19(1):42–49

Lin WT, Chao CM, Lin HL, Hung MC, Lai CC (2015) Emergence of antibiotic-resistant bacteria in patients with Fournier gangrene. Surg Infect (Larchmt) 16(2):165–168

Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine-leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128–1132

Linder BJ, Granberg CF (2016) Pediatric renal abscesses: a contemporary series. J Pediatr Urol 12 (2):99.e1–e15

Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HF, Infectious Diseases Society of America (2011) Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin Infect Dis 52(3):e18–e55

Lo BM, Erwin EA (2008) Missed epidural brain abscess after furunculosis. Am J Emerg Med 26 (4):522.e3–e4

Low DE, File TM Jr, Eckburg PB, Talbot GH, David Friedland H, Lee J, Llorens L, Critchley IA, Thye DA, FOCUS 2 investigators (2011) FOCUS 2: a randomized, double-blinded, multicentre, Phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. J Antimicrob Chemother 66(Suppl 3):iii33–iii44

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532

Mah FS, Sanfilippo CM (2016) Besifloxacin: efficacy and safety in treatment and prevention of ocular bacterial infections. Ophthalmol Ther 5(1):1–20

Maher C, McLaughlin J, Kelsey G, Cave D, Haran M (1993) Staphylococcal septicaemia after endometrial destruction. Aust N Z J Obstet Gynaecol 33(4):443–444

Mansur AJ, Grinberg M, da Luz PL, Bellotti G (1992) The complications of infective endocarditis. A reappraisal in the 1980s. Arch Intern Med 152(12):2428–2432

Martens MG, Faro S, Maccato M, Riddle G, Hammill HA (1991) Susceptibility of female pelvic pathogens to oral antibiotic agents in patients who develop postpartum endometritis. Am J Obstet Gynecol 164(5 Pt 2):1383–1386

McDougal LK, Fosheim GE, Nicholson A, Bulens SN, Limbago BM, Shearer JE, Summers AO, Patel JB (2010) Emergence of resistance among USA300 methicillin-resistant *Staphylococcus aureus* isolates causing invasive disease in the United States. Antimicrob Agents Chemother 54 (9):3804–3811

McHenry MC, Easley KA, Locker GA (2002) Vertebral osteomyelitis: long-term outcome for 253 patients from 7 Cleveland-area hospitals. Clin Infect Dis 34(10):1342–1350

Miller BA, Gray A, Leblanc TW, Sexton DJ, Martin AR, Slama TG (2010) Acute eosinophilic pneumonia secondary to daptomycin: a report of three cases. Clin Infect Dis 50(11):e63–e68

Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B (2005) Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 352(14):1445–1453

Miller LG, Daum RS, Creech CB, Young D, Downing MD, Eells SJ, Pettibone S, Hoagland RJ, Chambers HF, DMID 07-0051 Team (2015) Clindamycin versus

trimethoprim-sulfamethoxazole for uncomplicated skin infections. N Engl J Med 372 (12):1093–1103

Mohammed I, Hofstetter M (2004) Acute bacterial parotitis due to methicillin-resistant *Staphylococcus aureus*. South Med J 97(11):1139

Molloy A, Towersey G, Shackleton D, Aali A, Ash S (2012) The changing face of an old disease: case report of nonclassical Lemierre's syndrome caused by a Panton-Valentine leucocidin-positive methicillin-susceptible *Staphylococcus aureus* isolate. J Clin Microbiol 50(9):3144–3145

Moran GJ, Fang E, Corey GR, Das AF, De Anda C, Prokocimer P (2014) Tedizolid for 6 days versus linezolid for 10 days for acute bacterial skin and skin-structure infections (ESTABLISH-2): a randomised, double-blind, phase 3, non-inferiority trial. Lancet Infect Dis 14(8):696–705

Mubarak N, Sandaradura I, Isaia L, O'Sullivan M, Zhou F, Marriott D, Iredell JR, Harkness J, Andresen D (2015) Non-susceptibility to ceftaroline in healthcare-associated multiresistant MRSA in Eastern Australia. J Antimicrob Chemother 70(8):2413–2414

Munckhof WJ, Krishnan A, Kruger P, Looke D (2008) Cavernous sinus thrombosis and meningitis from community-acquired methicillin-resistant *Staphylococcus aureus* infection. Intern Med J 38(4):283–287

Mutale W, Sahay KM, Hartley J, Thompson D, Ratnasinghe D, Hudson L, Hulse E, Fellows G (2014) Community acquired Panton-Valentine Leukocidin (PVL) positive methicilin resistant Staphylococcal aureus cerebral abscess in an 11-month old boy: a case study. BMC Res Notes 7:862

Naesens R, Ronsyn M, Druwé P, Denis O, Ieven M, Jeurissen A (2009) Central nervous system invasion by community-acquired meticillin-resistant *Staphylococcus aureus*. J Med Microbiol 58(Pt 9):1247–1251

Narayanan M, Mookherjee S, Spector TB, White AA (2013) MSSA brain abscess and pyomyositis presenting as brain tumour and DVT. BMJ Case Rep 2013. pii: bcr2013009380

Nathwani D, Morgan M, Masterton RG, Dryden M, Cookson BD, French G, Lewis D, British Society for Antimicrobial Chemotherapy Working Party on Community-onset MRSA Infections (2008) Guidelines for UK practice for the diagnosis and management of methicillin-resistant *Staphylococcus aureus* (MRSA) infections presenting in the community. J Antimicrob Chemother 61(5):976–994

Newitt S, Myles PR, Birkin JA, Maskell V, Slack RC, Nguyen-Van-Tam JS, Szatkowski L (2015) Impact of infection control interventions on rates of *Staphylococcus aureus* bacteraemia in National Health Service acute hospitals, East Midlands, UK, using interrupted time-series analysis. J Hosp Infect 90(1):28–37

Nickerson M, Bhargava A, Kale-Pradhan PB (2017) Daptomycin-associated eosinophilic pneumonia with rechallenge: a case report. Int J Clin Pharmacol Ther 55(6):521–524

Nicolasora NP, Zacharek MA, Malani AN (2009) Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging cause of acute bacterial parotitis. South Med J 102 (2):208–210

Nigo M, Diaz L, Carvajal LP, Tran TT, Rios R, Panesso D, Garavito JD, Miller WR, Wanger A, Weinstock G, Munita JM, Arias CA, Chambers HF (2017 Feb 23) Ceftaroline-resistant, daptomycin-tolerant, and heterogeneous vancomycin-intermediate methicillin-resistant *Staphylococcus aureus* causing infective endocarditis. Antimicrob Agents Chemother 61(3), e-published ahead-of-print

Ntziora F, Falagas ME (2007) Linezolid for the treatment of patients with central nervous system infection. Ann Pharmacother 41(2):296–308

Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR, Infectious Diseases Society of America (2013) Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56(1):e1–e25

Ozuna RM, Delamarter RB (1996) Pyogenic vertebral osteomyelitis and postsurgical disc space infections. Orthop Clin North Am 27(1):87–94

Pagani L, Bonnin P, Janssen C, Desjoyaux E, Vitrat V, Bru JP (2014) Ceftaroline for the treatment of prosthetic valve endocarditis due to methicillin-resistant *Staphylococcus aureus*. J Heart Valve Dis 23(2):219–221

Paladino JA, Jacobs DM, Shields RK, Taylor J, Bader J, Adelman MH, Wilton GJ, Crane JK, Schentag JJ (2014) Use of ceftaroline after glycopeptide failure to eradicate meticillin-resistant *Staphylococcus aureus* bacteraemia with elevated vancomycin minimum inhibitory concentrations. Int J Antimicrob Agents 44(6):557–563

Pannaraj PS, Hulten KG, Gonzalez BE, Mason EO, Kaplan SL (2006) Infective pyomyositis and myositis in children in the era of community-acquired, methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 43:953–960

Park HK, Kim YS, Oh SH, Lee HJ (2015) Successful treatment with ultrasound-guided aspiration of intractable methicillin-resistant *Staphylococcus aureus* brain abscess in an extremely low birth weight infant. Pediatr Neurosurg 50(4):210–215. Erratum in: Pediatr Neurosurg 2015;50 (4):215

Patel JJ, Antony A, Herrera M, Lipchik RJ (2014) Daptomycin-induced acute eosinophilic pneumonia. WMJ 113(5):199–201

Peacock SJ, Paterson GK (2015) Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annu Rev Biochem 84:577–601

Perbet S, Soummer A, Vinsonneau C, Vandebrouck A, Rackelboom T, Etienne J, Cariou A, Chiche JD, Mira JP, Charpentier J (2010) Multifocal community-acquired necrotizing fasciitis caused by a Panton-Valentine leukocidin-producing methicillin-sensitive *Staphylococcus aureus*. Infection 38(3):223–225

Pereira NM, Shah I, Ohri A, Shah F (2015) Methicillin resistant *Staphylococcus aureus* meningitis. Oxf Med Case Reports 11:364–366

Pérez A, Orta L, Padilla E, Mesquida X (2013) CA-MRSA puerperal mastitis and breast abscess: a potential problem emerging in Europe with many unanswered questions. J Matern Fetal Neonatal Med 26(9):949–951

Perletti G, Marras E, Wagenlehner FM, Magri V (2013) Antimicrobial therapy for chronic bacterial prostatitis. Cochrane Database Syst Rev (8):CD009071

Pigrau C, Almirante B, Flores X, Falco V, Rodríguez D, Gasser I, Villanueva C, Pahissa A (2005) Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med 118(11):128

Pintado V, Pazos R, Jiménez-Mejías ME, Rodríguez-Guardado A, Gil A, García-Lechuz JM, Cabellos C, Chaves F, Domingo P, Ramos A, Pérez-Cecilia E, Domingo D (2012) Methicillin-resistant *Staphylococcus aureus* meningitis in adults: a multicenter study of 86 cases. Medicine (Baltimore) 91(1):10–17

Prokocimer P, De Anda C, Fang E, Mehra P, Das A (2013) Tedizolid phosphate vs linezolid for treatment of acute bacterial skin and skin structure infections: the ESTABLISH-1 randomized trial. JAMA 309(6):559–569

Rájová J, Pantůček R, Petráš P, Varbanovová I, Mašlaňová I, Beneš J (2016) Necrotizing pneumonia due to clonally diverse *Staphylococcus aureus* strains producing Panton-Valentine leukocidin: the Czech experience. Epidemiol Infect 144(3):507–515

Ramos A, Ley L, Muñez E, Videl A, Sánchez I (2009) Brain abscess due to Panton-Valentine leukocidin-positive *Staphylococcus aureus*. Infection 37(4):365–367

Raychaudhuri M, Chew PR (2012) A case of epididymitis associated with Panton-Valentine leukocidin *Staphylococcus aureus*. Sex Transm Infect 88(5):355–356

Redden MD, Chin TY, van Driel ML (2017) Surgical versus non-surgical management for pleural empyema. Cochrane Database Syst Rev 3:CD010651

Rhee Y, Aroutcheva A, Hota B, Weinstein RA, Popovich KJ (2015) Evolving epidemiology of *Staphylococcus aureus* bacteremia. Infect Control Hosp Epidemiol 36(12):1417–1422

Rodvold KA (2015) Telavancin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 61(Suppl 2):S35–S37

Rose WE, Rybak MJ (2006) Tigecycline: first of a new class of antimicrobial agents. Pharmacotherapy 26(8):1099–1110

Ross J, Abate MA (1990) Topical vancomycin for the treatment of *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus* conjunctivitis. DICP 24(11):1050–1053

Rubinstein E, Prokocimer P, Talbot GH (1999) Safety and tolerability of quinupristin/dalfopristin: administration guidelines. J Antimicrob Chemother 44(Suppl A):37–46

Rubinstein E, Lalani T, Corey GR, Kanafani ZA, Nannini EC, Rocha MG, Rahav G, Niederman MS, Kollef MH, Shorr AF, Lee PC, Lentnek AL, Luna CM, Fagon JY, Torres A, Kitt MM, Genter FC, Barriere SL, Friedland HD, Stryjewski ME, ATTAIN Study Group (2011) Telavancin versus vancomycin for hospital-acquired pneumonia due to Gram-positive pathogens. Clin Infect Dis 52(1):31–40

Ruggero MA, Peaper DR, Topal JE (2015) Telavancin for refractory methicillin-resistant *Staphylococcus aureus* bacteremia and infective endocarditis. Infect Dis (Lond) 47(6):379–384

Ruhe JJ, Menon A (2007) Tetracyclines as an oral treatment option for patients with community-onset methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infections. Antimicrob. Agents Chermother 51:3298–3303

Ruhe JJ, Smith N, Bradsher RW, Menon A (2007) Community-onset methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections: impact of antimicrobial therapy on outcome. Clin Infect Dis 44:777–784

Ruiz ME, Yohannes S, Wladyka CG (2005) Pyomyositis caused by methicillin-resistant *Staphylococcus aureus*. N Engl J Med 352(14):1488–1489

Russell CD, Ramaesh R, Kalima P, Murray A, Gaston MS (2015) Microbiological characteristics of acute osteoarticular infections in children. J Med Microbiol 64(Pt 4):446–453

Rutar T, Chambers HF, Crawford JB, Perdreau-Remington F, Zwick OM, Karr M, Diehn JJ, Cockerham KP (2006) Ophthalmic manifestations of infections caused by the USA300 clone of community-associated methicillin-resistant *Staphylococcus aureus*. Ophthalmology 113 (8):1455–1462

Rybak JM, Roberts K (2015 Feb 24) Tedizolid phosphate: a next-generation oxazolidinone. Infect Dis Ther, e-published ahead of print

Rybak JM, Marx K, Martin CA (2014) Early experience with tedizolid: clinical efficacy, pharmacodynamics, and resistance. Pharmacotherapy 34(11):1198–1208

Saavedra-Lozano J, Mejías A, Ahmad N, Peromingo E, Ardura MI, Guillen S, Syed A, Cavuoti D, Ramilo O (2008) Changing trends in acute osteomyelitis in children: impact of methicillin-resistant *Staphylococcus aureus* infections. J Pediatr Orthop 28(5):569–575

Saito N, Aoki K, Sakurai T, Ito K, Hayashi M, Hirata Y, Sato K, Harashina J, Akahata M, Iwabuchi S (2010) Linezolid treatment for intracranial abscesses caused by methicillin-resistant *Staphylococcus aureus*–two case reports. Neurol Med Chir (Tokyo) 50(6):515–517

Sakoulas G, Moise PA, Casapao AM, Nonejuie P, Olson J, Okumura CY, Rybak MJ, Kullar R, Dhand A, Rose WE, Goff DA, Bressler AM, Lee Y, Pogliano J, Johns S, Kaatz GW, Ebright JR, Nizet V (2014) Antimicrobial salvage therapy for persistent staphylococcal bacteremia using daptomycin plus ceftaroline. Clin Ther 36(10):1317–1333

Sandrock CE, Shorr AF (2015) The role of telavancin in hospital-acquired pneumonia and ventilator-associated pneumonia. Clin Infect Dis 61(Suppl 2):S79–S86

Saravolatz LD, Stein GE, Johnson LB (2011) Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 52(9):1156–1163

Sato K (2015) External ocular infections due to methicillin-resistant *Staphylococcus aureus* and medical history. Can J Ophthalmol 50(5):e97–e99

Schaumburg F, Peters G, Alabi A, Becker K, Idelevich EA (2016) Missense mutations of PBP2a are associated with reduced susceptibility to ceftaroline and ceftobiprole in African MRSA. J Antimicrob Chemother 71(1):41–44

Schmidt H, Lissner R, Struff W, Thamm O, Karch H (2002) Antibody reactivity of a standardized human serum protein solution against a spectrum of microbial pathogens and toxins: comparison with fresh frozen plasma. Ther Apher 6(2):145–153

Schoenfeld EM, McKay MP (2010) Mastitis and methicillin-resistant *Staphylococcus aureus* (MRSA): the calm before the storm? J Emerg Med 38(4):e31–e34

Schwartz KL, Nourse C (2012) Panton-Valentine leukocidin-associated *Staphylococcus aureus* necrotizing pneumonia in infants: a report of four cases and review of the literature. Eur J Pediatr 171(4):711–717

Shah NS, Greenberg JA, McNulty MC, Gregg KS, Riddell J, Mangino JE, Weber DM, Hebert CL, Marzec NS, Barron MA, Chaparro-Rojas F, Restrepo A, Hemmige V, Prasidthrathsint K, Cobb S, Herwaldt L, Raabe V, Cannavino CR, Hines AG, Bares SH, Antiporta PB, Scardina T, Patel U, Reid G, Mohazabnia P, Kachhdiya S, Le BM, Park CJ, Ostrowsky B, Robicsek A, Smith BA, Schied J, Bhatti MM, Mayer S, Sikka, M, Murphy-Aguilu I, Patwari P, Abeles SR, Torriani FJ, Abbas Z, Toya S, Doktor K, Chakrabarti A, Doblecki-Lewis S, Looney DJ, David MZ (2015) Severe influenza in 33 US hospitals, 2013–2014: complications and risk factors for death in 507 patients. Infect Control Hosp Epidemiol 36(11):1251–1260

Shane AL, Hansen NI, Stoll BJ, Bell EF, Sánchez PJ, Shankaran S, Laptook AR, Das A, Walsh MC, Hale EC, Newman NS, Schrag SJ, Higgins RD, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2012) Methicillin-resistant and susceptible *Staphylococcus aureus* bacteremia and meningitis in preterm infants. Pediatrics 129(4):e914–e922

Sharma S, Josephson GD (2014) Orbital complications of acute sinusitis in infants: a systematic review and report of a case. JAMA Otolaryngol Head Neck Surg 140(11):1070–1073

Scheeren TW (2015) Ceftobiprole medocaril in the treatment of hospital-acquired pneumonia. Future Microbiol 10(12):1913–1928

Shioya N, Ishibe Y, Kan S, Masuda T, Matsumoto N, Takahashi G, Makabe H, Yamada Y, Endo S (2012) Sternoclavicular joint septic arthritis following paraspinal muscle abscess and septic lumbar spondylodiscitis with epidural abscess in a patient with diabetes: a case report. BMC Emerg Med 12:7

Shyam DC, Rapsang AG (2013) Fournier's gangrene. Surgeon 11(4):222–232

Sifri CD, Park J, Helm GA, Stemper ME, Shukla SK (2007) Fatal brain abscess due to community-associated methicillin-resistant *Staphylococcus aureus* strain USA300. Clin Infect Dis 45(9):e113–e117

Silverman JA, Mortin LI, Vanpraagh AD, Li T, Alder J (2005) Inhibition of daptomycin by pulmonary surfactant: in vitro modeling and clinical impact. J Infect Dis 191(12):2149–2152

Simpfendorfer CS (2017) Radiologic approach to musculoskeletal infections. Infect Dis Clin North Am 31(2):299–324

Singh JA, Yu S (2017a) Septic arthritis in the emergency departments in the U.S.: a national study of healthcare utilization and time-trends. Arthritis Care Res (Hoboken), e-published ahead-of-print

Singh N, Vogelgesang SA (2017b) Monoarticular arthritis. Med Clin North Am 101(3):607–613

Sipahi OR, Bardak-Ozcem S, Turhan T, Arda B, Ruksen M, Pullukcu H, Aydemir S, Dalbasti T, Yurtseven T, Sipahi H, Zileli M, Ulusoy S (2013) Vancomycin versus linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis. Surg Infect (Larchmt) 14 (4):357–362

Small LN, Ross JJ (2005) Tropical and temperate pyomyositis. Infect Dis Clin North Am 19 (4):981–989, x–xi

Smetana M, Picard K, Boehm KM (2013) An acute ibuprofen overdose masking a severe *Staphylococcus aureus* meningitis: a case report. Case Rep Emerg Med 2013:603251

Smith JR, Roberts KD, Rybak MJ (2015) Dalbavancin: a novel lipoglycopeptide antibiotic with extended activity against Gram-positive infections. Infect Dis Ther 4(3):245–258

Spencer DA, Thomas MF (2014) Necrotising pneumonia in children. Paediatr Respir Rev 15 (3):240–245

Spernovasilis N, Demetriou S, Bachlitzanaki M, Gialamas I, Alpantaki K, Hamilos G, Karantanas A, Gikas A (2017) Characteristics and predictors of outcome of spontaneous spinal epidural abscesses treated conservatively: a retrospective cohort study in a referral center. Clin Neurol Neurosurg 156:11–17

Stein GE, Babinchak T (2013) Tigecycline: an update. Diagn Microbiol Infect Dis 75(4):331–336

Stevens DL, Herr D, Lampiris H, Hunt JL, Batts DH, Hafkin B (2002) Linezolid versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 34(11):1481–1490

Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC, Infectious Diseases Society of America (2014) Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clin Infect Dis 59(2):e10–e52

Stewart CL, Turner MS, Frens JJ, Snider CB, Smith JR (2017 Apr 6) Real-world experience with Oritavancin therapy in invasive Gram-positive infections. Infect Dis Ther 6(2):277–289

Stewart P, Khatami A, Loughran-Fowlds A, Isaacs D (2015) Methicillin-resistant *Staphylococcus aureus* bacteraemia and epidural abscess in a neonate. J Paediatr Child Health 51(4):458–460

Street M, Puna R, Huang M, Crawford H (2015) Pediatric acute hematogenous osteomyelitis. J Pediatr Orthop 35(6):634–639

Strommenger B, Layer F, Klare I, Werner G (2015) Pre-use susceptibility to ceftaroline in clinical *Staphylococcus aureus* isolates from Germany: is there a non-susceptible pool to be selected? PLoS One 10(5):e0125864

Stryjewski ME, Graham DR, Wilson SE, O'Riordan W, Young D, Lentnek A, Ross DP, Fowler VG, Hopkins A, Friedland HD, Barriere SL, Kitt MM, Corey GR, Assessment of Telavancin in Complicated Skin and Skin-Structure Infections Study (2008) Telavancin versus vancomycin for the treatment of complicated skin and skin-structure infections caused by Gram-positive organisms. Clin Infect Dis 46(11):1683–1693

Stryjewski ME, Lentnek A, O'Riordan W, Pullman J, Tambyah PA, Miró JM, Fowler VG Jr, Barriere SL, Kitt MM, Corey GR (2014) A randomized Phase 2 trial of telavancin versus standard therapy in patients with uncomplicated *Staphylococcus aureus* bacteremia: the ASSURE study. BMC Infect Dis 14:289

Stryjewski ME, Jones RN, Corey GR (2015) Ceftaroline: clinical and microbiology experience with focus on methicillin-resistant *Staphylococcus aureus* after regulatory approval in the USA. Diagn Microbiol Infect Dis 81(3):183–188

Sultan HY, Boyle AA, Sheppard N (2012) Necrotising fasciitis. BMJ 345:e4274

Suppiah S, Meng Y, Fehlings MG, Massicotte EM, Yee A, Shamji MF (2016) How best to manage the spinal epidural abscess? A current systematic review. World Neurosurg 93:20–28

Susaníbar Napurí LF, Simón Rodríguez C, López Martín L, Monzó Gardinier J, Cabello Benavente R, González Enguita C (2011) Prostatic abscess: diagnosis and treatment of an infrequent urological entity. Arch Esp Urol 64(1):62–66

Szumowski JD, Cohen DE, Kanaya F, Mayer KH (2007) Treatment and outcomes of MRSA at an ambulatory clinic. Antimicrob Agents Chemother 51:423–428

Talan DA, Mower WR, Krishnadasan A, Abrahamian FM, Lovecchio F, Karras DJ, Steele MT, Rothman RE, Hoagland R, Moran GJ (2016) Trimethoprim-sulfamethoxazole versus placebo for uncomplicated skin abscess. N Engl J Med 374(9):823–832

Tarff A, Behrens A (2017) Ocular emergencies: red eye. Med Clin North Am 101(3):615–639

Tattevin P, Boutoille D, Vitrat V, Van Grunderbeeck N, Revest M, Dupont M, Alfandari S, Stahl JP (2014) Salvage treatment of methicillin-resistant staphylococcal endocarditis with ceftaroline: a multicentre observational study. J Antimicrob Chemother 69(7):2010–2013

Thapaliya D, O'Brien AM, Wardyn SE, Smith TC (2015) Epidemiology of necrotizing infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes* at an Iowa hospital. J Infect Public Health 8(6):634–641

Thwaites GE, Edgeworth JD, Gkrania-Klotsas E, Kirby A, Tilley R, Török ME, Walker S, Wertheim HF, Wilson P, Llewelyn MJ, UK Clinical Infection Research Group (2011) Clinical management of *Staphylococcus aureus* bacteraemia. Lancet Infect Dis 11(3):208–222

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661

Townsend ML, Pound MW, Drew RH (2011) Potential role of tigecycline in the treatment of community-acquired bacterial pneumonia. Infect Drug Resist 4:77–86

Tsitsilonis S, Druschel C, Wichlas F, Haas NP, Schwabe P, Bail HJ, Schaser KD (2013) Necrotizing fasciitis: is the bacterial spectrum changing? Langenbecks Arch Surg 398(1):153–159

Tsoulas C, Nathwani D (2015) Review of meta-analyses of vancomycin compared with new treatments for Gram-positive skin and soft-tissue infections: are we any clearer? Int J Antimicrob Agents 46(1):1–7

Tyagi R (2016) Spinal infections in children: a review. J Orthop 13(4):254–258

Udassi S, Udassi JP, Giordano BP, Lew JF (2015) An unusual cause of neonatal meningitis. J Pediatr Health Care 29(6):547–550

Unnikrishnan PN, Perry DC, George H, Bassi R, Bruce CE (2010) Tropical primary pyomyositis in children of the UK: an emerging medical challenge. Int Orthop 34(1):109–113

Valentini P, Parisi G, Monaco M, Crea F, Spanu T, Ranno O, Tronci M, Pantosti A (2008) An uncommon presentation for a severe invasive infection due to methicillin-resistant *Staphylococcus aureus* clone USA300 in Italy: a case report. Ann Clin Microbiol Antimicrob 7:11

Vallejo JG, Cain AN, Mason EO, Kaplan SL, Hultén KG (2017) *Staphylococcus aureus* central nervous system infections in children. Pediatr Infect Dis J, e-published ahead-of-print

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy M, Etienne J (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984

van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB (2012) Predictors of mortality in *Staphylococcus aureus* bacteremia. Clin Microbiol Rev 25(2):362–386

Vazquez JA, Maggiore CR, Cole P, Smith A, Jandourek A, Friedland HD (2015) Ceftaroline fosamil for the treatment of *Staphylococcus aureus* bacteremia secondary to acute bacterial skin and skin structure infections or community-acquired bacterial pneumonia. Infect Dis Clin Pract (Baltim Md) 23(1):39–43

Vourganti S, Agarwal PK, Bodner DR, Dogra VS (2006) Ultrasonographic evaluation of renal infections. Radiol Clin North Am 44(6):763–775

Vuong C, Yeh AJ, Cheung GY, Otto M (2016) Investigational drugs to treat methicillin-resistant *Staphylococcus aureus*. Expert Opin Investig Drugs 25(1):73–93

Wada K, Uehara S, Yamamoto M, Sadahira T, Mitsuhata R, Araki M, Kobayashi Y, Ishii A, Kariyama R, Watanabe T, Nasu Y, Kumon H (2016) Clinical analysis of bacterial strain profiles isolated from urinary tract infections: a 30-year study. J Infect Chemother 22(7):478–482

Wahby KA, Alangaden GJ (2012) Daptomycin failure in a neutropenic leukemia patient with *Staphylococcus aureus* meningitis. Leuk Lymphoma. 53(8):1610–1612

Waldron C, Solon JG, O'Gorman J, Humphreys H, Burke JP, McNamara DA (2015) Necrotizing fasciitis: the need for urgent surgical intervention and the impact of intravenous drug use. Surgeon 13(4):194–199

Whitby CR, Kaplan SL, Mason EO Jr, Carrillo-Marquez M, Lamberth LB, Hammerman WA, Hultén KG (2011) *Staphylococcus aureus* sinus infections in children. Int J Pediatr Otorhinolaryngol 75(1):118–121

White BP, Barber KE, Stover KR (2017) Ceftaroline for the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Am J Health Syst Pharm 74(4):201–208

Wilcox MH, Corey GR, Talbot GH, Thye D, Friedland D, Baculik T, CANVAS 2 investigators (2010) CANVAS 2: the second Phase III, randomized, double-blind study evaluating ceftaroline fosamil for the treatment of patients with complicated skin and skin structure infections. J Antimicrob Chemother 65(Suppl 4):iv53–iv65

Wong JK, Ranganathan SC, Hart E, Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) (2013) *Staphylococcus aureus* in early cystic fibrosis lung disease. Pediatr Pulmonol 48(12):1151–1159

Wood JB, Smith DB, Baker EH, Brecher SM, Gupta K (2012) Has the emergence of community-associated methicillin-resistant *Staphylococcus aureus* increased

trimethoprim-sulfamethoxazole use and resistance?: a 10-year time series analysis. Antimicrob Agents Chemother 56(11):5655–5660

Wood SC (2015) Clinical manifestations and therapeutic management of vulvar cellulitis and abscess: methicillin-resistant *Staphylococcus aureus*, necrotizing fasciitis, Bartholin abscess, Crohn disease of the vulva, hidradenitis suppurativa. Clin Obstet Gynecol 58(3):503–511

Ye R, Zhao L, Wang C, Wu X, Yan H (2014) Clinical characteristics of septic pulmonary embolism in adults: a systematic review. Respir Med 108(1):1–8

Yock LC, Boyce TG (2015) Fever and abdominal pain following incision and drainage of a cutaneous abscess. Clin Pediatr (Phila) 54(3):296–298

Yonezawa R, Kuwana T, Kawamura K, Inamo Y (2015) Invasive community-acquired methicillin-resistant *Staphylococcus aureus* in a Japanese girl with disseminating multiple organ infection: a case report and review of Japanese pediatric cases. Case Rep Pediatr 2015:291025

Young LM, Price CS (2008) Community-associated methicillin-resistant *Staphylococcus aureus* emerging as important cause of necrotizing fasciitis. Surg Infect 9:469–474

Yun HC, Ellis MW, Jorgensen JH (2007) Activity of ceftobiprole against community-associated methicillin-resistant *Staphylococcus aureus* isolates recently recovered from US military trainees. Diagn Microbiol Infect Dis 59(4):463–466

Zahedi Bialvaei A, Rahbar M, Yousefi M, Asgharzadeh M, Samadi Kafil H (2017) Linezolid: a promising option in the treatment of Gram-positives. J Antimicrob Chemother 72(2):354–364

Zhanel GG, Love R, Adam H, Golden A, Zelenitsky S, Schweizer F, Gorityala B, Lagacé-Wiens PR, Rubinstein E, Walkty A, Gin AS, Gilmour M, Hoban DJ, Lynch JP 3rd, Karlowsky JA (2015) Tedizolid: a novel oxazolidinone with potent activity against multidrug-resistant Gram-positive pathogens. Drugs 75(3):253–270

Zimbelman J, Palmer A, Todd J (1999) Improved outcome of clindamycin compared with beta-lactam antibiotic treatment for invasive *Streptococcus pyogenes* infection. Pediatr Infect Dis J 18(12):1096–1100

Zurenko G, Bien P, Bensaci M, Patel HN, Thorne G (2014) Use of linezolid susceptibility test results as a surrogate for the susceptibility of Gram-positive pathogens to tedizolid, a novel oxazolidinone. Ann Clin Microbiol Antimicrob 13:46

The Innate Immune Response Against *Staphylococcus aureus*

Isabelle Bekeredjian-Ding, Christoph Stein and Julia Uebele

Abstract The innate immune system harbors a multitude of different receptor systems and cells that are constantly prepared to sense and eliminate invading microbial pathogens. *Staphylococcus aureus* enters the body on its exposed epithelial surfaces, e.g., on skin and mucosa. The initial interaction with epithelial cells is governed by Toll-like receptor (TLR)-2-mediated local production of soluble mediators, including cytokines, chemokines, and antimicrobial peptides. The overall goal is to achieve a steady state of immune mediators and colonizing bacteria. Following cell and tissue invasion clearance of bacteria depends on intracellular microbial sensors and subsequent activation of the inflammasomes. Tissue-resident mast cells and macrophages recruit neutrophils, macrophages, and NK cells. This inflammatory response supports the generation of IL-17 producing NKT, γδ T cells, and T helper cells. Local dendritic cells migrate to the lymph nodes and fine-tune the adaptive immune response. The scope of this chapter is to provide an overview on the major cell types and receptors involved in innate immune defense against *S. aureus*. By segregating the different stages of infection from epithelial barrier to intracellular and systemic infection, this chapter highlights the different qualities of the innate immune response to *S. aureus* at different stages of invasiveness.

Contents

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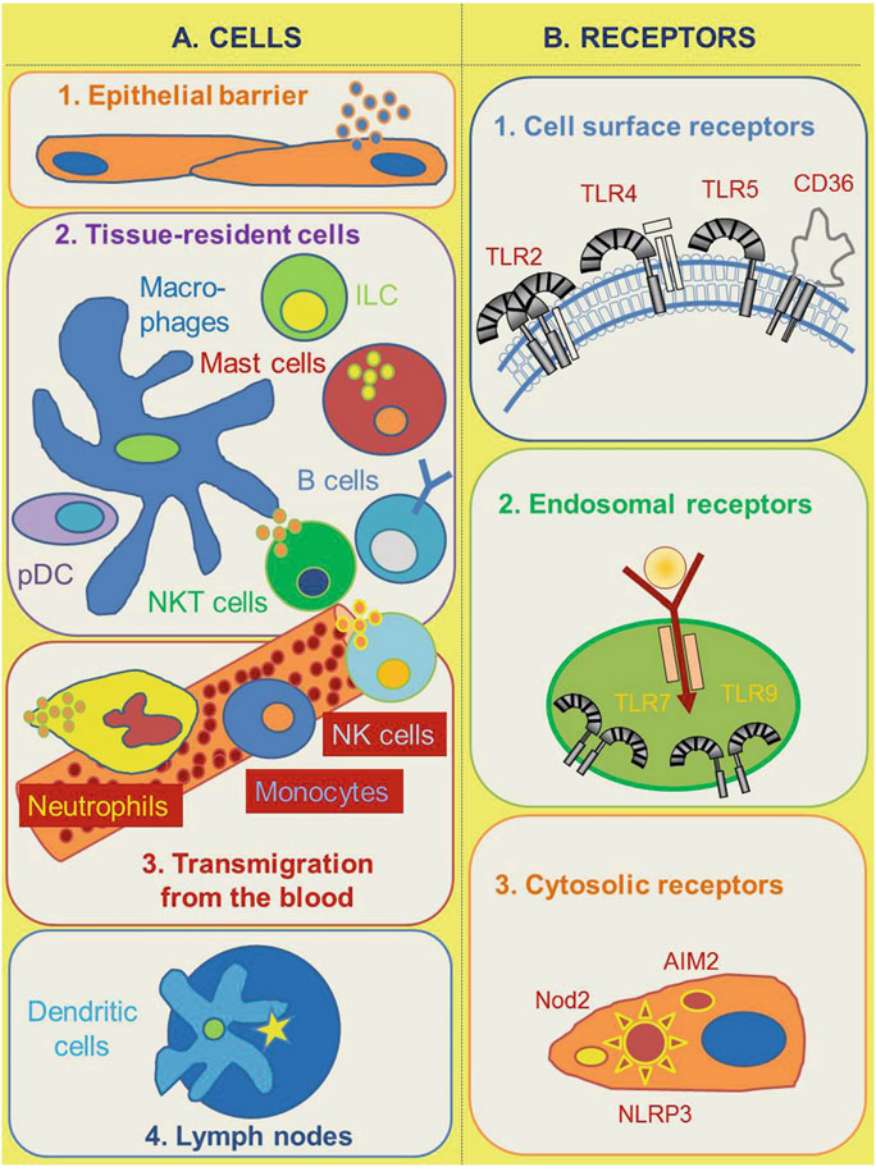
1 Introduction

In the last two decades, our understanding of the cellular and molecular mechanisms regulating innate immune defense has been refined. The researchers working in this field have not only cloned and described a broad variety of receptors responsible for the sensing of microbial pathogens but we have also learned how the different immune cell subsets contribute to immune defense and shape the effector function of the adaptive immunity. Despite all of these efforts, we have only started to understand how all of these cells and receptors integrate to mount a pathogen-specific protective immune response and which factors determine the differences in the outcome and quality of the immune response observed among individuals.

Numerous studies have dealt with the innate immune response against *S. aureus*. They have defined the receptors and cells required for innate immune protection against infection with this pathogen. It has even been postulated that clearance of *S. aureus* infection can solely rely on the innate immune system (Schmaler et al. 2011; Josefsson and Tarkowski 1999). By contrast, activation of adaptive immune cells in *S. aureus* infection has rather been associated with exacerbated inflammation and development of arthritis (Josefsson and Tarkowski 1999) and is most likely due to aberrant activation of B and T lymphocytes by *S. aureus* superantigens (reviewed in Broker et al. 2014). Nevertheless, a multitude of studies also highlighted that *S. aureus* also possesses a broad variety of virulence factors that promote its superb ability to adapt to a hostile environment and successfully evade the innate immune defense (reviewed elsewhere in this volume).

This chapter provides an overview on the innate immune mechanisms involved in physiological recognition and immune defense against *S. aureus* in carriers and local and systemic infections. Unfortunately, to date, there are no studies available that systemically compare the immune response to colonizing, invasive, and


A. CELLS
B. RECEPTORS
1. Epithelial barrier
2. Tissue-resident cells
Macro-
phages
ILC
Mast cells
B cells
pDC
NKT cells
NK cells
Neutrophils
Monocytes
3. Transmigration
from the blood
Dendritic
cells
4. Lymph nodes
1. Cell surface receptors
TLR4
TLR5
CD36
TLR2
2. Endosomal receptors
TLR7
TLR9
3. Cytosolic receptors
AIM2
Nod2
NLRP3

◀ **Fig. 1** The different layers of innate immune defense. **A**: **Antibacterial defense on a cellular level**: **1**. **Epithelial barrier**: keratinocytes and mucosal epithelial cells are the first to encounter bacteria. Bacteria are sensed by surface receptors and intracellular pattern recognition receptors (*PRRs*). These cells produce antimicrobial peptides (*blue*) and cytokines in response to stimulation of TLR2 or Nod-like receptors in response to *S. aureus* lipoproteins and/or peptidoglycan. **2**. **Tissue-resident cells**: mast cells, tissue-resident macrophages, innate lymphoid cells (*ILCs*), plasmacytoid dendritic cells (*pDCs*), NKT, and innate B cell subsets present in the tissues are prepared to fight invading pathogens. They express a broad variety of scavenger receptors and PRR that enable bacterial recognition, phagocytosis, and release of chemokines and proinflammatory cytokines, which attract leukocytes from blood. **3**. **Transmigration of cells from blood**: neutrophils, monocytes, and NK cells immigrate from blood and participate in phagocytosis and clearance of bacteria and abscess formation. **4**. **Lymph nodes**: Cell migration from the infected site initiates the antigen-specific adaptive immune response. Dendritic cells migrate to lymph nodes and present antigen to T cells. Neutrophils and macrophages interact with B cells. **B**: **Cellular levels of innate immune receptors**. **1**. **Surface receptors** on epithelial cells and leukocytes mediate the recognition of bacterial cell wall components. TLR2 recognizes staphylococcal lipoproteins and plays a central role in phagocyte activation. Scavenger receptors such as CD36 and SR-A recognize *S. aureus* and promote phagocytosis and bacterial clearance. **2**. **Endosomal TLRs** recognize microbial nucleic acids. They are activated upon degradation of the bacterium and acidification of the phagosome. **3**. **Cytosolic receptors** sense liberated bacterial degradation products such as peptidoglycan and microbial DNA. In infections with *S. aureus,* they activate the NLRP3, NLRC5, and AIM2 inflammasomes and promote caspase-1-dependent activation of IL-1β and IL-18, thus driving the formation of IL-17-producing T cells

intracellularly persisting *S. aureus* strains. The authors have, therefore, defined different layers of antibacterial immune defense on a cellular (Fig. 1A) and a receptor level (Fig. 1B) and assigned these to the different stages and types of infection.

2 The Encounter at the Epithelial Barrier

When encountering the body surfaces, *S. aureus* cells interact with and adhere to epithelial surfaces in skin and mucosa. The presence of adherent bacterial cells is sensed by epithelial surface receptors that recognize microbial components derived from the bacterial cell wall. These include wall teichoic acid (WTA), lipoteichoic acid (LTA), peptidoglycan (PG), and lipoproteins (Lpp). Additionally, secreted bacterial toxins such as alpha toxin, beta hemolysin, and Panton-Valentine leukocidin (PVL) are neutralized by preformed IgA present in the mucosa (Verkaik et al. 2009). Notably, to date, there is nearly no information available that allows a comparison of the epithelial and leukocyte responses upon initial encounter of the pathogen to those maintained in chronic colonization with *S. aureus*.

2.1 The Sentinel Function of Toll-like Receptor-2: Permitting Colonization and Preventing Invasion

The major surface receptor implicated in epithelial recognition of Gram-positive commensals such as *S. aureus* is Toll-like receptor (TLR)-2, a pattern recognition receptor (PRR) expressed on cells of epithelial, endothelial, and leukocyte origin. It senses di- and triacylated bacterial Lpp by forming heterodimers together with TLR6 and TLR1, respectively (Jin et al. 2007; Kang et al. 2009). Its engagement by *S. aureus*-derived TLR2 ligands contributes to the integrity of the epithelial barrier by supporting tight junctions and skin wound repair (Kuo et al. 2013). In vivo studies demonstrated that mice lacking TLR2 (or the central TLR adaptor molecules MyD88 and IRAK4) are highly susceptible to *S. aureus* infection (Takeuchi et al. 2000; Kielian et al. 2005; Yimin Kohanawa et al. 2013; Suzuki et al. 2002). In infection with this pathogen, these mice exhibit reduced cytokine responses and a higher bacterial burden in the affected organs. Well in line with these findings, patients with genetic mutations in the *irak4* and *myd88* loci display increased frequencies with pyogenic infections, among them *S. aureus* (reviewed in von Bernuth et al. 2012). However, the requirement for TLR2 in the human is less clear.

Staphylococcal Lpp account for approximately 2 % of the proteome and function as regulators of the iron transport through the cytoplasmic membrane (Schmaler et al. 2010; Babu et al. 2006; Maresso and Schneewind 2006). *S. aureus* expresses more than 50 lipoproteins, among them SitC or the tandem lipoproteins encoded in the pathogenicity island vSaα (Stoll et al. 2005; Hashimoto et al. 2006; Kurokawa et al. 2011; Nguyen et al. 2015). Maturation of Lpp involves several steps of posttranslational modification, the most important one being the transfer of a diacylglycerol group by the phosphatidyl glycerol diacylglyceryl transferase (*lgt*) (Sankaran et al. 1995; Sankaran and Wu 1995). This modification is essential for recognition via TLR2 (Stoll et al. 2005; Hashimoto et al. 2006; Kurokawa et al. 2011; Schmaler et al. 2009).

Activation of TLR2 on keratinocytes leads to the release of antimicrobial peptides (AMP) and proinflammatory cytokines and neutrophil-attracting chemokines (Niebuhr et al. 2010a; Wanke et al. 2011; Olaru and Jensen 2010). Well in line with a central role of TLR2 in the recognition of *S. aureus* in the skin, unresponsiveness of keratinocytes from patients with atopic dermatitis to TLR2 stimulation might contribute to colonization of affected skin with *S. aureus* (Niebuhr et al. 2011).

Similarly, expression of TLR2 protected against nasal colonization with *S. aureus* (Gonzalez-Zorn et al. 2005). Moreover, deficient secretion of nasal AMP and *S. aureus*-triggered downregulation of TLR2 have been proposed to support *S. aureus* nasal carriage (Gonzalez-Zorn et al. 2005; Nurjadi et al. 2013; Zanger et al. 2011; Quinn and Cole 2007) and adherence of *S. aureus* on bronchial epithelial cells that express low to absent levels of TLR2 (Mayer et al. 2007). Furthermore, recent reports proposed that *S. aureus* induces expression of TLR2 on salivary epithelial cells (Negrini et al. 2014) and triggers the release of the chemoattractant cytokine IL-8 from colonic epithelial cells in a TLR2-dependent manner (Kang

et al. 2015), a finding important in light of the gastrointestinal tract serving as an important reservoir for colonizing *S. aureus* strains (Nowrouzian et al. 2011; Gries et al. 2005; Kato-Matsunaga and Okonogi 1996; Bhalla et al. 2007; Klotz et al. 2005).

Notably, sensing via TLR2 cannot distinguish between viable and dead bacteria. Furthermore, TLR2 activity is subject to strain variation, e.g., it varies depending on the proliferative and metabolic state (Hilmi et al. 2014). TLR2 recognition, therefore, exerts an immune stimulatory effect on epithelial cells and a broad range of immune cells, but it is not solely responsible for bacterial clearance or containment of adherent *S. aureus* on the epithelial surfaces. Not surprisingly, it was, thus, not possible to prove an association of single nucleotide polymorphisms in the *tlr2* gene with either *S. aureus* carriage and/or associated rhinopolyposis (Sachse et al. 2010; Tewfik et al. 2008) or infection with *S. aureus* (El-Helou et al. 2011; Moore et al. 2004).

Keeping in mind that sensing of TLR2-active lipoproteins is coupled to bacterial proliferation and loss of cell wall integrity that uncover the cytoplasmic membrane (Hilmi et al. 2014; Wolf et al. 2011), the central role of TLR2 sensing might be attributed to two key determinants: Firstly, TLR2-dependent induction of AMP that degrade bacterial cells achieves a steady-state condition in the quantity of colonizing bacterial cells, thus making further recruitment and activation of phagocytes unnecessary: This principle has been described in drosophila but very probably is also valid for mammalian immune defense (Zaidman-Remy et al. 2006). Secondly, TLR2 exerts an important priming effect on epithelial cells and immune cells, thus preparing these cells to recognize invading *S. aureus* via intracellular sensors that subsequently induce its elimination by intracellular bacterial lysis in phagocytes or death of infected epithelial cells.

These events prepare the grounds for the polarization of T cell responses, most importantly the generation of Th17 cells in *S. aureus* skin infections, which are reviewed in Miller and Cho (2011). Nonetheless, *S. aureus* and diacylated TLR2 Lpp derived thereof also induce thymic stromal lymphopoietin (TSLP) (Takai et al. 2014; Vu et al. 2010), a cytokine expressed by human epidermal keratinocytes and mucosal epithelial cells that favors Th2 cell responses and blocks Th1/Th17 differentiation (Ziegler et al. 2013). In addition, *S. aureus* and TLR2 ligands have been implicated in the promotion of tolerance established via TLR2-dependent IL-10-mediated suppression of T cell responses induced by *S. aureus* or TLR2-dependent infiltration of colonized skin with myeloid suppressor cells (Skabytska et al. 2014; Chau et al. 2009).

Altogether, these findings highlight the central role of TLR2 in the recognition of *S. aureus* on epithelial cells from different body origins. Notably, the stimulatory function of TLR2 on epithelial cells is supplemented by its effects on professional phagocytes, which are discussed later in this chapter. The current findings further illustrate that TLR2 balances pro- and anti-inflammatory immune responses, thus permitting colonization but preventing infection.

2.2 Bacterial Invasion: Immune Defense Relies on Intracellular Sensors and Inflammasome Activation

Upon invasion of epithelial cells (or endosomal escape in professional phagocytes), cytosolic pattern recognition receptors (PRRs) secure immune detection of invading pathogens. In this context, recognition of *S. aureus* occurs via peptidoglycan binding by Nod receptors. The Nod1 receptor senses meso-diaminopimelic acid (*meso*DAP) in PG, which is present in peptidoglycan of Gram-negative species; the Nod2 receptor recognizes the muramyl dipeptide present in peptidoglycan of Gram-positive and Gram-negative organisms (Girardin et al. 2003). Nod2$^{-/-}$ cells were irresponsive to *S. aureus* PG (Volz et al. 2010). Nevertheless, one report claimed that both Nod receptors contribute to the recognition of *S. aureus* (Kapetanovic et al. 2007). A central role of Nod2 in vivo was demonstrated in a skin infection model using in *nod2*-deficient mice (Hruz et al. 2009). In this study, production of α-toxin facilitated Nod2 recognition by promoting access to the cytosol through pore formation in the plasma membrane.

TLR2 stimulation represents an important costimulus for Nod2-dependent recognition of peptidoglycan and vice versa (Volz et al. 2010; Schaffler et al. 2014). Furthermore, staphylococcal lipoteichoic acid (LTA) enhances recognition of TLR2 ligands and PG by a, so far, ill-defined mechanism. Additionally, Nod2 ligand MDP enhances LTA-triggered induction of cyclooxygenase-2 in macrophages (Ahn et al. 2014), which increases bactericidal activity against *S. aureus* (Bernard and Gallo 2010).

Notably, recognition of PG is greatly facilitated by uncovering the minimal PG recognition motifs: PG digestion by PG recognition proteins (PGRP) facilitates Toll signaling in drosophila and phagocytosis of *S. aureus* in human cells (De Marzi et al. 2015; Garver et al. 2006). Moreover, PG digestion by lysozyme is an important prerequisite for efficient activation of the NLRP3 inflammasome (Shimada et al. 2010). However, the PG-hydrolyzing activity of the major autolysin (*atl*) and D-alanylated wall teichoic acid interferes with recognition of *S. aureus* PG by drosophila PGRP (Kurokawa et al. 2011; Atilano et al. 2011, 2014) and O-acetylation of muramic acid (*oat*) typically found in *S. aureus* makes PG resistant to the hydrolytic activity of lysozyme (Shimada et al. 2010; Bera et al. 2007). This results in a pathogenicity factor-mediated suppression of proinflammatory cytokine production and subsequent inflammasome activation, facilitates colonization of skin and mucosa, and enables prolonged intracellular persistence. Relevance of these mechanisms was further provided by studies on *S. aureus* mutants with minimized PG synthesis, i.e., absence of nonessential peptidoglycan binding proteins, which rendered *S. aureus* less virulent while more susceptible to antibiotics and resulted in better bacterial clearance in infection (Reed et al. 2015).

Subsequent to engagement of cytosolic PRR, *S. aureus* induces activation of the inflammasomes. These cytosolic molecular complexes mediate the activation of caspase-1, which triggers the release of biologically active IL-1β and IL-18 and cell death. Notably, next to TLR activation, inflammasome activation depends on the

presence of ATP, which is required for the induction of an intracellular potassium flux (Franchi et al. 2007; Arlehamn et al. 2010; Petrilli et al. 2007). *S. aureus* establishes this intracellular potassium gradient via *agr*-dependent expression of pore-forming toxins, e.g., α-hemolysin, Panton-Valentine leucocidin (PVL), and phenol-soluble modulins; this results in NLRP3 (NACHT, leucine-rich repeat (LRR), and pyrin domain-containing protein 3)-dependent caspase-1 activation in a variety of cell types, ultimately leading to pyroptosis and secretion of IL-1β and IL-18 (Soong et al. 2015; Accarias et al. 2015; McGilligan et al. 2013; Holzinger et al. 2012; Perret et al. 2012; Craven et al. 2009; Munoz-Planillo et al. 2009; Chi et al. 2014; DuMont and Torres 2014; Queck et al. 2008; Bocker et al. 2001; Gurcel et al. 2006). By contrast, the absence of *agr* and/or pore-forming toxins promotes intracellular persistence and survival of *S. aureus* by inducing autophagy, a process counteracting NLRP3 and caspase-1 activation and associated pyroptosis and IL-1β production (Soong et al. 2015). Nevertheless, the role of autophagy and Nod/NLRP3 activation in immune recognition of intracellularly persisting metabolically inactive small colony variants has not been sufficiently addressed to date.

Moreover, an earlier study suggested that low MOIs of *S. aureus* promote IL-1β and IL-18 cleavage via activation of NLRC5 (Davis et al. 2011). In addition, a recent study suggested a specific role of the DNA-activated absent in melanoma-2 (AIM2) inflammasome in IL-1β-mediated bacterial immune defense in *S. aureus* CNS infection (Hanamsagar et al. 2014). To date, however, there is no evidence for involvement of the cytosolic RNA-sensing RIG-I-like receptors (RLR) in immune defense against *S. aureus*.

Notably, in murine *S. aureus* infection models, neutrophil recruitment, cutaneous abscess formation, and clearance of *S. aureus* in pneumonia were shown to be dependent on IL-1β levels (Labrousse et al. 2014; Cho et al. 2012; Miller et al. 2007). However, based on a murine model of α-toxin-dependent severe necrotizing pneumonia, pharmacological intervention with NLRP3 activation was also discussed to ameliorate the course of disease (Kebaier et al. 2012). Moreover, a study in patients with atopic dermatitis suggested that the Th2-derived cytokines IL-4, IL-5, and IL-13 counteract activation of *S. aureus* α-toxin-induced activation of NLRP3 and ASC (Niebuhr et al. 2014). This finding supported the hypothesis that susceptibility of Th2-prototypical DBA/2 mice to *S. aureus* infection could be attributed to the failure to activate the NLRP3 inflammasome, while *S. aureus*-resistant C57BL/6 mice with an inherent Th1-profile clear *S. aureus* by activating the inflammasome (Accarias et al. 2015).

2.3 *Linking Inflammasomes to Protective T Cell Responses: The Role of NLRP3 in Th17 Differentiation*

Interestingly, a recent study proposed that NLRP3/IL-1β-dependent recruitment of IL-17-producing γδ-T cells is required for recruitment of neutrophils to the infection site (Maher et al. 2013). Further in vitro studies showed that IL-1β and IL-23

support a T cell receptor and CD1d-dependent IL-17A response to heat-killed *S. aureus* in invariant NK T cells (Doisne et al. 2011). This T cell subset expresses a T cell receptor that recognizes lipid antigens in a CD1d-restricted manner and NK cell markers. It is typically found in barrier tissues where it assumes a protective role in colonization with *S. aureus* (Nieuwenhuis et al. 2009) and a murine *S. aureus* sepsis model (Kwiecinski et al. 2013). Alternatively, induction of Th17 cells was reported to be induced by dendritic cells; here, IL-1β production and IL-23 production were triggered by dual activation of surface TLRs (TLR2/4/5) and concomitant engagement of FcγRIIA by staphylococcal immune complexes with IgG (den Dunnen et al. 2012).

Taken together, these findings argue for the coexistence of redundant innate immune mechanisms for the induction of Th17-mediated immune defense. Future work is needed to understand whether certain mechanisms are more relevant in distinct disease entities.

3 Professional Phagocytes and Their Effector Functions

3.1 Phagocytosis: Linking Intracellular Lysis to Antigen Presentation

Engulfment, ingestion, and phagosomal degradation of microorganisms by processes such as acidification and enzyme digestion are essential for killing of the microbes and are required for activation of cells of the adaptive immune response via antigen presentation by MHCII molecules. This process is initiated by cellular recognition of *S. aureus* PAMPs and opsonins. One important opsonin is mannose-binding lectin (MBL), a collection that contributes to bacterial recognition and mediates activation of complement and innate immune cells. Its binding to *S. aureus* facilitates phagocytosis by enhancing opsonization by complement components and synergy with TLR2/6 ligands in the phagosome (Neth et al. 2000, 2002; Ip et al. 2008). Survival of mice lacking MBL is severely impaired after intraperitoneal injection of *S. aureus* (Shi et al. 2004). MBL has been shown to increase uptake of *S. aureus* via upregulation of the scavenger receptor SR-A (Ono et al. 2006). However, strain-specific differences in MBL binding have been attributed to differences in the surface carbohydrate structures (Shang et al. 2005).

It should, however, not be disregarded that the processes of internalization and phagosome maturation are important prerequisites for MyD88/TLR-dependent recognition of *S. aureus* (Wolf et al. 2011; Ip et al. 2010). Albeit TLR2 itself does not mediate phagocytosis, sensing of *S. aureus* and induction of the proinflammatory cytokine response only occur upon recruitment of TLR2 to the phagosome (Underhill et al. 1999). Degradation of bacteria in the phagosome facilitates recognition of PG via Nod receptors and, thus, activation of the inflammasome (Wolf et al. 2011; Shimada et al. 2010; Ip et al. 2010; Sokolovska et al. 2013;

Kaplan et al. 2012). Interestingly, inflammasome-mediated activation of caspase-1 not only activates IL-1 and IL-18 precursors but also participates in the acidification of the phagosome (Sokolovska et al. 2013), which enables recognition of microbial nucleic acids by TLRs (Parcina et al. 2008, 2013).

Staphylococcal ribosomal (23S) RNA is recognized in a sequence-specific manner by TLR13 expressed in murine conventional $CD8_{HIGH}$ DC but not plasmacytoid dendritic cells (pDCs) (Oldenburg et al. 2012). This receptor is not expressed in humans, but staphylococcal RNA was recently shown to mediate human monocyte activation by TLR8 (Bergstrom et al. 2015). By contrast, in human B cells, pDC activation by *S. aureus* is blocked by inhibitory oligonucleotides blocking TLR7 and TLR9, the only TLRs expressed in these cell types (Parcina et al. 2008, 2013; Hornung et al. 2002).

Further support for a role of phagocytosis in cellular defense against *S. aureus* is provided by the importance of scavenger receptors in infection. These receptors mediate recognition and clearance of *S. aureus* and are typically expressed on phagocytes:

Most prominently, mice lacking CD36, a receptor expressed in macrophages, monocytes, endothelial, and epithelial cells, are highly susceptible to *S. aureus* infections (Hoebe et al. 2005; Stuart et al. 2005; Blanchet et al. 2014). Of note, mice with a double deficiency in CD36 and SR (scavenger receptor)-A, another scavenger receptor required for phagocytosis of *S. aureus* (Amiel et al. 2009), died of pneumonia but were protected from *S. aureus* peritonitis (Blanchet et al. 2014).

Interestingly, CD36 has been implicated in the recognition of phosphatidylserine on apoptotic cells (Fadok et al. 1998). The same receptor specifically recognizes *S. aureus* via the diacylglycerol moiety in LTA and acts as a coreceptor for TLR2 (Hoebe et al. 2005; Nilsen et al. 2008; Baranova et al. 2008). Supporting its association with TLR2, expression of CD36 was higher in patients with atopic dermatitis carrying a TLR2 polymorphism (R753Q) that leads to reduced TLR2 reactivity (Niebuhr et al. 2010b; Mrabet-Dahbi et al. 2008).

Moreover, PIR-B (paired immunoglobulin-like receptor) was also found to bind *S. aureus* LTA (Nakayama et al. 2012). A protective function in *S. aureus* pneumonia was found to be due to suppression of the inflammatory response (Banerjee et al. 2010).

Notably, CD36 and $\alpha_5\beta_1$ integrin, the receptor for vitronectin, a plasma protein that binds to apoptotic cells, collaborate in apoptotic cell recognition (Fadok et al. 1998). *S. aureus* binds vitronectin via extracellular adherence protein (Eap) (Hussain et al. 2008). It is, therefore, likely that cells involved in apoptotic cell clearance also participate in the recognition of *S. aureus.* Well in line with the tolerogenic response induced by apoptotic cells, in murine peritonitis the presence of Eap on *S. aureus* had an anti-inflammatory effect that led to a reduction in infiltrating neutrophils (Chavakis et al. 2002).

3.2 Tissue-resident Phagocytes

3.2.1 Mast Cells: Well-prepared Guardians of Skin and Mucosa

Mast cells are important sentinels in the skin and mucosa. These phagocytic cells are prepared to kill bacteria with intracellular ROS or release extracellular traps and AMP. They further have the unique ability to rapidly release cytoplasmic granules storing preformed vasoactive and immune stimulatory mediators, e.g., histamine, TNF, and the proteases tryptase and chymase into the extracellular environment. In bacterial infections, they have mainly been implicated in defense against respiratory pathogens in pneumonia (reviewed in Urb and Sheppard 2012). Since they predominantly reside at sites where encounters with invading pathogens are to be expected due to constant contact with the exterior environment, it should not be over interpreted that mast cells were not required for bacterial clearance or host survival in an in vivo *S. aureus* peritonitis model (Ronnberg et al. 2014).

Mast cells contribute to the elimination of *S. aureus* by release of extracellular traps, proinflammatory cytokines, and AMP (Abel et al. 2011; Rocha-de-Souza et al. 2008; von Kockritz-Blickwede et al. 2008a). *S. aureus*-derived PG, LTA, protein A, and TLR2 ligands have been implicated in mast cell activation (Jawdat et al. 2006; Matsui and Nishikawa 2002, 2005; Terada et al. 2006; McCurdy et al. 2003). Degranulation was recently postulated to be specifically induced by *S. aureus* δ-toxin, which links colonization to allergic reactions in atopic dermatitis (Nakamura et al. 2013). However, recent studies highlight that *S. aureus* evades these bactericidal effects by invading mast cells via $\alpha_5\beta_1$ integrin, a reaction demonstrated in mucosal tissue from nasal polyposis (Abel et al. 2011; Hayes et al. 2015).

3.2.2 Macrophages: Tissue-Specific Vigilants Balancing the Local Immune Response

Macrophages are professional phagocytes that are either resident in the tissue or develop from monocytes that enter the tissue from the blood vessels. Their major function is the clearance of invading pathogens from the site of infection and antigen presentation to T cells in the periphery and in the lymphatic organs. Tissue-resident macrophages are often highly specialized on their specific environment, e.g., marginal zone macrophages in the spleen, microglia in the brain, alveolar macrophages, and macrophages in the thymus are each equipped to fulfill their specific tasks.

Again, TLR2 plays a central role in the release of soluble mediators mediating bacterial defense and in bacterial killing, e.g., in macrophages, staphylococcal lipoproteins induce proinflammatory cytokines, nitric oxide (NO), and reactive oxygen species (ROS) (Nguyen et al. 2015; Kim et al. 2015; Nandi et al. 2015; Bishayi et al. 2014). Moreover, in brain abscesses of TLR2-deficient mice,

secretion of proinflammatory cytokines and NO was abrogated, but IL-17 production was increased (Kielian et al. 2005). Furthermore, expression of *S. aureus*-binding scavenger receptor, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) (Shimaoka et al. 2001), and murine macrophage scavenger receptor (MSR)-AI/II was increased in response to *S. aureus* and TLR2 ligands in microglia arguing for a specific role of TLR2 and these scavenger receptors in CNS infection (Kielian et al. 2005).

By contrast, a recent study claimed that neither TLR2 expression on macrophages nor proinflammatory cytokine production by macrophages is essential for immune defense against *S. aureus* (Yimin Kohanawa et al. 2013). Nevertheless, the importance of macrophages in antistaphylococcal immune defense was recently demonstrated in a murine post-influenza pneumonia model where resistance to *S. aureus* infection was achieved by GM-CSF-mediated influx of neutrophils and alveolar macrophages and mainly established by the production of reactive oxygen species (ROS) in macrophages (Subramaniam et al. 2014). In an additional study, MyD88-dependent activation of dermal macrophages and their interaction with neutrophils was shown to be required for control of neutrophil-mediated inflammation in cutaneous *S. aureus* infection (Feuerstein et al. 2015).

In addition, several studies reported binding and uptake of *S. aureus* cells in marginal zone (MZ) macrophages (Palecanda et al. 1999; van der Laan et al. 1999; Westerberg et al. 2008; Birjandi et al. 2011). It was further suggested that in vivo uptake was mediated by macrophage receptor with collagenous structure (MARCO) (Palecanda et al. 1999; van der Laan et al. 1999). This receptor might, therefore, play a central role in MZ macrophage-mediated removal of *S. aureus* from the bloodstream.

Notably, the currently available studies do not differentiate between pro- and anti-inflammatory monocyte and macrophage subsets. However, macrophage polarization is highly dependent on the microenvironment, e.g., GM-CSF, TLR2 ligands, and Fcγ receptors differentially affect macrophage function (reviewed in Martinez and Gordon 2014). Contradictory findings, might therefore, arise from the tissue- and/or mouse strain-specific milieu and the predominance of functionally distinct subpopulations.

3.3 Blood-Derived Phagocytes

Both epithelial cells and tissue-resident innate immune cells release chemokines and cytokines upon bacterial encounter. These soluble mediators provoke transmigration of cells from the blood circulation. Activation induces differentiation peripheral blood monocytes to inflammatory monocytes, macrophages, and dendritic cells that transmigrate into the tissue and participate in bacterial clearance.

3.3.1 Neutrophils: Recruited to Resolve Uncontrolled Spread of Infection

Neutrophils are recruited to the site of infection and represent the hallmark of early antistaphylococcal defense in the invaded tissue (reviewed in Rigby and DeLeo 2012). They further participate in abscess formation, an event that is supported by *S. aureus*-derived toxins and their ability to induce a specific form of programmed cell necrosis (necroptosis) (reviewed in Kobayashi et al. 2015; Greenlee-Wacker et al. 2015). Indeed, phagocytosis and subsequent intracellular lysis of *S. aureus* in neutrophils are often prevented by staphylococcal virulence factors, and infected neutrophils containing viable bacteria evade efferocytosis by macrophages via an upregulation of CD47 expression, the phosphatidylserine "don't eat me" signal (Greenlee-Wacker et al. 2014).

Nevertheless, neutrophils are essential for antistaphylococcal innate immune defense, a fact that was demonstrated in mice depleted of neutrophils (Kohler et al. 2011; Robertson et al. 2008; Verdrengh and Tarkowski 1997) but can also be derived from increased susceptibility to *S. aureus* seen in neutropenic patients and in patients with genetic defects in neutrophil function, i.e., chronic granulomatosis disease (CGD) (Hartl et al. 2008; McNeil 2014) and mouse models thereof (Kohler et al. 2011; Pizzolla et al. 2012). Furthermore, increased mortality in *S. aureus* septicemia after depletion of complement was mainly attributed to insufficient recruitment of neutrophils (Sakiniene et al. 1999). In addition, in mice, MyD88-dependent IL-1 and IL-18 signalings are required for mobilization of neutrophils and resolution of staphylococcal skin abscesses (Miller et al. 2006) and post-burn infection (Kinoshita et al. 2011). Therefore, neutrophils remain central cells in immune defense against *S. aureus* and other compensatory mechanisms such as the release of bactericidal antimicrobial peptides most likely govern neutrophil-dependent first-line immune defense against *S. aureus*.

Despite their synthesis in many different types of phagocytes, e.g., monocytes, macrophages, and DC, the highest levels of defensins as HNP1-3 are expressed by neutrophils (Ryu et al. 2014). AMP, such as cathelicidin (LL-37) and α-defensin, contribute to bacterial killing and degradation in the phagosome (Jann et al. 2009), neutralize toxins (Cardot-Martin et al. 2015), induce the production of cytokines (Chaly et al. 2000), exert chemotactic activity (Yang et al. 2001), and facilitate the formation of neutrophil extracellular traps (NETs) (Neumann et al. 2014). However, again, *S. aureus* virulence factors shield the pathogen from AMP. This occurs through AMP-inactivating enzymes (Braff et al. 2007; Jin et al. 2004; Sieprawska-Lupa et al. 2004) and modification of charge of surface structures, e.g., via D-alanylation of WTA and LTA (Collins et al. 2002; Peschel et al. 1999; Simanski et al. 2013).

A recent study further highlights that in vivo recruitment of neutrophils to the site of infection occurs in two waves, e.g., initially from the blood, and secondly, they are mobilized from the bone marrow (Kamenyeva et al. 2015). The authors propose that in *S. aureus* infection, neutrophils enter the lymph node medulla and interfollicular areas where they interact with B cells to interfere with antibody

production (Kamenyeva et al. 2015). The significance of these findings remains unclear but is well compatible with a previously described anti-inflammatory role of neutrophils in staphylococcal arthritis (Verdrengh and Tarkowski 1997). Concomitant with the release of neutrophil myeloid-related proteins (MRP) -8 and -14 into the bronchoalveolar fluid exerts an additional protective effect in pneumonia by mediating transmigration of neutrophils, this phenomenon might be necessary for resolution of inflammation (Achouiti et al. 2015).

3.4 Dendritic Cells: Orchestrating the Adaptive Immune Response in Tissue and Lymph Nodes

3.4.1 Myeloid Dendritic Cells: Expert Control of T Cell Responses

Dendritic cells regulate the T cell response in infection via presentation of antigen and concomitant expression of T cell polarizing costimulatory ligands and release of cytokines and other soluble mediators.

Myeloid dendritic cells (mDCs) are specialized cells whose major function is to present antigen to T cells after migration to the local lymph nodes. They play a key role in the clearance of *S. aureus,* and their depletion increases mortality in an in vivo model of bacteremia (Schindler et al. 2012). Furthermore, Jin et al. highlighted that mDCs expressing the C-type lectin BDCA1+ are highly responsive to *S. aureus* and support differentiation of T cells into IFNγ-producing CD4+ (Th1) and CD8+ (Tc1) cells (Jin et al. 2014). Interestingly, this property was attributed to high expression levels for TLR2 and the scavenger receptor SR-A whose expression levels were low on mDCs with a BDCA3+ CD16+ phenotype.

Another study differentiated the effects of *S. aureus* Lpp and PG on monocytes and on in vitro generated monocyte-derived dendritic cells: While PI3K-dependent production of anti-inflammatory IL-10 is induced on monocytes, production of Th1/Th17 polarizing cytokines IL-12 and IL-23 is triggered in monocyte-derived dendritic cells (Frodermann et al. 2011). Well in line with this report, it was demonstrated that IRAK4 reverts IL-10-dominated tolerogenic MyD88-dependent signaling in *S. aureus*- or TLR2/4-stimulated human monocytes from PKB/Akt/mTOR-dependent IL-10 to Th1-promoting IL-12 secretion (Over et al. 2013). However, release of phenol-soluble modulins by *S. aureus* results in the loss of T cell priming capacity and the induction of T regulatory cells (Schreiner et al. 2013).

In the skin, *S. aureus* is phagocytosed by Langerhans cells, a specialized DC subset found in dermis (Reis e Sousa et al. 1993). Furthermore, in a murine atopic dermatitis model, dual activation of DC via TLR2 ligands and IL-4 induced the progression of self-limited Th2-mediated dermatitis to chronic cutaneous inflammation (Kaesler et al. 2014).

Myeloid-derived suppressor cells, on the contrary, suppress T cell responses and thereby contribute to the persistence of *S. aureus* in chronic infections (Tebartz et al. 2015). They further halt monocyte and macrophage-mediated clearance of *S. aureus* biofilm (Heim et al. 2014), and once induced by TLR2/6 ligands are recruited to *S. aureus* colonized skin where they suppress T cell responses to the pathogen (Skabytska et al. 2014).

3.4.2 Plasmacytoid Dendritic Cells and Type I Interferons: Fine-Tuning of Innate and Adaptive Immune Responses

Plasmacytoid dendritic cells are considered to be DC of lymphoid origin. They represent the major producers of systemically active interferon-α in the human body and have been implicated in tolerance to oral antigens (Goubier et al. 2008). They are present in the blood, in the lymphatic organs, and probably all peripheral tissues. *S. aureus* induces pDC-derived secretion of IFN-α, TNF, and IL-6 (Parcina et al. 2008; Michea et al. 2013). However, depending on the tissue environment, soluble mediators such as prostaglandins and TGF-β alter pDC-derived cytokine secretion panels and their function (Michea et al. 2013; Bekeredjian-Ding et al. 2009; Contractor et al. 2007). Their role in infection, particularly *S. aureus* infection, was extensively reviewed in Bekeredjian-Ding et al. (2014).

One of their most important characteristics is the expression of Fcγ receptor IIA and Fcε receptor that exert positive and negative regulatory effects, respectively, on pDC activation and release of IFN-α. Therefore, pDC most likely orchestrate the secondary immune response to *S. aureus* (Parcina et al. 2008), and their presumptive role in first-line immune defense is limited to invasion by protein A-bearing *S. aureus* strains and pDC-mediated support of polyclonal B cell activation (Parcina et al. 2013).

Additionally, pDC might be involved regulation of the antistaphylococcal immune response by type I interferon. In vivo, induction of IFN-α production in pDC by TLR9 ligand CpG ODN was protective against *S. aureus* pneumonia in a hemorrhagic shock model (Roquilly et al. 2010) and IFN-α-mediated resistance of host cells against *S. aureus* alpha toxin (Lizak and Yarovinsky 2012). Similarly, IFN-β increased clearance of *S. aureus* from murine BMDC and human monocytes in vitro and in an cutaneous infection model in vivo (Kaplan et al. 2012), and deficiency in IFN-α/β receptor or TLR9 expression resulted in improved clearance of bacteria from mice with *S. aureus* pneumonia (Parker and Prince 2012). However, IFN-β production was associated with increased inflammation and cellular necrosis in murine skin infection (Kaplan et al. 2012).

Notably, several studies showed that IFN-α- and IFN-α-inducing TLR7/9 agonists suppressed the formation of Th17 cells under healthy conditions, in infection, and autoimmune disease (Cui et al. 2014; Hirohata et al. 2010; Liu et al. 2011; Meyers et al. 2006; Vultaggio et al. 2011). The inhibitory effect of IFN-α on Th17 responses can be attributed to the induction of the IL-17-suppressing cytokine IL-27 (reviewed in Goriely et al. 2009). In accordance with these findings, lack of IL-27

receptor expression increases Th17 cell numbers and decreases the bacterial burden in post-influenza *S. aureus* pneumonia (Robinson et al. 2015).

Moreover, a recent report showed that *S. aureus*-induced expression of osteopontin in DC was responsible for increased Th17 induction (Salvi et al. 2013). Upregulation of osteopontin occurred upon TLR2 activation and concomitant absence of IFN-β induction, which was observed with Gram-negative bacteria after engagement of LPS/TLR4 and subsequent recruitment of TRIF (Salvi et al. 2013). Notably, *S. aureus*-induced IFN-β release is further prevented by lysozyme resistance of *S. aureus* and its resistance to degradation in the phagosome (Kaplan et al. 2012). Nevertheless, protective effects of IFN-I-mediated suppression of Th17 responses on DC have also been described: In a murine EAE model, IFN-I suppressed the expression of an intracellular translational isoform of osteopontin (iOPN) in myeloid DC; this enabled IL-27 synthesis and, in turn, decreased Th17-mediated inflammation, which ultimately slowed down the progression of EAE (Shinohara et al. 2008; Guo et al. 2008).

Furthermore, generation of monocyte-derived dendritic cells in the presence of IL-27 resulted in increased suppression of intracellular growth of *S. aureus*, upregulation of MHC II, and increased IL-12 secretion that shifted the T cell response to a Th1 phenotype (Jung et al. 2015). IL-27 further induced the synthesis of IFN-α and IFNλ1 in PBMC and DC derived from healthy donors, thus promoting an autoregulatory negative feedback loop (Cao et al. 2014). Thus, intertwined regulation of IFN-α and IL-17 determines the polarization of CD4+ T cell responses, the efficacy of bacterial clearance, and the degree of inflammation in autoimmune disease and infection. A fine-tuned balancing of these two cytokines is, therefore, most likely very critical for the resolution of *S. aureus* infection and immunopathology.

4 The Last Frontiers Before Adaptive Immunity

4.1 Innate Immune B Cells: Rapid Supply of Antibacterial Antibodies

In murine peritonitis and sepsis, rapid accumulation of IgM-secreting plasmablasts is observed within 48 h after bacterial challenge (Martin et al. 2001). The cells responsible for this early IgM secretion are B cell subsets that express B cell receptors that recognize thymus-independent (TI) antigens, e.g., bacterial PAMPs such as capsular polysaccharides, LPS, and phosphocholine and phosphatidylserine, which are also present on apoptotic cells. Together with PRR, stimulation targeting of these B cell receptors elicits B cell proliferation and differentiation to plasmablast in a T cell-independent manner. Release of these antibacterial IgM molecules (also called natural IgM) enables bacterial opsonization and subsequent complement activation. It has further been postulated that cellular uptake of *S.*

aureus immune complexes with IgM is promoted by an Fcα/μ receptor (Shibuya and Honda 2006).

Among these, specialized B cell subsets are CD5-positive B1a and CD5-negative B1b cells that reside in the peritoneal and pleural cavities in mice. B1b cells are capable of phagocytosis of *S. aureus*, intracellular bacterial killing, and antigen presentation to T cells (Gao et al. 2012). Albeit this response is weaker than in macrophages, it underlines the phylogenetic relationship of innate immune B cells and macrophages.

Notably, *S. aureus* targets V_H3+ B cells via protein A, which results in long-term depletion of the B1a and MZ B cell pools (Goodyear and Silverman 2004; Viau et al. 2005). In the presence of pDC and costimulation via TLR2-active Lpp and endosomal TLRs, protein A-activated B cells are not directly harmed by programmed cell death but undergo differentiation to IL-10-secreting B regulatory cells and IgM-producing plasmablasts (Parcina et al. 2013; Bekeredjian-Ding et al. 2007). This process enables IL-10-mediated suppression of T cell responses before ultimately resulting in cell death due to the physiologically short life span of plasmablasts and, again, extinction of these B cell subsets.

4.2 *Natural Killer Cells: Neglected Sensors for Intracellular Persisting* S. aureus*?*

NK cells have mainly been studied in viral infection and malignancy. They are attracted to the infected tissue and enter by transmigration from the bloodstream. Their ability to recognize infected and damaged cells independent of MHC-restricted antigen presentation of antibodies makes them very flexible and invaluable cells for the rapid defense against invading pathogens. Upon encounter of suspicious cells, the release of IFNγ and granules containing cytotoxic proteases (granzymes) and perforine induces apoptosis of the encountered cell.

Although resistance Rag2-IL2R$\gamma^{-/-}$ C57BL/6 mice against *S. aureus* infection suggested that NK cells are not essential for innate immune defense against *S. aureus* (von Kockritz-Blickwede et al. 2008b), the interaction of natural killer cells with alveolar macrophages was shown to be beneficial in murine *S. aureus* pneumonia models (Zhao et al. 2014; Small et al. 2008). Similarly, upon exposure to *S. aureus* interaction with monocytes was required for activation of human NK cells in vitro (Haller et al. 2002). A protective role of NK cells was further described in the development of *S. aureus* arthritis (Nilsson et al. 1999). Detection of NK cells in human joint infections caused by chronically persistent *S. aureus,* indeed, argues for a potential role of NK cells at the site of infection (Wagner et al. 2006). Nevertheless, the obvious role of NK cells in the detection intracellularly persisting *S. aureus* and the elimination of infected cells have not yet been demonstrated.

4.3 *Innate Lymphoid Cells: Confinement of* S. aureus *to Its Niche?*

In tissues exposed to the environment and colonized by commensals, innate lymphoid cells (ILCs) prevent bacterial translocation beyond the epithelial barrier, trigger mucosal IgA production, and function as important regulators of immune homeostasis (reviewed in Philip and Artis 2013; Tait Wojno and Artis 2012; Diefenbach et al. 2014). Notably, commensals trigger the development of ILC, and ILCs phenotypically and functionally adapt to changes in the composition of the local microbiome (Tait Wojno and Artis 2012). NK cell receptors (NCR) such as NKp44/46 and expression of TLR2 and its coreceptors enable binding and sensing of the local microbiota (Philip and Artis 2013).

Three different classes of murine ILC have been defined by the expression of prototypical transcription factors and T helper cell-like cytokine secretion profiles, e.g., Tbet+ ILC (group 1) produce IFNγ, GATA3+ ILC (group 2) secrete IL-5, IL-9, and IL-13 and RORγt+ ILC (group 3) release IL-17A, IFNγ, and IL-22 (Diefenbach et al. 2014; Robinette et al. 2015). Notably, NK cells have recently been allocated to group 1 ILC (Philip and Artis 2013; Robinette et al. 2015; Monticelli et al. 2012). In the human, similar ILC subsets have been described, including different group 2-like ILCs in the respiratory tract and skin or different CD127+ group 3-like ILCs in tonsils, appendix, and Peyer's patches (Tait Wojno and Artis 2012; Monticelli et al. 2012; Mjosberg and Eidsmo 2014). Nevertheless, despite their presence at the main sites of *S. aureus* colonization, at present there is no available information on their role in preventing invasion. We can only speculate that in chronic carriers, ILC might confine *S. aureus* to its niche, thus preventing systemic immune responses.

5 Conclusion

A broad variety of receptor systems cooperates in sensing *S. aureus* and regulating the host immune response to this pathogen (see Table 1 for summary). TLR2-dependent recognition of *S. aureus* by epithelial cells limits the spread of colonizing *S. aureus* on the skin and mucosal surfaces. Upon cell and tissue invasion activation of resident innate immune cells by *S. aureus* fosters a proinflammatory environment that attracts neutrophils, NK cells, and monocytes from the blood. Notably, degradation of bacteria in the phagosomes is essential for bacterial recognition via TLR, activation of the inflammasomes, and subsequent bacterial clearance. However, evolution has selected *S. aureus* strains that are resistant to these processes.

At present, we know that innate immune cells and PRR also participate in the resolution of *S. aureus* infections. However, the exact mechanisms involved remain to be investigated and are potentially exploited by *S. aureus* to promote tolerance.

Table 1 Staphylococcal recognition motifs in innate immunity

Cellular level	Receptor system	PRR	Cell type	Staphylococcal recognition motif	Species	References
Soluble molecules	Collectins	Mannose-binding lectin (MBL)	S	Mannose-fucose spacing[a]	Mouse	Ip et al. (2008), Shi et al. (2004)
					Human	Neth et al. (2000, 2002), Shang et al. (2005)
	Antimicrobial peptides (AMP)	α-defensin, LL-37[b]	E, M	Surface structures such as lipids and wall teichoic acid	Human	Cardot-Martin et al. (2015), Yang et al. (2001), Neumann et al. (2014)
Surface receptors	Integrins	$\alpha_5\beta_1$ integrin	M, L, E	Surface proteins (MSCRAMMs, i.e., FnBP)	Mouse	Abel et al. (2011)
					Chronic rhinosinusitis patients	Hayes et al. (2015)
	Toll-like receptors (TLR)	TLR2	M, L, E	di- and triacylated lipoproteins	Mouse	Takeuchi et al. (2000), Kielian et al. (2005), Yimin Kohanawa et al. (2013), Stoll et al. (2005), Hashimoto et al. (2006), Nguyen et al. (2015), Schmaler et al. (2009), Underhill et al. (1999), Kim et al. (2015), Nandi et al. (2015), Bishayi et al. (2014)
					Human	Nguyen et al. (2015), Hilmi et al. (2014)
					patients with atopic dermatitis	Niebuhr et al. (2010), Mrabet-Dahbi et al. (2008)
	Scavenger receptors	CD36[a]	M, E	Lipoteichoic acid (LTA)	Mouse	Hoebe et al. (2005), Stuart et al. (2005), Blanchet et al. (2014)
		PIR-B	L, M	Lipoteichoic acid (LTA)	Mouse	Nakayama et al. (2012)
		SR-A	M, E	Broad but ill-defined specificity	Mouse	Ono et al. (2006), Stuart et al. (2005), Jin et al. (2014)
		MARCO	M, E		Mouse	Nakayama et al. (2012)

(continued)

Table 1 (continued)

Cellular level	Receptor system	PRR	Cell type	Staphylococcal recognition motif	Species	References
		(MSR)-AI/II[a]	M		Mouse	Kielian et al. (2005)
		LOX-1[a]	M, En		Hamster and Bovine cell lines	Shimaoka et al. (2001)
Endosomal receptors	Endosomal Toll-like receptors (TLR)	TLR8	M	Ribosomal RNA	Human	Bergstrom et al. (2015)
		TLR13	M	Ribosomal RNA	Mouse	Oldenburg et al. (2012)
		TLR9	M, L, E	DNA	Mouse	Roquilly et al. (2010), Lizak and Yarovinsky (2012), Parker and Prince (2012)
					Human	Parcina et al. (2008, 2013)
Cytosolic receptors	NLR	NOD1	M, L, E	Peptidoglycan (*meso*DAP)	Mouse	Kapetanovic et al. (2007)
		NOD2[a]	M, L, E	Peptidoglycan (muramyl dipeptide)	Mouse	Girardin et al. (2003), Volz et al. (2010), Kapetanovic et al. (2007), Hruz et al. (2009), Schaffler et al. (2014)
		NLRP3	M, L, E	α-hemolysin, Panton-Valentine-leukocidin	Mouse	Shimada et al. (2010), Soong et al. (2015), Accarias et al. (2015), McGilligan et al. (2013), Holzinger et al. (2012), Perret et al. (2012), Craven et al. (2009), Munoz-Planillo et al. (2009), Chi et al. (2014), DuMont and Torres (2014), Bocker et al. (2001), Gurcel et al. (2006), Kebaier et al. (2012), Niebuhr et al. (2014), Maher et al. (2013), Sokolovska et al. (2013)
		NLRC5	M, L	α-hemolysin	Human	Davis et al. (2011)
		AIM2	M, L	DNA	Mouse	Hanamsagar et al. (2014)

Cell types: *M* (myeloid), *L* (lymphoid), *E* (epithelial), *En* (endothelial); *S* (serum)
[a]Synergism with TLR2; [b]Synergism with TLR9

Finally, future work is needed to clarify the role of innate immunity and, in particular, NK cells in the recognition of intracellular persisting *S. aureus* and the role of ILC in immune homeostasis in chronic carriage.

References

Abel J, Goldmann O, Ziegler C, Holtje C, Smeltzer MS, Cheung AL, Bruhn D, Rohde M, Medina E (2011) Staphylococcus aureus evades the extracellular antimicrobial activity of mast cells by promoting its own uptake. J Innate Immun 3(5):495–507. doi:10.1159/000327714

Accarias S, Lugo-Villarino G, Foucras G, Neyrolles O, Boullier S, Tabouret G (2015) Pyroptosis of resident macrophages differentially orchestrates inflammatory responses to Staphylococcus aureus in resistant and susceptible mice. Eur J Immunol 45(3):794–806. doi:10.1002/eji.201445098

Achouiti A, Vogl T, Van der Meer AJ, Stroo I, Florquin S, de Boer OJ, Roth J, Zeerleder S, van't Veer C, de Vos AF, van der Poll T (2015) Myeloid-related protein-14 deficiency promotes inflammation in staphylococcal pneumonia. Eur Respir J. doi:10.1183/09031936.00183814

Ahn KB, Jeon JH, Baik JE, Park OJ, Kang SS, Yun CH, Park JH, Han SH (2014) Muramyl dipeptide potentiates staphylococcal lipoteichoic acid induction of cyclooxygenase-2 expression in macrophages. Microbes Infect 16(2):153–160. doi:10.1016/j.micinf.2013.10.018

Amiel E, Alonso A, Uematsu S, Akira S, Poynter ME, Berwin B (2009) Pivotal advance: Toll-like receptor regulation of scavenger receptor-A-mediated phagocytosis. J Leukoc Biol 85(4):595–605. doi:10.1189/jlb.1008631

Arlehamn CS, Petrilli V, Gross O, Tschopp J, Evans TJ (2010) The role of potassium in inflammasome activation by bacteria. J Biol Chem 285(14):10508–10518. doi:10.1074/jbc.M109.067298

Atilano ML, Pereira PM, Vaz F, Catalao MJ, Reed P, Grilo IR, Sobral RG, Ligoxygakis P, Pinho MG, Filipe SR (2014) Bacterial autolysins trim cell surface peptidoglycan to prevent detection by the Drosophila innate immune system. Elife 3:e02277. doi:10.7554/eLife.02277

Atilano ML, Yates J, Glittenberg M, Filipe SR, Ligoxygakis P (2011) Wall teichoic acids of Staphylococcus aureus limit recognition by the drosophila peptidoglycan recognition protein-SA to promote pathogenicity. PLoS Pathog 7(12):e1002421. doi:10.1371/journal.ppat.1002421

Babu MM, Priya ML, Selvan AT, Madera M, Gough J, Aravind L, Sankaran K (2006) A database of bacterial lipoproteins (DOLOP) with functional assignments to predicted lipoproteins. J Bacteriol 188(8):2761–2773. doi:188/8/2761 [pii] 10.1128/JB.188.8.2761-2773.2006

Banerjee A, Stevenaert F, Pande K, Haghjoo E, Antonenko S, Gorman DM, Sathe M, McClanahan TK, Pierce R, Turner SP, Bigler ME, Phillips JH, Heyworth PG (2010) Modulation of paired immunoglobulin-like type 2 receptor signaling alters the host response to Staphylococcus aureus-induced pneumonia. Infect Immun 78(3):1353–1363. doi:10.1128/iai.00969-09

Baranova IN, Kurlander R, Bocharov AV, Vishnyakova TG, Chen Z, Remaley AT, Csako G, Patterson AP, Eggerman TL (2008) Role of human CD36 in bacterial recognition, phagocytosis, and pathogen-induced JNK-mediated signaling. J Immunol 181(10):7147–7156

Bekeredjian-Ding I, Greil J, Ammann S, Parcina M (2014) Plasmacytoid dendritic cells: neglected regulators of the immune response to Staphylococcus aureus. Front Immunol 5:238. doi:10.3389/fimmu.2014.00238

Bekeredjian-Ding I, Inamura S, Giese T, Moll H, Endres S, Sing A, Zahringer U, Hartmann G (2007) Staphylococcus aureus protein A triggers T cell-independent B cell proliferation by sensitizing B cells for TLR2 ligands. J Immunol 178(5):2803–2812

Bekeredjian-Ding I, Schafer M, Hartmann E, Pries R, Parcina M, Schneider P, Giese T, Endres S, Wollenberg B, Hartmann G (2009) Tumour-derived prostaglandin E and transforming growth factor-beta synergize to inhibit plasmacytoid dendritic cell-derived interferon-alpha. Immunology 128(3):439–450. doi:10.1111/j.1365-2567.2009.03134.x

Bera A, Biswas R, Herbert S, Kulauzovic E, Weidenmaier C, Peschel A, Gotz F (2007) Influence of wall teichoic acid on lysozyme resistance in Staphylococcus aureus. J Bacteriol 189(1):280–283. doi:JB.01221-06 [pii] 10.1128/JB.01221-06

Bergstrom B, Aune MH, Awuh JA, Kojen JF, Blix KJ, Ryan L, Flo TH, Mollnes TE, Espevik T, Stenvik J (2015) TLR8 senses Staphylococcus aureus RNA in human primary monocytes and macrophages and induces IFN-beta production via a TAK1-IKKbeta-IRF5 signaling pathway. J Immunol. doi:10.4049/jimmunol.1403176

Bernard JJ, Gallo RL (2010) Cyclooxygenase-2 enhances antimicrobial peptide expression and killing of Staphylococcus aureus. J Immunol 185(11):6535–6544. doi:10.4049/jimmunol.1002009

Bhalla A, Aron DC, Donskey CJ (2007) Staphylococcus aureus intestinal colonization is associated with increased frequency of S. aureus on skin of hospitalized patients. BMC Infect Dis 7:105. doi:10.1186/1471-2334-7-105

Birjandi SZ, Ippolito JA, Ramadorai AK, Witte PL (2011) Alterations in marginal zone macrophages and marginal zone B cells in old mice. J Immunol 186(6):3441–3451. doi:10.4049/jimmunol.1001271 jimmunol.1001271 [pii]

Bishayi B, Bandyopadhyay D, Majhi A, Adhikary R (2014) Possible role of Toll-like receptor-2 in the intracellular survival of Staphylococcus aureus in murine peritoneal macrophages: involvement of cytokines and anti-oxidant enzymes. Scand J Immunol 80(2):127–143. doi:10.1111/sji.12195

Blanchet C, Jouvion G, Fitting C, Cavaillon JM, Adib-Conquy M (2014) Protective or deleterious role of scavenger receptors SR-A and CD36 on host resistance to Staphylococcus aureus depends on the site of infection. PLoS ONE 9(1):e87927. doi:10.1371/journal.pone.0087927

Bocker U, Manigold T, Watson JM, Singer MV, Rossol S (2001) Regulation of Staphylococcus aureus-mediated activation of interleukin-18 in peripheral blood mononuclear cells. Eur Cytokine Netw 12(4):631–638

Braff MH, Jones AL, Skerrett SJ, Rubens CE (2007) Staphylococcus aureus exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis. J Infect Dis 195(9):1365–1372. doi:10.1086/513277

Broker BM, Holtfreter S, Bekeredjian-Ding I (2014) Immune control of Staphylococcus aureus—regulation and counter-regulation of the adaptive immune response. Int J Med Microbiol 304(2):204–214. doi:10.1016/j.ijmm.2013.11.008

Cao Y, Zhang R, Zhang W, Zhu C, Yu Y, Song Y, Wang Q, Bai L, Liu Y, Wu K, Wu J (2014) IL-27, a cytokine, and IFN-lambda1, a type III IFN, are coordinated to regulate virus replication through type I IFN. J Immunol 192(2):691–703. doi:10.4049/jimmunol.1300252

Cardot-Martin E, Casalegno JS, Badiou C, Dauwalder O, Keller D, Prevost G, Rieg S, Kern WV, Cuerq C, Etienne J, Vandenesch F, Lina G, Dumitrescu O (2015) alpha-Defensins partially protect human neutrophils against Panton-Valentine leukocidin produced by Staphylococcus aureus. Lett Appl Microbiol. doi:10.1111/lam.12438

Chaly YV, Paleolog EM, Kolesnikova TS, Tikhonov II, Petratchenko EV, Voitenok NN (2000) Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells. Eur Cytokine Netw 11(2):257–266

Chau TA, McCully ML, Brintnell W, An G, Kasper KJ, Vines ED, Kubes P, Haeryfar SM, McCormick JK, Cairns E, Heinrichs DE, Madrenas J (2009) Toll-like receptor 2 ligands on the staphylococcal cell wall downregulate superantigen-induced T cell activation and prevent toxic shock syndrome. Nat Med 15(6):641–648

Chavakis T, Hussain M, Kanse SM, Peters G, Bretzel RG, Flock JI, Herrmann M, Preissner KT (2002) Staphylococcus aureus extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. Nat Med 8(7):687–693. doi:10.1038/nm728

Chi CY, Lin CC, Liao IC, Yao YC, Shen FC, Liu CC, Lin CF (2014) Panton-Valentine leukocidin facilitates the escape of Staphylococcus aureus from human keratinocyte endosomes and induces apoptosis. J Infect Dis 209(2):224–235. doi:10.1093/infdis/jit445

Cho JS, Guo Y, Ramos RI, Hebroni F, Plaisier SB, Xuan C, Granick JL, Matsushima H, Takashima A, Iwakura Y, Cheung AL, Cheng G, Lee DJ, Simon SI, Miller LS (2012) Neutrophil-derived IL-1beta is sufficient for abscess formation in immunity against Staphylococcus aureus in mice. PLoS Pathog 8(11):e1003047. doi:10.1371/journal.ppat.1003047

Collins LV, Kristian SA, Weidenmaier C, Faigle M, Van Kessel KP, Van Strijp JA, Gotz F, Neumeister B, Peschel A (2002) Staphylococcus aureus strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis 186(2):214–219. doi:10.1086/341454

Contractor N, Louten J, Kim L, Biron CA, Kelsall BL (2007) Cutting edge: Peyer's patch plasmacytoid dendritic cells (pDCs) produce low levels of type I interferons: possible role for IL-10, TGFbeta, and prostaglandin E2 in conditioning a unique mucosal pDC phenotype. J Immunol 179(5):2690–2694

Craven RR, Gao X, Allen IC, Gris D, Bubeck Wardenburg J, McElvania-Tekippe E, Ting JP, Duncan JA (2009) Staphylococcus aureus alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. PLoS ONE 4(10):e7446. doi:10.1371/journal.pone.0007446

Cui F, Meng J, Luo P, Chen P (2014) IFN- alpha blocks IL-17 production by peripheral blood mononuclear cells in patients with chronic active hepatitis B Infection. BMC Infect Dis 14:55. doi:10.1186/1471-2334-14-55

Davis BK, Roberts RA, Huang MT, Willingham SB, Conti BJ, Brickey WJ, Barker BR, Kwan M, Taxman DJ, Accavitti-Loper MA, Duncan JA, Ting JP (2011) Cutting edge: NLRC5-dependent activation of the inflammasome. J Immunol 186(3):1333–1337. doi:10.4049/jimmunol.1003111

De Marzi MC, Todone M, Ganem MB, Wang Q, Mariuzza RA, Fernandez MM, Malchiodi EL (2015) Peptidoglycan recognition protein-peptidoglycan complexes increase monocyte/macrophage activation and enhance the inflammatory response. Immunology. doi:10.1111/imm.12460

den Dunnen J, Vogelpoel LT, Wypych T, Muller FJ, de Boer L, Kuijpers TW, Zaat SA, Kapsenberg ML, de Jong EC (2012) IgG opsonization of bacteria promotes Th17 responses via synergy between TLRs and FcgammaRIIa in human dendritic cells. Blood 120(1):112–121. doi:10.1182/blood-2011-12-399931

Diefenbach A, Colonna M, Koyasu S (2014) Development, differentiation, and diversity of innate lymphoid cells. Immunity 41(3):354–365. doi:10.1016/j.immuni.2014.09.005

Doisne JM, Soulard V, Becourt C, Amniai L, Henrot P, Havenar-Daughton C, Blanchet C, Zitvogel L, Ryffel B, Cavaillon JM, Marie JC, Couillin I, Benlagha K (2011) Cutting edge: crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1-invariant NKT cells to bacteria. J Immunol 186(2):662–666. doi:10.4049/jimmunol.1002725

DuMont AL, Torres VJ (2014) Cell targeting by the Staphylococcus aureus pore-forming toxins: it's not just about lipids. Trends Microbiol 22(1):21–27. doi:10.1016/j.tim.2013.10.004 S0966-842X(13)00202-3 [pii]

El-Helou O, Berbari EF, Brown RA, Gralewski JH, Osmon DR, Razonable RR (2011) Functional assessment of Toll-like receptor 2 and its relevance in patients with Staphylococcus aureus infection of joint prosthesis. Hum Immunol 72(1):47–53. doi:10.1016/j.humimm.2010.10.001

Fadok VA, Warner ML, Bratton DL, Henson PM (1998) CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor (alpha v beta 3). J Immunol 161(11):6250–6257

Feuerstein R, Seidl M, Prinz M, Henneke P (2015) MyD88 in macrophages is critical for abscess resolution in staphylococcal skin infection. J Immunol 194(6):2735–2745. doi:10.4049/jimmunol.1402566 jimmunol.1402566 [pii]

Franchi L, Kanneganti TD, Dubyak GR, Nunez G (2007) Differential requirement of P2X7 receptor and intracellular K+ for caspase-1 activation induced by intracellular and extracellular bacteria. J Biol Chem 282(26):18810–18818. doi:10.1074/jbc.M610762200

Frodermann V, Chau TA, Sayedyahossein S, Toth JM, Heinrichs DE, Madrenas J (2011) A modulatory interleukin-10 response to staphylococcal peptidoglycan prevents Th1/Th17 adaptive immunity to Staphylococcus aureus. J Infect Dis 204(2):253–262. doi:10.1093/infdis/jir276

Gao J, Ma X, Gu W, Fu M, An J, Xing Y, Gao T, Li W, Liu Y (2012) Novel functions of murine B1 cells: active phagocytic and microbicidal abilities. Eur J Immunol 42(4):982–992. doi:10.1002/eji.201141519

Garver LS, Wu J, Wu LP (2006) The peptidoglycan recognition protein PGRP-SC1a is essential for Toll signaling and phagocytosis of Staphylococcus aureus in Drosophila. Proc Natl Acad Sci U S A 103(3):660–665. doi:10.1073/pnas.0506182103

Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278(11):8869–8872. doi:10.1074/jbc.C200651200

Gonzalez-Zorn B, Senna JP, Fiette L, Shorte S, Testard A, Chignard M, Courvalin P, Grillot-Courvalin C (2005) Bacterial and host factors implicated in nasal carriage of methicillin-resistant Staphylococcus aureus in mice. Infect Immun 73(3):1847–1851. doi:73/3/1847 [pii] 10.1128/IAI.73.3.1847-1851.2005

Goodyear CS, Silverman GJ (2004) Staphylococcal toxin induced preferential and prolonged in vivo deletion of innate-like B lymphocytes. Proc Natl Acad Sci U S A 101(31):11392–11397. doi:10.1073/pnas.0404382101 0404382101 [pii]

Goriely S, Cavoy R, Goldman M (2009) Interleukin-12 family members and type I interferons in Th17-mediated inflammatory disorders. Allergy 64(5):702–709. doi:10.1111/j.1398-9995.2009.02039.x

Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, Trinchieri G, Kaiserlian D (2008) Plasmacytoid dendritic cells mediate oral tolerance. Immunity 29(3):464–475. doi:S1074-7613(08)00372-5 [pii] 10.1016/j.immuni.2008.06.017

Greenlee-Wacker M, DeLeo FR, Nauseef WM (2015) How methicillin-resistant Staphylococcus aureus evade neutrophil killing. Curr Opin Hematol 22(1):30–35. doi:10.1097/moh.0000000000000096

Greenlee-Wacker MC, Rigby KM, Kobayashi SD, Porter AR, DeLeo FR, Nauseef WM (2014) Phagocytosis of Staphylococcus aureus by human neutrophils prevents macrophage efferocytosis and induces programmed necrosis. J Immunol 192(10):4709–4717. doi:10.4049/jimmunol.1302692

Gries DM, Pultz NJ, Donskey CJ (2005) Growth in cecal mucus facilitates colonization of the mouse intestinal tract by methicillin-resistant Staphylococcus aureus. J Infect Dis 192(9):1621–1627. doi:JID34054 [pii] 10.1086/491737

Guo B, Chang EY, Cheng G (2008) The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. J Clin Invest 118(5):1680–1690. doi:10.1172/jci33342

Gurcel L, Abrami L, Girardin S, Tschopp J, van der Goot FG (2006) Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. Cell 126(6):1135–1145. doi:10.1016/j.cell.2006.07.033

Haller D, Serrant P, Granato D, Schiffrin EJ, Blum S (2002) Activation of human NK cells by staphylococci and lactobacilli requires cell contact-dependent costimulation by autologous monocytes. Clin Diagn Lab Immunol 9(3):649–657

Hanamsagar R, Aldrich A, Kielian T (2014) Critical role for the AIM2 inflammasome during acute CNS bacterial infection. J Neurochem 129(4):704–711. doi:10.1111/jnc.12669

Hartl D, Lehmann N, Hoffmann F, Jansson A, Hector A, Notheis G, Roos D, Belohradsky BH, Wintergerst U (2008) Dysregulation of innate immune receptors on neutrophils in chronic granulomatous disease. J Allergy Clin Immunol 121(2):375–382 e379. doi:10.1016/j.jaci.2007.10.037

Hashimoto M, Tawaratsumida K, Kariya H, Kiyohara A, Suda Y, Krikae F, Kirikae T, Gotz F (2006) Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in Staphylococcus aureus. J Immunol 177(5):3162–3169

Hayes SM, Howlin R, Johnston DA, Webb JS, Clarke SC, Stoodley P, Harries PG, Wilson SJ, Pender SL, Faust SN, Hall-Stoodley L, Salib RJ (2015) Intracellular residency of Staphylococcus aureus within mast cells in nasal polyps: a novel observation. J Allergy Clin Immunol. doi:10.1016/j.jaci.2014.12.1929

Heim CE, Vidlak D, Scherr TD, Kozel JA, Holzapfel M, Muirhead DE, Kielian T (2014) Myeloid-derived suppressor cells contribute to Staphylococcus aureus orthopedic biofilm infection. J Immunol 192(8):3778–3792. doi:10.4049/jimmunol.1303408

Hilmi D, Parcina M, Stollewerk D, Ostrop J, Josten M, Meilaender A, Zaehringer U, Wichelhaus TA, Bierbaum G, Heeg K, Wolz C, Bekeredjian-Ding I (2014) Heterogeneity of host TLR2 stimulation by Staphylocoocus aureus isolates. PLoS One 9(5):e96416. doi:10.1371/journal.pone.0096416 PONE-D-13-55098 [pii]

Hirohata S, Shibuya H, Tejima S (2010) Suppressive influences of IFN-alpha on IL-17 expression in human CD4+ T cells. Clin Immunol 134(3):340–344. doi:10.1016/j.clim.2009.11.012

Hoebe K, Georgel P, Rutschmann S, Du X, Mudd S, Crozat K, Sovath S, Shamel L, Hartung T, Zahringer U, Beutler B (2005) CD36 is a sensor of diacylglycerides. Nature 433(7025):523–527. doi:nature03253 [pii] 10.1038/nature03253

Holzinger D, Gieldon L, Mysore V, Nippe N, Taxman DJ, Duncan JA, Broglie PM, Marketon K, Austermann J, Vogl T, Foell D, Niemann S, Peters G, Roth J, Loffler B (2012) Staphylococcus aureus Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. J Leukoc Biol 92(5):1069–1081. doi:10.1189/jlb.0112014

Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G (2002) Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168(9):4531–4537

Hruz P, Zinkernagel AS, Jenikova G, Botwin GJ, Hugot JP, Karin M, Nizet V, Eckmann L (2009) NOD2 contributes to cutaneous defense against Staphylococcus aureus through alpha-toxin-dependent innate immune activation. Proc Natl Acad Sci U S A 106(31):12873–12878. doi:10.1073/pnas.0904958106

Hussain M, Haggar A, Peters G, Chhatwal GS, Herrmann M, Flock JI, Sinha B (2008) More than one tandem repeat domain of the extracellular adherence protein of Staphylococcus aureus is required for aggregation, adherence, and host cell invasion but not for leukocyte activation. Infect Immun 76(12):5615–5623. doi:10.1128/iai.00480-08

Ip WK, Sokolovska A, Charriere GM, Boyer L, Dejardin S, Cappillino MP, Yantosca LM, Takahashi K, Moore KJ, Lacy-Hulbert A, Stuart LM (2010) Phagocytosis and phagosome acidification are required for pathogen processing and MyD88-dependent responses to Staphylococcus aureus. J Immunol 184(12):7071–7081. doi:10.4049/jimmunol.1000110

Ip WK, Takahashi K, Moore KJ, Stuart LM, Ezekowitz RA (2008) Mannose-binding lectin enhances Toll-like receptors 2 and 6 signaling from the phagosome. J Exp Med 205(1):169–181. doi:10.1084/jem.20071164

Jann NJ, Schmaler M, Kristian SA, Radek KA, Gallo RL, Nizet V, Peschel A, Landmann R (2009) Neutrophil antimicrobial defense against Staphylococcus aureus is mediated by phagolysosomal but not extracellular trap-associated cathelicidin. J Leukoc Biol 86(5):1159–1169. doi:10.1189/jlb.0209053

Jawdat DM, Rowden G, Marshall JS (2006) Mast cells have a pivotal role in TNF-independent lymph node hypertrophy and the mobilization of Langerhans cells in response to bacterial peptidoglycan. J Immunol 177(3):1755–1762

Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A (2004) Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J Immunol 172(2):1169–1176

Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO (2007) Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 130 (6):1071–1082

Jin JO, Zhang W, Du JY, Yu Q (2014) BDCA1-positive dendritic cells (DCs) represent a unique human myeloid DC subset that induces innate and adaptive immune responses to Staphylococcus aureus Infection. Infect Immun 82(11):4466–4476. doi:10.1128/iai.01851-14

Josefsson E, Tarkowski A (1999) Staphylococcus aureus-induced inflammation and bone destruction in experimental models of septic arthritis. J Periodontal Res 34(7):387–392

Jung JY, Roberts LL, Robinson CM (2015) The presence of interleukin-27 during monocyte-derived dendritic cell differentiation promotes improved antigen processing and stimulation of T cells. Immunology 144(4):649–660. doi:10.1111/imm.12417

Kaesler S, Volz T, Skabytska Y, Koberle M, Hein U, Chen KM, Guenova E, Wolbing F, Rocken M, Biedermann T (2014) Toll-like receptor 2 ligands promote chronic atopic dermatitis through IL-4-mediated suppression of IL-10. J Allergy Clin Immunol 134(1):92–99. doi:10.1016/j.jaci.2014.02.017 S0091-6749(14)00267-X [pii]

Kamenyeva O, Boularan C, Kabat J, Cheung GY, Cicala C, Yeh AJ, Chan JL, Periasamy S, Otto M, Kehrl JH (2015) Neutrophil recruitment to lymph nodes limits local humoral response to Staphylococcus aureus. PLoS Pathog 11(4):e1004827. doi:10.1371/journal.ppat.1004827

Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity 31(6):873–884

Kang SS, Noh SY, Park OJ, Yun CH, Han SH (2015) Staphylococcus aureus induces IL-8 expression through its lipoproteins in the human intestinal epithelial cell, Caco-2. Cytokine. doi:10.1016/j.cyto.2015.04.017

Kapetanovic R, Nahori MA, Balloy V, Fitting C, Philpott DJ, Cavaillon JM, Adib-Conquy M (2007) Contribution of phagocytosis and intracellular sensing for cytokine production by Staphylococcus aureus-activated macrophages. Infect Immun 75(2):830–837. doi:10.1128/iai.01199-06

Kaplan A, Ma J, Kyme P, Wolf AJ, Becker CA, Tseng CW, Liu GY, Underhill DM (2012) Failure to induce IFN-beta production during Staphylococcus aureus infection contributes to pathogenicity. J Immunol 189(9):4537–4545. doi:10.4049/jimmunol.1201111

Kato-Matsunaga N, Okonogi K (1996) Gastrointestinal colonization by methicillin-resistant Staphylococcus aureus in immunosuppressed mice. Infect Immun 64(10):4231–4235

Kebaier C, Chamberland RR, Allen IC, Gao X, Broglie PM, Hall JD, Jania C, Doerschuk CM, Tilley SL, Duncan JA (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. doi:10.1093/infdis/jir846

Kielian T, Haney A, Mayes PM, Garg S, Esen N (2005) Toll-like receptor 2 modulates the proinflammatory milieu in Staphylococcus aureus-induced brain abscess. Infect Immun 73 (11):7428–7435. doi:73/11/7428 [pii] 10.1128/IAI.73.11.7428-7435.2005

Kim NJ, Ahn KB, Jeon JH, Yun CH, Finlay BB, Han SH (2015) Lipoprotein in the cell wall of Staphylococcus aureus is a major inducer of nitric oxide production in murine macrophages. Mol Immunol 65(1):17–24. doi:10.1016/j.molimm.2014.12.016 S0161-5890(14)00358-7 [pii]

Kinoshita M, Miyazaki H, Ono S, Inatsu A, Nakashima H, Tsujimoto H, Shinomiya N, Saitoh D, Seki S (2011) Enhancement of neutrophil function by interleukin-18 therapy protects burn-injured mice from methicillin-resistant Staphylococcus aureus. Infect Immun 79(7):2670–2680. doi:10.1128/iai.01298-10

Klotz M, Zimmermann S, Opper S, Heeg K, Mutters R (2005) Possible risk for re-colonization with methicillin-resistant Staphylococcus aureus (MRSA) by faecal transmission. Int J Hyg Environ Health 208(5):401–405. doi:10.1016/j.ijheh.2005.05.004

Kobayashi SD, Malachowa N, DeLeo FR (2015) Pathogenesis of Staphylococcus aureus abscesses. Am J Pathol 185(6):1518–1527. doi:10.1016/j.ajpath.2014.11.030

Kohler J, Breitbach K, Renner C, Heitsch AK, Bast A, van Rooijen N, Vogelgesang S, Steinmetz I (2011) NADPH-oxidase but not inducible nitric oxide synthase contributes to resistance in a murine Staphylococcus aureus Newman pneumonia model. Microbes Infect 13(11):914–922. doi:10.1016/j.micinf.2011.05.004

Kuo IH, Carpenter-Mendini A, Yoshida T, McGirt LY, Ivanov AI, Barnes KC, Gallo RL, Borkowski AW, Yamasaki K, Leung DY, Georas SN, De Benedetto A, Beck LA (2013) Activation of epidermal toll-like receptor 2 enhances tight junction function: implications for atopic dermatitis and skin barrier repair. J Invest Dermatol 133(4):988–998. doi:10.1038/jid.2012.437

Kurokawa K, Gong JH, Ryu KH, Zheng L, Chae JH, Kim MS, Lee BL (2011) Biochemical characterization of evasion from peptidoglycan recognition by Staphylococcus aureus D-alanylated wall teichoic acid in insect innate immunity. Dev Comp Immunol 35(8):835–839. doi:10.1016/j.dci.2011.03.001

Kwiecinski J, Rhost S, Lofbom L, Blomqvist M, Mansson JE, Cardell SL, Jin T (2013) Sulfatide attenuates experimental Staphylococcus aureus sepsis through a CD1d-dependent pathway. Infect Immun 81(4):1114–1120. doi:10.1128/iai.01334-12

Labrousse D, Perret M, Hayez D, Da Silva S, Badiou C, Couzon F, Bes M, Chavanet P, Lina G, Vandenesch F, Croisier-Bertin D, Henry T (2014) Kineret(R)/IL-1ra blocks the IL-1/IL-8 inflammatory cascade during recombinant Panton Valentine Leukocidin-triggered pneumonia but not during S. aureus infection. PLoS ONE 9(6):e97546. doi:10.1371/journal.pone.0097546

Liu X, Yang P, Wang C, Li F, Kijlstra A (2011) IFN-alpha blocks IL-17 production by peripheral blood mononuclear cells in Behcet's disease. Rheumatology (Oxford) 50(2):293–298. doi:10.1093/rheumatology/keq330

Lizak M, Yarovinsky TO (2012) Phospholipid scramblase 1 mediates type i interferon-induced protection against staphylococcal alpha-toxin. Cell Host Microbe 11(1):70–80. doi:10.1016/j.chom.2011.12.004 S1931-3128(11)00405-7 [pii]

Maher BM, Mulcahy ME, Murphy AG, Wilk M, O'Keeffe KM, Geoghegan JA, Lavelle EC, McLoughlin RM (2013) Nlrp-3-driven interleukin 17 production by gammadeltaT cells controls infection outcomes during Staphylococcus aureus surgical site infection. Infect Immun 81(12):4478–4489. doi:10.1128/iai.01026-13

Maresso AW, Schneewind O (2006) Iron acquisition and transport in Staphylococcus aureus. Biometals 19(2):193–203. doi:10.1007/s10534-005-4863-7

Martin F, Oliver AM, Kearney JF (2001) Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. Immunity 14(5):617–629. doi:S1074-7613(01)00129-7 [pii]

Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6:13. doi:10.12703/p6-13

Matsui K, Nishikawa A (2002) Lipoteichoic acid from Staphylococcus aureus induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. Clin Exp Allergy 32(5):783–788

Matsui K, Nishikawa A (2005) Percutaneous application of peptidoglycan from Staphylococcus aureus induces an increase in mast cell numbers in the dermis of mice. Clin Exp Allergy 35(3):382–387. doi:10.1111/j.1365-2222.2005.02190.x

Mayer AK, Muehmer M, Mages J, Gueinzius K, Hess C, Heeg K, Bals R, Lang R, Dalpke AH (2007) Differential recognition of TLR-dependent microbial ligands in human bronchial epithelial cells. J Immunol 178(5):3134–3142

McCurdy JD, Olynych TJ, Maher LH, Marshall JS (2003) Cutting edge: distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. J Immunol 170(4):1625–1629

McGilligan VE, Gregory-Ksander MS, Li D, Moore JE, Hodges RR, Gilmore MS, Moore TC, Dartt DA (2013) Staphylococcus aureus activates the NLRP3 inflammasome in human and rat conjunctival goblet cells. PLoS ONE 8(9):e74010. doi:10.1371/journal.pone.0074010

McNeil JC (2014) Staphylococcus aureus—antimicrobial resistance and the immunocompromised child. Infect Drug Resist 7:117–127. doi:10.2147/idr.s39639

Meyers JA, Mangini AJ, Nagai T, Roff CF, Sehy D, van Seventer GA, van Seventer JM (2006) Blockade of TLR9 agonist-induced type I interferons promotes inflammatory cytokine IFN-gamma and IL-17 secretion by activated human PBMC. Cytokine 35(5–6):235–246. doi:10.1016/j.cyto.2006.09.001

Michea P, Vargas P, Donnadieu MH, Rosemblatt M, Bono MR, Dumenil G, Soumelis V (2013) Epithelial control of the human pDC response to extracellular bacteria. Eur J Immunol 43 (5):1264–1273. doi:10.1002/eji.201242990

Miller LS, Cho JS (2011) Immunity against Staphylococcus aureus cutaneous infections. Nat Rev Immunol 11(8):505–518. doi:10.1038/nri3010

Miller LS, O'Connell RM, Gutierrez MA, Pietras EM, Shahangian A, Gross CE, Thirumala A, Cheung AL, Cheng G, Modlin RL (2006) MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity 24 (1):79–91. doi:10.1016/j.immuni.2005.11.011

Miller LS, Pietras EM, Uricchio LH, Hirano K, Rao S, Lin H, O'Connell RM, Iwakura Y, Cheung AL, Cheng G, Modlin RL (2007) Inflammasome-mediated production of IL-1beta is required for neutrophil recruitment against Staphylococcus aureus in vivo. J Immunol 179 (10):6933–6942

Mjosberg J, Eidsmo L (2014) Update on innate lymphoid cells in atopic and non-atopic inflammation in the airways and skin. Clin Exp Allergy 44(8):1033–1043. doi:10.1111/cea.12353

Monticelli LA, Sonnenberg GF, Artis D (2012) Innate lymphoid cells: critical regulators of allergic inflammation and tissue repair in the lung. Curr Opin Immunol 24(3):284–289. doi:10.1016/j.coi.2012.03.012

Moore CE, Segal S, Berendt AR, Hill AV, Day NP (2004) Lack of association between Toll-like receptor 2 polymorphisms and susceptibility to severe disease caused by Staphylococcus aureus. Clin Diagn Lab Immunol 11(6):1194–1197. doi:10.1128/cdli.11.6.1194-1197.2004

Mrabet-Dahbi S, Dalpke AH, Niebuhr M, Frey M, Draing C, Brand S, Heeg K, Werfel T, Renz H (2008) The Toll-like receptor 2 R753Q mutation modifies cytokine production and Toll-like receptor expression in atopic dermatitis. J Allergy Clin Immunol 121(4):1013–1019. doi:10.1016/j.jaci.2007.11.029

Munoz-Planillo R, Franchi L, Miller LS, Nunez G (2009) A critical role for hemolysins and bacterial lipoproteins in Staphylococcus aureus-induced activation of the Nlrp3 inflammasome. J Immunol 183(6):3942–3948. doi:jimmunol.0900729 [pii] 10.4049/jimmunol.0900729

Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Munoz-Planillo R, Hasegawa M, Villaruz AE, Cheung GY, McGavin MJ, Travers JB, Otto M, Inohara N, Nunez G (2013) Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. Nature 503(7476):397–401. doi:10.1038/nature12655

Nakayama M, Kurokawa K, Nakamura K, Lee BL, Sekimizu K, Kubagawa H, Hiramatsu K, Yagita H, Okumura K, Takai T, Underhill DM, Aderem A, Ogasawara K (2012) Inhibitory receptor paired Ig-like receptor B is exploited by Staphylococcus aureus for virulence. J Immunol 189(12):5903–5911. doi:10.4049/jimmunol.1201940

Nandi A, Dey S, Biswas J, Jaiswal P, Naaz S, Yasmin T, Bishayi B (2015) Differential induction of inflammatory cytokines and reactive oxygen species in murine peritoneal macrophages and resident fresh bone marrow cells by acute staphylococcus aureus infection: contribution of toll-like receptor 2 (TLR2). Inflammation 38(1):224–244. doi:10.1007/s10753-014-0026-8

Negrini TC, Arthur RA, Waeiss RA, Carlosa IZ, Srinivasan M (2014) Salivary epithelial cells as model to study immune response against cutaneous pathogens. Clin Transl Sci 7(1):48–51. doi:10.1111/cts.12113

Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW (2000) Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun 68(2):688–693

Neth O, Jack DL, Johnson M, Klein NJ, Turner MW (2002) Enhancement of complement activation and opsonophagocytosis by complexes of mannose-binding lectin with mannose-binding lectin-associated serine protease after binding to Staphylococcus aureus. J Immunol 169(8):4430–4436

Neumann A, Berends ET, Nerlich A, Molhoek EM, Gallo RL, Meerloo T, Nizet V, Naim HY, von Kockritz-Blickwede M (2014) The antimicrobial peptide LL-37 facilitates the formation of neutrophil extracellular traps. Biochem J 464(1):3–11. doi:10.1042/bj20140778

Nguyen MT, Kraft B, Yu W, Demicrioglu DD, Hertlein T, Burian M, Schmaler M, Boller K, Bekeredjian-Ding I, Ohlsen K, Schittek B, Gotz F (2015) The nuSaalpha specific lipoprotein like cluster (lpl) of S. aureus USA300 contributes to immune stimulation and invasion in human cells. PLoS Pathog 11(6):e1004984. doi:10.1371/journal.ppat.1004984 PPATHOGENS-D-15-00227 [pii]

Niebuhr M, Baumert K, Heratizadeh A, Satzger I, Werfel T (2014) Impaired NLRP3 inflammasome expression and function in atopic dermatitis due to Th2 milieu. Allergy 69 (8):1058–1067. doi:10.1111/all.12428

Niebuhr M, Baumert K, Werfel T (2010a) TLR-2-mediated cytokine and chemokine secretion in human keratinocytes. Exp Dermatol 19(10):873–877. doi:10.1111/j.1600-0625.2010.01140.x

Niebuhr M, Langnickel J, Sigel S, Werfel T (2010b) Dysregulation of CD36 upon TLR-2 stimulation in monocytes from patients with atopic dermatitis and the TLR2 R753Q polymorphism. Exp Dermatol 19(8):e296–e298. doi:10.1111/j.1600-0625.2009.00989.x

Niebuhr M, Heratizadeh A, Wichmann K, Satzger I, Werfel T (2011) Intrinsic alterations of pro-inflammatory mediators in unstimulated and TLR-2 stimulated keratinocytes from atopic dermatitis patients. Exp Dermatol 20(6):468–472. doi:10.1111/j.1600-0625.2011.01277.x

Nieuwenhuis EE, Matsumoto T, Lindenbergh D, Willemsen R, Kaser A, Simons-Oosterhuis Y, Brugman S, Yamaguchi K, Ishikawa H, Aiba Y, Koga Y, Samsom JN, Oshima K, Kikuchi M, Escher JC, Hattori M, Onderdonk AB, Blumberg RS (2009) Cd1d-dependent regulation of bacterial colonization in the intestine of mice. J Clin Invest 119(5):1241–1250. doi:10.1172/jci36509

Nilsen NJ, Deininger S, Nonstad U, Skjeldal F, Husebye H, Rodionov D, von Aulock S, Hartung T, Lien E, Bakke O, Espevik T (2008) Cellular trafficking of lipoteichoic acid and Toll-like receptor 2 in relation to signaling: role of CD14 and CD36. J Leukoc Biol 84(1):280–291

Nilsson N, Bremell T, Tarkowski A, Carlsten H (1999) Protective role of NK1.1+ cells in experimental Staphylococcus aureus arthritis. Clin Exp Immunol 117(1):63–69

Nowrouzian FL, Dauwalder O, Meugnier H, Bes M, Etienne J, Vandenesch F, Lindberg E, Hesselmar B, Saalman R, Strannegard IL, Aberg N, Adlerberth I, Wold AE, Lina G (2011) Adhesin and superantigen genes and the capacity of Staphylococcus aureus to colonize the infantile gut. J Infect Dis 204(5):714–721. doi:10.1093/infdis/jir388

Nurjadi D, Herrmann E, Hinderberger I, Zanger P (2013) Impaired beta-defensin expression in human skin links DEFB1 promoter polymorphisms with persistent Staphylococcus aureus nasal carriage. J Infect Dis 207(4):666–674. doi:10.1093/infdis/jis735

Olaru F, Jensen LE (2010) Staphylococcus aureus stimulates neutrophil targeting chemokine expression in keratinocytes through an autocrine IL-1alpha signaling loop. J Invest Dermatol 130(7):1866–1876. doi:10.1038/jid.2010.37

Oldenburg M, Kruger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, Bathke B, Lauterbach H, Suter M, Dreher S, Koedel U, Akira S, Kawai T, Buer J, Wagner H, Bauer S, Hochrein H, Kirschning CJ (2012) TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. Science 337(6098):1111–1115. doi:10.1126/science.1220363 science.1220363 [pii]

Ono K, Nishitani C, Mitsuzawa H, Shimizu T, Sano H, Suzuki H, Kodama T, Fujii N, Fukase K, Hirata K, Kuroki Y (2006) Mannose-binding lectin augments the uptake of lipid A, Staphylococcus aureus, and Escherichia coli by Kupffer cells through increased cell surface expression of scavenger receptor A. J Immunol 177(8):5517–5523

Over B, Ziegler S, Foermer S, Weber AN, Bode KA, Heeg K, Bekeredjian-Ding I (2013) IRAK4 turns IL-10+ phospho-FOXO+ monocytes into pro-inflammatory cells by suppression of protein kinase B. Eur J Immunol 43(6):1630–1642. doi:10.1002/eji.201243217

Palecanda A, Paulauskis J, Al-Mutairi E, Imrich A, Qin G, Suzuki H, Kodama T, Tryggvason K, Koziel H, Kobzik L (1999) Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles. J Exp Med 189(9):1497–1506

Parcina M, Miranda-Garcia MA, Durlanik S, Ziegler S, Over B, Georg P, Foermer S, Ammann S, Hilmi D, Weber KJ, Schiller M, Heeg K, Schneider-Brachert W, Gotz F, Bekeredjian-Ding I (2013) Pathogen-triggered activation of plasmacytoid dendritic cells induces IL-10-producing B cells in response to Staphylococcus aureus. J Immunol 190(4):1591–1602. doi:10.4049/jimmunol.1201222

Parcina M, Wendt C, Goetz F, Zawatzky R, Zahringer U, Heeg K, Bekeredjian-Ding I (2008) Staphylococcus aureus-induced plasmacytoid dendritic cell activation is based on an IgG-mediated memory response. J Immunol 181(6):3823–3833. doi:181/6/3823 [pii]

Parker D, Prince A (2012) Staphylococcus aureus induces type I IFN signaling in dendritic cells via TLR9. J Immunol 189(8):4040–4046. doi:10.4049/jimmunol.1201055

Perret M, Badiou C, Lina G, Burbaud S, Benito Y, Bes M, Cottin V, Couzon F, Juruj C, Dauwalder O, Goutagny N, Diep BA, Vandenesch F, Henry T (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. doi:10.1111/j.1462-5822.2012.01772.x

Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F (1999) Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem 274(13):8405–8410

Petrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J (2007) Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Differ 14 (9):1583–1589. doi:10.1038/sj.cdd.4402195

Philip NH, Artis D (2013) New friendships and old feuds: relationships between innate lymphoid cells and microbial communities. Immunol Cell Biol 91(3):225–231. doi:10.1038/icb.2013.2

Pizzolla A, Hultqvist M, Nilson B, Grimm MJ, Eneljung T, Jonsson IM, Verdrengh M, Kelkka T, Gjertsson I, Segal BH, Holmdahl R (2012) Reactive oxygen species produced by the NADPH oxidase 2 complex in monocytes protect mice from bacterial infections. J Immunol 188 (10):5003–5011. doi:10.4049/jimmunol.1103430

Queck SY, Jameson-Lee M, Villaruz AE, Bach TH, Khan BA, Sturdevant DE, Ricklefs SM, Li M, Otto M (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. doi:10.1016/j.molcel.2008.08.005 S1097-2765(08)00537-6 [pii]

Quinn GA, Cole AM (2007) Suppression of innate immunity by a nasal carriage strain of Staphylococcus aureus increases its colonization on nasal epithelium. Immunology 122 (1):80–89

Reed P, Atilano ML, Alves R, Hoiczyk E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe SR, Pereira-Leal JB, Ligoxygakis P, Pinho MG (2015) Staphylococcus aureus survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. PLoS Pathog 11(5):e1004891. doi:10.1371/journal.ppat.1004891

Reis e Sousa C, Stahl PD, Austyn JM (1993) Phagocytosis of antigens by Langerhans cells in vitro. J Exp Med 178(2):509–519

Rigby KM, DeLeo FR (2012) Neutrophils in innate host defense against Staphylococcus aureus infections. Semin Immunopathol 34(2):237–259. doi:10.1007/s00281-011-0295-3

Robertson CM, Perrone EE, McConnell KW, Dunne WM, Boody B, Brahmbhatt T, Diacovo MJ, Van Rooijen N, Hogue LA, Cannon CL, Buchman TG, Hotchkiss RS, Coopersmith CM (2008) Neutrophil depletion causes a fatal defect in murine pulmonary Staphylococcus aureus clearance. J Surg Res 150(2):278–285. doi:10.1016/j.jss.2008.02.009

Robinette ML, Fuchs A, Cortez VS, Lee JS, Wang Y, Durum SK, Gilfillan S, Colonna M (2015) Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. Nat Immunol 16(3):306–317. doi:10.1038/ni.3094

Robinson KM, Lee B, Scheller EV, Mandalapu S, Enelow RI, Kolls JK, Alcorn JF (2015) The role of IL-27 in susceptibility to post-influenza Staphylococcus aureus pneumonia. Respir Res 16 (1):10. doi:10.1186/s12931-015-0168-8

Rocha-de-Souza CM, Berent-Maoz B, Mankuta D, Moses AE, Levi-Schaffer F (2008) Human mast cell activation by Staphylococcus aureus: interleukin-8 and tumor necrosis factor alpha release and the role of Toll-like receptor 2 and CD48 molecules. Infect Immun 76(10):4489–4497. doi:10.1128/iai.00270-08

Ronnberg E, Johnzon CF, Calounova G, Garcia Faroldi G, Grujic M, Hartmann K, Roers A, Guss B, Lundequist A, Pejler G (2014) Mast cells are activated by Staphylococcus aureus in vitro but do not influence the outcome of intraperitoneal S. aureus infection in vivo. Immunology 143(2):155–163. doi:10.1111/imm.12297

Roquilly A, Gautreau L, Segain JP, de Coppet P, Sebille V, Jacqueline C, Caillon J, Potel G, Lejus C, Josien R, Asehnoune K (2010) CpG-ODN and MPLA prevent mortality in a murine model of post-hemorrhage-Staphyloccocus aureus pneumonia. PLoS One 5(10):e13228. doi:10.1371/journal.pone.0013228 e13228 [pii]

Ryu S, Song PI, Seo CH, Cheong H, Park Y (2014) Colonization and infection of the skin by S. aureus: immune system evasion and the response to cationic antimicrobial peptides. Int J Mol Sci 15(5):8753–8772. doi:10.3390/ijms15058753 ijms15058753 [pii]

Sachse F, Becker K, Rudack C (2010) Incidence of staphylococcal colonization and of the 753Q Toll-like receptor 2 variant in nasal polyposis. Am J Rhinol Allergy 24(1):e10–e13. doi:10.2500/ajra.2010.24.3416

Sakiniene E, Bremell T, Tarkowski A (1999) Complement depletion aggravates Staphylococcus aureus septicaemia and septic arthritis. Clin Exp Immunol 115(1):95–102

Salvi V, Scutera S, Rossi S, Zucca M, Alessandria M, Greco D, Bosisio D, Sozzani S, Musso T (2013) Dual regulation of osteopontin production by TLR stimulation in dendritic cells. J Leukoc Biol 94(1):147–158. doi:10.1189/jlb.0412194

Sankaran K, Gupta SD, Wu HC (1995) Modification of bacterial lipoproteins. Methods Enzymol 250:683–697. doi:0076-6879(95)50105-3 [pii]

Sankaran K, Wu HC (1995) Bacterial prolipoprotein signal peptidase. Methods Enzymol 248:169–180

Schaffler H, Demircioglu DD, Kuhner D, Menz S, Bender A, Autenrieth IB, Bodammer P, Lamprecht G, Gotz F, Frick JS (2014) NOD2 stimulation by Staphylococcus aureus-derived peptidoglycan is boosted by Toll-like receptor 2 costimulation with lipoproteins in dendritic cells. Infect Immun 82(11):4681–4688. doi:10.1128/iai.02043-14

Schindler D, Gutierrez MG, Beineke A, Rauter Y, Rohde M, Foster S, Goldmann O, Medina E (2012) Dendritic cells are central coordinators of the host immune response to Staphylococcus aureus bloodstream infection. Am J Pathol 181(4):1327–1337. doi:10.1016/j.ajpath.2012.06.039

Schmaler M, Jann NJ, Ferracin F, Landolt LZ, Biswas L, Götz F, Landmann R (2009) Lipoproteins in *Staphylococcus aureus* mediate inflammation by TLR2 and iron-dependent growth *in vivo*. J Immunol 182(11):7110–7118. doi:182/11/7110 [pii] 10.4049/jimmunol.0804292

Schmaler M, Jann NJ, Ferracin F, Landmann R (2011) T and B cells are not required for clearing Staphylococcus aureus in systemic infection despite a strong TLR2-MyD88-dependent T cell activation. J Immunol 186(1):443–452. doi:10.4049/jimmunol.1001407

Schmaler M, Jann NJ, Gotz F, Landmann R (2010) Staphylococcal lipoproteins and their role in bacterial survival in mice. IntJMedMicrobiol 300(2–3):155–160. doi:S1438-4221(09)00111-8 [pii]; 10.1016/j.ijmm.2009.08.018 [doi]

Schreiner J, Kretschmer D, Klenk J, Otto M, Buhring HJ, Stevanovic S, Wang JM, Beer-Hammer S, Peschel A, Autenrieth SE (2013) Staphylococcus aureus phenol-soluble modulin peptides

modulate dendritic cell functions and increase in vitro priming of regulatory T cells. J Immunol 190(7):3417–3426. doi:10.4049/jimmunol.1202563

Shang SQ, Chen GX, Shen J, Yu XH, Wang KY (2005) The binding of MBL to common bacteria in infectious diseases of children. J Zhejiang Univ Sci B 6(1):53–56. doi:10.1631/jzus.2005.B0053

Shi L, Takahashi K, Dundee J, Shahroor-Karni S, Thiel S, Jensenius JC, Gad F, Hamblin MR, Sastry KN, Ezekowitz RA (2004) Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. J Exp Med 199(10):1379–1390. doi:10.1084/jem.20032207

Shibuya A, Honda S (2006) Molecular and functional characteristics of the Fcalpha/muR, a novel Fc receptor for IgM and IgA. Springer Semin Immunopathol 28(4):377–382. doi:10.1007/s00281-006-0050-3

Shimada T, Park BG, Wolf AJ, Brikos C, Goodridge HS, Becker CA, Reyes CN, Miao EA, Aderem A, Gotz F, Liu GY, Underhill DM (2010) Staphylococcus aureus evades lysozyme-based peptidoglycan digestion that links phagocytosis, inflammasome activation, and IL-1beta secretion. Cell Host Microbe 7(1):38–49. doi:10.1016/j.chom.2009.12.008

Shimaoka T, Kume N, Minami M, Hayashida K, Sawamura T, Kita T, Yonehara S (2001) LOX-1 supports adhesion of Gram-positive and Gram-negative bacteria. J Immunol 166(8):5108–5114

Shinohara ML, Kim JH, Garcia VA, Cantor H (2008) Engagement of the type I interferon receptor on dendritic cells inhibits T helper 17 cell development: role of intracellular osteopontin. Immunity 29(1):68–78. doi:10.1016/j.immuni.2008.05.008

Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, Suder P, Silberring J, Reed M, Pohl J, Shafer W, McAleese F, Foster T, Travis J, Potempa J (2004) Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrob Agents Chemother 48(12):4673–4679. doi:48/12/4673 [pii] 10.1128/AAC.48.12.4673-4679.2004

Simanski M, Glaser R, Koten B, Meyer-Hoffert U, Wanner S, Weidenmaier C, Peschel A, Harder J (2013) Staphylococcus aureus subverts cutaneous defense by D-alanylation of teichoic acids. Exp Dermatol 22(4):294–296. doi:10.1111/exd.12114

Skabytska Y, Wolbing F, Gunther C, Koberle M, Kaesler S, Chen KM, Guenova E, Demircioglu D, Kempf WE, Volz T, Rammensee HG, Schaller M, Rocken M, Gotz F, Biedermann T (2014) Cutaneous innate immune sensing of Toll-like receptor 2-6 ligands suppresses T cell immunity by inducing myeloid-derived suppressor cells. Immunity 41(5):762–775. doi:10.1016/j.immuni.2014.10.009

Small CL, McCormick S, Gill N, Kugathasan K, Santosuosso M, Donaldson N, Heinrichs DE, Ashkar A, Xing Z (2008) NK cells play a critical protective role in host defense against acute extracellular Staphylococcus aureus bacterial infection in the lung. J Immunol 180(8):5558–5568

Sokolovska A, Becker CE, Ip WK, Rathinam VA, Brudner M, Paquette N, Tanne A, Vanaja SK, Moore KJ, Fitzgerald KA, Lacy-Hulbert A, Stuart LM (2013) Activation of caspase-1 by the NLRP3 inflammasome regulates the NADPH oxidase NOX2 to control phagosome function. Nat Immunol 14(6):543–553. doi:10.1038/ni.2595

Soong G, Paulino F, Wachtel S, Parker D, Wickersham M, Zhang D, Brown A, Lauren C, Dowd M, West E, Horst B, Planet P, Prince A (2015) Methicillin-resistant Staphylococcus aureus adaptation to human keratinocytes. MBio 6(2). doi:10.1128/mBio.00289-15

Stoll H, Dengjel J, Nerz C, Gotz F (2005) Staphylococcus aureus deficient in lipidation of prelipoproteins is attenuated in growth and immune activation. Infect Immun 73(4):2411–2423

Stuart LM, Deng J, Silver JM, Takahashi K, Tseng AA, Hennessy EJ, Ezekowitz RA, Moore KJ (2005) Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. J Cell Biol 170(3):477–485. doi:jcb.200501113 [pii] 10.1083/jcb.200501113

Subramaniam R, Barnes PF, Fletcher K, Boggaram V, Hillberry Z, Neuenschwander P, Shams H (2014) Protecting against post-influenza bacterial pneumonia by increasing phagocyte recruitment and ROS production. J Infect Dis 209(11):1827–1836. doi:10.1093/infdis/jit830

Suzuki N, Suzuki S, Duncan GS, Millar DG, Wada T, Mirtsos C, Takada H, Wakeham A, Itie A, Li S, Penninger JM, Wesche H, Ohashi PS, Mak TW, Yeh WC (2002) Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature 416(6882):750–756. doi:10.1038/nature736

Tait Wojno ED, Artis D (2012) Innate lymphoid cells: balancing immunity, inflammation, and tissue repair in the intestine. Cell Host Microbe 12(4):445–457. doi:10.1016/j.chom.2012.10.003

Takai T, Chen X, Xie Y, Vu AT, Le TA, Kinoshita H, Kawasaki J, Kamijo S, Hara M, Ushio H, Baba T, Hiramatsu K, Ikeda S, Ogawa H, Okumura K (2014) TSLP expression induced via Toll-like receptor pathways in human keratinocytes. Methods Enzymol 535:371–387. doi:10.1016/b978-0-12-397925-4.00021-3

Takeuchi O, Hoshino K, Akira S (2000) Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol 165(10):5392–5396

Tebartz C, Horst SA, Sparwasser T, Huehn J, Beineke A, Peters G, Medina E (2015) A major role for myeloid-derived suppressor cells and a minor role for regulatory T cells in immunosuppression during Staphylococcus aureus infection. J Immunol 194(3):1100–1111. doi:10.4049/jimmunol.1400196

Terada M, Tsutsui H, Imai Y, Yasuda K, Mizutani H, Yamanishi K, Kubo M, Matsui K, Sano H, Nakanishi K (2006) Contribution of IL-18 to atopic-dermatitis-like skin inflammation induced by Staphylococcus aureus product in mice. Proc Natl Acad Sci U S A 103(23):8816–8821. doi:10.1073/pnas.0602900103

Tewfik MA, Bosse Y, Hudson TJ, Vallee-Smejda S, Al-Shemari H, Desrosiers M (2008) Assessment of Toll-like receptor 2 gene polymorphisms in severe chronic rhinosinusitis. J Otolaryngol Head Neck Surg 37(4):552–558

Urb M, Sheppard DC (2012) The role of mast cells in the defence against pathogens. PLoS Pathog 8(4):e1002619. doi:10.1371/journal.ppat.1002619 PPATHOGENS-D-12-00120 [pii]

Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, Aderem A (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 401(6755):811–815. doi:10.1038/44605

van der Laan LJ, Dopp EA, Haworth R, Pikkarainen T, Kangas M, Elomaa O, Dijkstra CD, Gordon S, Tryggvason K, Kraal G (1999) Regulation and functional involvement of macrophage scavenger receptor MARCO in clearance of bacteria in vivo. J Immunol 162(2):939–947

Verdrengh M, Tarkowski A (1997) Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus. Infect Immun 65(7):2517–2521

Verkaik NJ, de Vogel CP, Boelens HA, Grumann D, Hoogenboezem T, Vink C, Hooijkaas H, Foster TJ, Verbrugh HA, van Belkum A, van Wamel WJ (2009) Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of Staphylococcus aureus. J Infect Dis 199(5):625–632

Viau M, Longo NS, Lipsky PE, Zouali M (2005) Staphylococcal protein a deletes B-1a and marginal zone B lymphocytes expressing human immunoglobulins: an immune evasion mechanism. J Immunol 175(11):7719–7727

Volz T, Nega M, Buschmann J, Kaesler S, Guenova E, Peschel A, Rocken M, Gotz F, Biedermann T (2010) Natural Staphylococcus aureus-derived peptidoglycan fragments activate NOD2 and act as potent costimulators of the innate immune system exclusively in the presence of TLR signals. FASEB J 24(10):4089–4102. doi:10.1096/fj.09-151001

von Bernuth H, Picard C, Puel A, Casanova JL (2012) Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. Eur J Immunol 42(12):3126–3135. doi:10.1002/eji.201242683

von Kockritz-Blickwede M, Goldmann O, Thulin P, Heinemann K, Norrby-Teglund A, Rohde M, Medina E (2008a) Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. Blood 111(6):3070–3080. doi:10.1182/blood-2007-07-104018

von Kockritz-Blickwede M, Rohde M, Oehmcke S, Miller LS, Cheung AL, Herwald H, Foster S, Medina E (2008b) Immunological mechanisms underlying the genetic predisposition to severe Staphylococcus aureus infection in the mouse model. Am J Pathol 173(6):1657–1668. doi:10.2353/ajpath.2008.080337

Vu AT, Baba T, Chen X, Le TA, Kinoshita H, Xie Y, Kamijo S, Hiramatsu K, Ikeda S, Ogawa H, Okumura K, Takai T (2010) Staphylococcus aureus membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. J Allergy Clin Immunol 126(5):985–993, 993 e981-983. doi:10.1016/j.jaci.2010.09.002

Vultaggio A, Nencini F, Pratesi S, Maggi L, Guarna A, Annunziato F, Romagnani S, Parronchi P, Maggi E (2011) The TLR7 ligand 9-benzyl-2-butoxy-8-hydroxy adenine inhibits IL-17 response by eliciting IL-10 and IL-10-inducing cytokines. J Immunol 186(8):4707–4715. doi:10.4049/jimmunol.1002398

Wagner C, Iking-Konert C, Hug F, Stegmaier S, Heppert V, Wentzensen A, Hansch GM (2006) Cellular inflammatory response to persistent localized Staphylococcus aureus infection: phenotypical and functional characterization of polymorphonuclear neutrophils (PMN). Clin Exp Immunol 143(1):70–77. doi:10.1111/j.1365-2249.2005.02963.x

Wanke I, Steffen H, Christ C, Krismer B, Gotz F, Peschel A, Schaller M, Schittek B (2011) Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. J Invest Dermatol 131(2):382–390. doi:10.1038/jid.2010.328

Westerberg LS, de la Fuente MA, Wermeling F, Ochs HD, Karlsson MC, Snapper SB, Notarangelo LD (2008) WASP confers selective advantage for specific hematopoietic cell populations and serves a unique role in marginal zone B-cell homeostasis and function. Blood 112(10):4139–4147. doi:10.1182/blood-2008-02-140715 blood-2008-02-140715 [pii]

Wolf AJ, Arruda A, Reyes CN, Kaplan AT, Shimada T, Shimada K, Arditi M, Liu G, Underhill DM (2011) Phagosomal degradation increases TLR access to bacterial ligands and enhances macrophage sensitivity to bacteria. J Immunol 187(11):6002–6010. doi:10.4049/jimmunol.1100232

Yang D, Chertov O, Oppenheim JJ (2001) Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). J Leukoc Biol 69(5):691–697

Yimin Kohanawa M, Zhao S, Ozaki M, Haga S, Nan G, Kuge Y, Tamaki N (2013) Contribution of toll-like receptor 2 to the innate response against Staphylococcus aureus infection in mice. PLoS ONE 8(9):e74287. doi:10.1371/journal.pone.0074287

Zaidman-Remy A, Herve M, Poidevin M, Pili-Floury S, Kim MS, Blanot D, Oh BH, Ueda R, Mengin-Lecreulx D, Lemaitre B (2006) The Drosophila amidase PGRP-LB modulates the immune response to bacterial infection. Immunity 24(4):463–473

Zanger P, Nurjadi D, Vath B, Kremsner PG (2011) Persistent nasal carriage of Staphylococcus aureus is associated with deficient induction of human beta-defensin 3 after sterile wounding of healthy skin in vivo. Infect Immun 79(7):2658–2662. doi:10.1128/iai.00101-11

Zhao H, Li W, Gao Y, Li J, Wang H (2014) Exposure to particular matter increases susceptibility to respiratory Staphylococcus aureus infection in rats via reducing pulmonary natural killer cells. Toxicology 325:180–188. doi:10.1016/j.tox.2014.09.006

Ziegler SF, Roan F, Bell BD, Stoklasek TA, Kitajima M, Han H (2013) The biology of thymic stromal lymphopoietin (TSLP). Adv Pharmacol 66:129–155. doi:10.1016/b978-0-12-404717-4.00004-4

Adaptive Immunity Against *Staphylococcus aureus*

Hatice Karauzum and Sandip K. Datta

Abstract A complex interplay between host and bacterial factors allows *Staphylococcus aureus* to occupy its niche as a human commensal and a major human pathogen. The role of neutrophils as a critical component of the innate immune response against *S. aureus*, particularly for control of systemic infection, has been established in both animal models and in humans with acquired and congenital neutrophil dysfunction. The role of the adaptive immune system is less clear. Although deficiencies in adaptive immunity do not result in the marked susceptibility to *S. aureus* infection that neutrophil dysfunction imparts, emerging evidence suggests both T cell- and B cell-mediated adaptive immunity can influence host susceptibility and control of *S. aureus*. The contribution of adaptive immunity depends on the context and site of infection and can be either beneficial or detrimental to the host. Furthermore, *S. aureus* has evolved mechanisms to manipulate adaptive immune responses to its advantage. In this chapter, we will review the evidence for the role of adaptive immunity during *S. aureus* infections. Further elucidation of this role will be important to understand how it influences susceptibility to infection and to appropriately design vaccines that elicit adaptive immune responses to protect against subsequent infections.

Contents

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1 Introduction

Staphylococcus aureus is a major human pathogen. Data from the USA and Europe indicate it is the predominant cause of both cutaneous and invasive infections and is the leading cause of infectious morbidity and mortality in the industrialized world (Tong et al. 2015). Strain-specific virulence strategies and acquisition of resistance against a variety of antibiotics reflect the adaptive capabilities that have shaped its ability to cause continually shifting patterns of disease (Chambers and Deleo 2009; Tong et al. 2015). Despite its clear pathogenic potential, *S. aureus* has the ability to coexist with its human host as a commensal, with 20–30 % of the population colonized at mucocutaneous surfaces and significantly higher proportions exposed at least intermittently (Verhoeven et al. 2014). The success of *S. aureus* as a human commensal and pathogen suggests the evolution of a complex and intricate interplay between host and bacterial factors.

S. aureus has a plethora of virulence factors that evade and modulate components of the human innate and adaptive immune system (Nizet 2007; Lowy 1998; Rooijakkers et al. 2005). Much attention has been rightly focused on interactions with the innate immune system, in particular neutrophils, which play a central role in host defense against *S. aureus*. However, the readily detectable antibody and T cell responses in humans and the extensive mechanisms for staphylococcal evasion of antibody and T cell-mediated host defense suggest an important contribution of adaptive immunity that may influence host susceptibility and will need to be invoked by a successful vaccine. In this chapter, we will highlight the major findings related to adaptive immune responses induced by *S. aureus* and the evasion mechanisms it uses to escape this aspect of host defense.

2 Immunological Overview

The immune response against *S. aureus* involves activation of both the innate and the adaptive immune systems. As the first line of defense against infections, the innate immune response is rapidly activated by pathways that detect pathogen-associated molecular patterns. A key result of this is activation of phagocytic cells such as macrophages and neutrophils. Neutrophils are recognized as a key component of the acute response and centrally important against *S. aureus*,

as declared by the susceptibility of humans and mice with inherited and acquired neutrophil defects to deep-seated infections. The adaptive immune response kicks in later during the course of infection, dependent on the presentation of bacterial antigens by antigen-presenting cells (APCs) and influenced by the cytokine milieu generated by the innate response. Through T cell activation and B cell production of antibodies, the adaptive immune response targets specific bacterial antigens and can be recalled during subsequent infections to provide 'memory' against that particular pathogen. Antibodies and T cells can have direct activity against bacteria, but also amplify the activity of innate immune cells, e.g., by increasing phagocyte killing and recruitment. The prevalence of recurrent infections with *S. aureus* suggests the adaptive memory response is not completely effective, although it could be argued that the relative paucity of systemic infections despite the high rate of colonization may be evidence for its protective role. Understanding the contribution of the adaptive immune response in determining *S. aureus* susceptibility may help identify risk factors and therapeutic strategies, and will be essential to harness for successful vaccine development.

3 Role of B Cells and Antibodies

The major function of B cells is to secrete immunoglobulins (antibodies) that neutralize the function of target proteins (e.g., toxins and other virulence factors) or opsonize pathogens to optimize phagocytosis and clearance. The importance of antibody-mediated protection against infectious agents is clearly demonstrated by patients with X-linked agammaglobulinemia (XLA), in whom lack of appropriate B cell maturation leads to susceptibility to infections with a variety of viruses and encapsulated bacteria that is largely reversed with the periodic administration of pooled donor immunoglobulins (Bruton 1952; Conley and Howard 1993). The apparent lack of increased susceptibility in this patient population to invasive *S. aureus* infection argues that antibodies are unimportant in protection against *S. aureus* infection. Although these patients have a recognized susceptibility to cellulitis, this has also not been clearly attributed to *S. aureus*. The lack of increased susceptibility to *S. aureus* infection in B cell- or antibody-deficient mice (Gjertsson et al. 2000; Schmaler et al. 2011; Gaidamakova et al. 2012) parallels the observations in patients with XLA. However, recent work has revealed that primary *S. aureus* cutaneous infection can induce antibody-mediated protection against a subsequent infection in certain mouse strains (Montgomery et al. 2014), and numerous preclinical studies have shown at least partial protection from subsequent infection after induction of antibodies by vaccination (see below). Furthermore, the ubiquitous presence of antibodies after *S. aureus* exposure in humans and animal models, and the virulence strategies of *S. aureus* that have evolved to evade antibodies, suggests antibodies may have a role in modulating susceptibility to infection. Evidence for this potential role will be examined in further detail here.

3.1 Preexisting Antibodies as Immunologic Correlates for Protection

The immune correlates of protection from and susceptibility to staphylococcal infections are still not well understood. A few reports have suggested that preexisting antibodies toward certain staphylococcal virulence factors can correlate with clinical outcome in humans. Adhikari et al. (2012a) measured serum antibodies to an array of staphylococcal exotoxins and observed that low antibody titers correlated with a higher risk for the development of sepsis. Another study found that elevated serum titers against *S. aureus* α-hemolysin (Hla) correlated with the protection from subsequent infection, and invasive infections elicited a more durable antibody response when compared to cutaneous infections (Fritz et al. 2013). This study also reported high titer anti-staphylococcal antibodies in colonized individuals without a history of overt infection (carriers), which may explain the enhanced recovery from infection observed in carriers despite their increased risk of developing infection compared to non-carriers (Wertheim et al. 2005; von Eiff et al. 2001a).

3.2 Role of Antibodies in Vaccine-Mediated Protection

S. aureus has been generally regarded as an extracellular pathogen. Consequently, complement and antibodies with neutralizing and opsonizing qualities were considered major players not only in mediating neutralization of secreted virulence factors, but also in facilitating uptake and clearance of the pathogen by innate immune cells (van Kessel et al. 2014; Verbrugh et al. 1982; Leijh et al. 1981). Because most vaccines in use today are thought to work through the elicitation of protective antibody responses, it is also not surprising that most of the vaccine candidates against *S. aureus* to date were chosen and evaluated based heavily on their ability to generate opsonizing and neutralizing antibodies (Pozzi et al. 2012; Fattom et al. 1990; Ohlsen and Lorenz 2010).

The conjugate vaccine StaphVax (Nabi Biopharmaceuticals) was the first *S. aureus* vaccine candidate to enter a phase III clinical trial. It targeted clinically prevalent capsular polysaccharide (CP) serotypes 5 (CP5) and 8 (CP8), emulating the successful strategy of targeting CPs to prevent infections with *Streptococcus pneumonia* and *Haemophilus influenzae*. Preclinical studies demonstrated that CP-specific antibodies protected mice from lethal *S. aureus* challenge and bacterial dissemination (Fattom et al. 1990, 1996), and an initial phase III clinical trial in hemodialysis patients suggested modest reductions in bacteremia early after vaccination (Shinefield et al. 2002). However, a booster dose in a subsequent phase III study failed to prevent bacteremia despite augmenting antibody titers (Fattom et al. 2004; Schaffer and Lee 2008). The reasons for this failure remain incompletely understood, but the outcome highlighted that *S. aureus* virulence is not solely dependent on CP production, a fact exemplified by the lack of capsule production in some highly virulent strains such as USA300.

A more recent vaccine candidate for which preclinical promise failed to translate into clinical trial success targeted the *S. aureus* iron-binding surface determinant B (IsdB). This protein was identified as a vaccine candidate by screening patients with high antibody titers against *S. aureus* surface antigens displayed by an *E. coli* expression library (Etz et al. 2002). Immunization with this protein in preclinical and phase I clinical studies showed protection in mouse models of sepsis and antibody induction in mice, macaques, and humans (Kuklin et al. 2006; Stranger-Jones et al. 2006; Kim et al. 2010; Harro et al. 2010, 2012). However, a phase IIB/III clinical trial with the IsdB vaccine (Merck V710) in cardiothoracic surgery patients was stopped prematurely when, despite induction of IsdB antibodies, excessive deaths were noted in the vaccine group among subjects who developed postoperative *S. aureus* infections (Fowler et al. 2013). Subsequent serum cytokine analysis showed that low IL-2 and IL-17 levels post-vaccination correlated with mortality in subjects who later developed *S. aureus* infections, consistent with a potential T cell-based mechanism although immune analysis after infection to further characterize the associated immune response has not been reported (McNeely et al. 2014).

Instead of targeting cell surface antigens to promote opsonophagocytic clearance of organisms, other vaccine approaches have attempted to generate neutralizing antibodies against secreted *S. aureus* virulence factors. Active and passive immunization studies have validated this strategy in experimental models. The clearest rationale for this approach has been against toxic shock syndrome, which is driven by superantigen toxins such as staphylococcal enterotoxin A (SEA), SEB, or toxic shock syndrome toxin 1 (TSST-1). Several studies have reported that immunization of mice and rhesus macaques with recombinant superantigen toxoids devoid of their superantigenic activity induces toxin-specific antibodies and protects from lethal shock induced by the targeted wild-type toxins (Bavari et al. 1996; Lowell et al. 1996; Stiles et al. 1995; Boles et al. 2003). Furthermore, active immunization with recombinant SEA and TSST-1 toxoid vaccines, as well as adoptive transfer of immune sera, protected mice from systemic *S. aureus* infection (Hu et al. 2003; Nilsson et al. 1999).

The option of targeting other secreted toxins that contribute to *S. aureus* infection became apparent when antibodies generated against Hla_{H35L}, a non-pore-forming mutant of Hla, were shown to protect mice from lethal pneumonia (Bubeck Wardenburg and Schneewind 2008) and from skin and soft tissue infections (Kennedy et al. 2010; Mocca et al. 2014). Similarly, antibodies raised against a recombinant fusion protein (AT-62) designed to mimic key topographic features of the Hla heptamer protected mice from bacteremia and lethal pneumonia (Adhikari et al. 2012b). Antibodies raised against attenuated recombinant LukF-PV and LukS-PV, subunits of the bicomponent Panton–Valentine leukocidin (PVL), showed protective efficacy in a mouse bacteremia model and appeared to have cross-neutralizing activity toward other leukocidins in PVL-deficient strains (Karauzum et al. 2013), a potentially important characteristic given the complex redundant and antagonistic interactions between these bicomponent toxins (Yoong and Torres 2015). Considering the multiple virulence strategies employed by

S. aureus, Spaulding et al. (2014) demonstrated that vaccination with a cocktail of 7 secreted virulence factors, consisting of superantigens and cytolysins, induced antibody-mediated protection against lethal pneumonia in a rabbit model. Interestingly, in the same report, vaccination with a cocktail of surface antigens enhanced lethality in a rabbit model of infective endocarditis, an outcome suggested to be due to antibody-mediated bacterial aggregation (Spaulding et al. 2014). This highlighted the potential for deleterious antibody responses that may be elicited depending on the antigenic targets and the model of infection. Based on the role of antibody shown in such active immunization studies, the therapeutic potential of passive immunization has also been demonstrated in mouse models using mouse, human, and/or chimeric monoclonal antibodies targeting secreted or surface-bound virulence factors such as clumping factor A (ClfA) (Domanski et al. 2005), lipoteichoic acid (LTA) (Weisman et al. 2009, 2011), Hla (Ragle and Bubeck Wardenburg 2009; Tkaczyk et al. 2012), and SEB (Larkin et al. 2010; Karauzum et al. 2012; Varshney et al. 2014).

3.3 *Evasion Mechanisms from the Humoral Immune Response*

S. aureus has developed evasion mechanisms that combat the B cell antibody response. In particular, staphylococcal protein A (SpA) and the second immunoglobulin binding protein (Sbi) (Zhang et al. 1998; Smith et al. 2011) are virulence factors that bind immunoglobulins. SpA is a highly expressed, cell wall-anchored surface protein that binds to the complement-binding Fcγ portion of mammalian IgG. Decoration of the staphylococcal surface with IgG molecules bound in this reverse manner interferes with the complement activation and opsonophagocytosis. In addition, SpA in its secreted form acts as a B cell superantigen, binding the $F(ab)_2$ portion of the B cell receptor to induce B cell proliferation and death (Kobayashi and DeLeo 2013). Beyond its direct effects on opsonophagocytosis and B cell survival, SpA activity has been shown to inhibit the development of antibody responses against other staphylococcal antigens in mouse models and in humans (Kim et al. 2011; Falugi et al. 2013; Pauli et al. 2014). In contrast to SpA, baseline expression of Sbi on the cell surface is low but increases in the presence of IgG, suggesting a highly specific mechanism of immune evasion (Zhang et al. 2000). Like SpA, Sbi can act as both a cell wall-anchored or secreted virulence factor, binding the Fcγ portion of IgG on the cell surface and the soluble complement factor C3, respectively (Smith et al. 2011).

In addition to these specific mechanisms, *S. aureus* can also abandon its usual niche as an extracellular pathogen and evade the humoral immune response as a facultative intracellular organism. For example, it can resist killing and grow within neutrophils (Voyich et al. 2005), or persist in epithelial cells in the form of small colony variants (SCVs). Persistence as SCVs enables the bacterium to avoid

antimicrobial treatment, promote disease pathogenesis, and facilitate recurrent infections (Tuchscherr et al. 2011; Proctor et al. 1995; von Eiff et al. 2001b; Gresham et al. 2000). Furthermore, certain antibody responses generated against *S. aureus* can promote its virulence. For example, treatment with anti-PVL antibodies increased bacterial loads in mouse skin abscesses and inhibited in vitro killing of *S. aureus* by human neutrophils (Yoong and Pier 2010). Further highlighting the unpredictable potential for negative effects of antibody responses, the combination of two antibodies against surface polysaccharides (CP and poly-N-acetyl glucosamine) interfered with the beneficial effects of each individually on opsonophagocytic activity and protection in mouse models of bacteremia and skin infection (Skurnik et al. 2010).

In sum, antibody deficiency in mice and humans shows us that antibodies are not necessary for protection against *S. aureus* infections. However, they may very well contribute to the protective response as suggested by the modulation of antibody responses by *S. aureus* virulence factors, the ubiquitous presence of anti-staphylococcal serum antibodies, antibody-mediated protection after active and passive immunization in preclinical models, and human data correlating antibody titers with protection. Published data also support the possibility of ineffective or deleterious antibody responses, emphasizing the need to better understand the characteristics of a protective antibody response in order to elucidate contributions to natural immunity and implications for vaccine design.

4 Role of T Cells

T cells are thymic-derived cells that express unique T cell receptors (TCRs) that recognize antigen-derived peptides in the context of major histocompatibility complex (MHC) molecules on APCs. Similar to B cells and antibodies, a case can be made for a role for T cells during *S. aureus* infection based on the presence of detectable T cell responses in humans (Zielinski et al. 2012; Kolata et al. 2015) and the ability of the bug to modulate T cells as exemplified by its expression of a multitude of T cell superantigens (Spaulding et al. 2013). However, it has been reported that T cells are not essential for protection against *S. aureus* in mice (Schmaler et al. 2011). Furthermore, *S. aureus* shows up only occasionally as a cause of infection in evaluations of humans with T cell deficiencies (Stephan et al. 1993), although the severe susceptibility of these patients to other organisms confounds our ability to fully assess the contribution of T cells to staphylococcal immunity in this context. Various subsets of T cells have differing functions, and a more nuanced role for these subsets has become evident in mouse studies and with the recognized susceptibility to staphylococcal infections of patients with HIV and other partial T cell disorders (Hidron et al. 2010; Cook and Tangye 2009). These will be discussed in further detail below.

The majority of T cells are comprised of CD4+ and CD8+ T cells that have long been recognized to be the major cellular arm of adaptive immunity. The major

function of CD8+ T cells is to target intracellular pathogens by cytolytic killing of the infected host cell. Consistent with *S. aureus* being a primarily extracellular pathogen, a clear role for CD8+ T cells has not been reported, although CD8+ T cell activation can be detected during *S. aureus* infection and staphylococcal superantigen exposure. Naïve CD4+ T cells are polarized toward different effector functions depending on the cytokine milieu in which activation of their TCR occurs. These helper T cell (Th) subsets are functionally characterized by their cytokine expression profiles, which will be detailed below. A percentage of these polarized cells will persist in the host as memory cells awaiting re-activation by subsequent antigen exposure. The role of these different subsets of effector CD4+ T cells in the context of *S. aureus* infection will be reviewed below. In addition to CD4+ and CD8+ T cells, more recently described subsets of T cells, such as γδ T cells, innate lymphoid cells (ILC), and NK T cells, contribute mainly to the innate immune response at mucosal sites rather than antigen-specific memory, although recent reports have suggested the potential for γδ T cells to contribute to a memory response under certain circumstances (Murphy et al. 2014).

4.1 Th1 Cells

TCR-mediated activation of naïve CD4+ T cells in the presence of IL-12 signaling via STAT4 leads to the generation of Th1 effector cells. Although capable of producing multiple inflammatory cytokines, including IL-2, TNFα, and GM-CSF, Th1 cells are defined by the secretion of their signature cytokine interferon (IFN)-γ and expression of the transcriptional regulator T-bet (O'Shea and Paul 2010; Schmitt and Ueno 2015; Raphael et al. 2014). Th1 cells are not the only source of IFNγ, with various innate immune cells, including NK cells and ILC being notable producers. Among its functions, IFNγ activates phagocytic cells such as macrophages and neutrophils to promote killing of intracellular pathogens. Its role in protection against these organisms is highlighted by the susceptibility of patients with hereditary defects in IFNγ signaling to infections with *Mycobacteria*, *Salmonella*, and certain viruses (Rosenzweig and Holland 2005). Unregulated IFNγ production can contribute to immunopathology and autoimmunity (Feldmann et al. 1998).

In the context of *S. aureus* infections, it appears Th1 cells and IFNγ can have both beneficial and detrimental roles. Guillen et al. reported a protective role of an enhanced Th1 response in a mouse model of septicemia and septic arthritis in mice transgenic for lactoferrin. The enhanced production of IFNγ and TNFα in these mice during infection resulted in higher bacterial clearance and lower mortality compared to their wild-type littermates (Guillen et al. 2002). An overproduction of this cytokine, however, can be associated with immunopathology. An early study evaluating the role of T cells in *S. aureus*-induced arthritis indicated that Th1 cells, stained positive for the IL2R and intracellular IFNγ, infiltrated the synovium of joints of infected mice, and depletion of CD4+ but not CD8+ T cells in the infected

animals ameliorated disease (Abdelnour et al. 1994). However, intravenous inoculation of mice deficient in T-bet, which may have deficiencies beyond a defect in Th1 cell IFNγ production (Lazarevic et al. 2013), had increased severity of septic arthritis that was associated with increased weight loss, mortality, and kidney bacterial burden (Hultgren et al. 2004). Consistent with the potential duality of roles for Th1 cells during *S. aureus* infection, Th1 cells and IFNγ production were reported to promote chemokine-mediated neutrophil recruitment in a wound infection model, but this resulted in a paradoxical increase of bacterial burden, potentially due to the ability of *S. aureus* to persist in neutrophils (McLoughlin et al. 2006, 2008).

Th1 cells appear to be able to contribute to vaccination-induced protection against subsequent *S. aureus* infection. CD4+ T cell IFNγ production was required for the protection against subsequent systemic infection after vaccination with a recombinant protein derived from Als3p, a *Candida* protein that cross-protected against *S. aureus* (Lin et al. 2009). Similarly, vaccination with extracellular vesicles released from *S. aureus* induced a Th1 response, and protection in a pneumonia model was dependent on CD4+ T cells and IFNγ (Choi et al. 2015). However, protection after vaccination against cutaneous infection with a lethally irradiated whole-cell vaccine was not associated with an IFNγ response (Gaidamakova et al. 2012), and increased mortality in similarly vaccinated mice after intravenous challenge was dependent on CD4+ T cell IFNγ production (Karauzum and Datta, unpublished data). Another study also hinted at potential detrimental effects of vaccine-induced Th1 responses by showing that mice vaccinated with heat-killed *S. aureus* had significant disease burden after intravenous infection despite detectable CD4+ T cell IFNγ production (Schmaler et al. 2011); however, lack of direct comparison to an unvaccinated control group prevents conclusive interpretation of these results.

In sum, it appears Th1 cells can have protective, detrimental, or non-contributory roles against *S. aureus* infection, likely dependent on factors such as route of infection, organism burden, antigenic targets, level of induction, and balance with other immune mechanisms. Clarification of the conditions under which Th1 cells exert these apparently contradictory effects will better guide approaches to interventions aimed at therapy and prevention.

4.2 Th2 Cells

Activation of naïve CD4 T cells in the presence of IL-4 via STAT6 signaling leads to the priming of Th2 cells. This subset of CD4 T cells is characterized by its signature transcription factor GATA-3, which promotes induction of Th2 cytokines that include IL-4, IL-5, and IL-13. Th2 cells play an important role in host defense against extracellular parasites, driving various aspects of cellular and humoral immunity to promote parasite clearance and tissue repair (Allen and Sutherland 2014). Their dysregulation contributes to allergic and atopic diseases (Raphael et al. 2014; Geginat et al. 2013). Of particular relevance to staphylococcal disease is

atopic dermatitis (AD), a prevalent inflammatory skin disorder that is characterized by the overexpression of Th2 cytokines (Hamid et al. 1994), which contribute to barrier permeability issues and other features of AD. Skin colonization and infection with *S. aureus* is almost a universal feature of AD (Boguniewicz and Leung 2011). The propensity of Th2 cytokines to inhibit antimicrobial gene programs, including induction and mobilization of antimicrobial peptides such as human beta-defensin (HBD)-3, are thought to contribute to this susceptibility (Kisich et al. 2008; Nomura et al. 2003; Howell et al. 2006). Th2 cytokines may not only drive aspects of AD and staphylococcal susceptibility, but *S. aureus* colonization may further promote this Th2-driven milieu. Staphylococcal cell wall components, such as peptidoglycan (Matsui and Nishikawa 2012) and lipoteichoic acid (Matsui and Nishikawa 2002), were shown to induce Th2 cells and may contribute along with other secreted toxins toward the inflammatory environment in the skin of AD patients (Schlievert et al. 2010; Nakamura et al. 2013; Brauweiler et al. 2014).

The role of Th2 responses during *S. aureus* infection outside the setting of AD is less clear. *S. aureus* footpad infection was less severe in the Th2-biased DBA and BALB/c mouse strains than in Th1-biased C57BL/6 mice (Nippe et al. 2011). A protective role for Th2 cells was also suggested in an ocular keratitis model where unexpectedly high Th2 responses in C57BL/6 mice correlated with the protection compared to less robust responses seen in more susceptible BALB/c mice (Hume et al. 2005). However, these correlative observations do not definitively address whether the Th2 response is driving resistance to infection or whether other immunological parameters are responsible. A recent study in a model of persistent biofilm infection did show STAT6-dependent clearance in BALB/c mice that suggested a contribution of Th2 responses to protection (Prabhakara et al. 2011). In the same study, neutrophilic inflammatory responses worsened infection and this effect could be reversed by neutralization of IL-12p40 or IL-6, treatments that would be predicted to dampen Th1 and Th17 responses, respectively, and skew toward Th2 responses (Prabhakara et al. 2011).

The complexity of Th2 responses and their downstream effects may potentially trigger both beneficial and detrimental responses. It seems clear that Th2 responses contribute to a vicious cycle of inflammation and *S. aureus* susceptibility at the skin in the context of AD. However, Th2 effects may play a role in achieving the appropriate balance between inflammatory and anti-inflammatory responses in other situations, particularly during chronic infection.

4.3 Th17 Cells

Th17 cells are a relatively recently recognized subset of effector CD4+ T cells. They are defined by their expression of Rorγt and secretion of inflammatory cytokines, including IL-17A, IL-17F, and IL-22 (Liang et al. 2006; Chung et al. 2006; Ivanov et al. 2006). These cytokines predominantly act on epithelial cells to enhance barrier function, antimicrobial properties, and neutrophil recruitment

(Ouyang et al. 2008). Initially discovered in the context of autoimmunity, they have been shown to play a protective role in mouse models of extracellular bacterial and fungal infections, especially at mucosal sites (Ouyang et al. 2008).

A protective role for Th17 cells against *S. aureus* was first suggested by a report that mice deficient in both IL-17A and IL-17F spontaneously developed mucocutaneous *S. aureus* infections (Ishigame et al. 2009). Subsequent work clarified that induction of IL-17A from γδ T cells in the skin played a critical role in controlling *S. aureus* burden and abscess size after subcutaneous inoculation (Cho et al. 2010). In this context, γδ T cells functioned in their traditional role as a cytokine-activated component of innate immunity, implicating an important role for innate immune cell-derived IL-17A. Hla-dependent Th17 induction (Frank et al. 2012) and influenza-mediated antagonism of Th17-dependent protection (Kudva et al. 2011) indicated a role for Th17 cells in a model of *S. aureus* pneumonia. Interestingly, CD4+ T cell depletion in the context of this Th17-inducing pneumonia model improved outcomes in another study, hinting at a delicate balance within the CD4+ T cell compartment between protective immunity and immunopathology (Parker et al. 2015). Deficiency in IL-17A also enhanced susceptibility to *S. aureus* joint infection, although the relevant cellular source was not identified (Henningsson et al. 2010). Deficiency in IL-17A did not increase susceptibility to systemic challenge with *S. aureus* in multiple studies (Ishigame et al. 2009; Lin et al. 2009; Narita et al. 2010; Henningsson et al. 2010), consistent with a primary role for this cytokine at skin and mucosal sites. However, a protective function for IL-17A from vaccination-induced Th17 cells has been shown against skin (Gaidamakova et al. 2012) and systemic *S. aureus* infections (Lin et al. 2009; Narita et al. 2010; Joshi et al. 2012). Consistent with this, antibody-dependent protection against multiple models of infection by a four-component vaccine was further enhanced by Th1 and Th17 cell induction with inclusion of a TLR7 agonist adjuvant (Bagnoli et al. 2015). The Merck IsdB vaccine also showed contribution of IL-17A, but not IL-22 or IFNγ, to protection in a mouse model of sepsis (Joshi et al. 2012), and, as mentioned previously, low IL-2 and IL-17 levels post-vaccination correlated with mortality in *S. aureus*-infected human subjects (McNeely et al. 2014). Of note, the Th17-associated cytokine IL-22 seems to have either no or minimal effects on the course of acute cutaneous infection in mice (Myles et al. 2013; Chan et al. 2015), but can independently contribute to protection against pneumonia (Kudva et al. 2011) and vaccine-induced protection against skin and systemic infection (Yeaman et al. 2014).

Autoantibody- and genetically mediated dysfunction of the IL-17 pathway predisposes to mucocutaneous *Candida* infections (Burbelo et al. 2010; Kisand et al. 2010; Puel et al. 2010, 2011). Only a striking minority of these patients reported *S. aureus* infections, making it unclear whether IL-17 is a critical element for human anti-staphylococcal responses. However, patients with HyperIgE (or Job's) Syndrome, who are susceptible to staphylococcal skin and lung infections, lack normal Th17 generation due to STAT3 dysfunction (Minegishi et al. 2007; Holland et al. 2007; Milner et al. 2008). The role for Th17 cytokines in promoting keratinocyte and epithelial antimicrobial function (Minegishi et al. 2009) is also consistent with an IL-17-dependent basis for their susceptibility specifically to skin and lung infections,

although other functions of STAT3, including its direct role in antimicrobial peptide production (Choi et al. 2013), very likely contribute. Consistent with a potential contribution of Th17 cells to human immunity is the Th17 depletion seen in HIV-infected patients early in the course of disease that correlates with their increased likelihood of *S. aureus* skin and soft tissue infections (Hidron et al. 2010; Prendergast et al. 2010). Patients with AD also have decreased IL-17 pathway cytokines and decreased antimicrobial peptides in lesional skin, potentially contributing to their staphylococcal susceptibility (Guttman-Yassky et al. 2008). The relative induction of these pathways in psoriasis has been postulated to contribute to the relative resistance of these patients to *S. aureus* (Guttman-Yassky et al. 2008).

In sum, IL-17 from either innate or adaptive sources plays an important role against *S. aureus* in mouse models of infection at skin and mucosal sites. Induction of Th17 cells by vaccination can enhance protection at these sites and also against bacteremia. Th17 cells appear to be potential key players in immunity against *S. aureus*; however, their exact contribution to the control of human staphylococcal infection remains to be fully elucidated and their potential for autoimmune inflammation will need to be kept in check if they are to be targeted by clinical vaccines.

4.4 Regulatory T Cells

Regulatory T cells (T_{reg}) display contact-dependent and cytokine-mediated immunosuppressive functions that counteract inflammatory responses and maintain immune homeostasis. *S. aureus* may exploit these immunosuppressive functions by inducing T_{reg} responses that contribute, along with other immunosuppressive mechanisms, to diminished effector T cell responses during models of persistent infection (Ziegler et al. 2011; Tebartz et al. 2015). Increased T_{reg} numbers may also contribute to the immune dysregulation and *S. aureus* susceptibility seen in the skin of patients with AD (Ou et al. 2004). However, depletion of T_{reg} exacerbated a model of chronic biofilm infection, suggesting that an appropriate balance between inflammatory and anti-inflammatory responses is needed for optimal bacterial control (Prabhakara et al. 2011). Further studies will be needed to increase our nascent understanding of the role of T_{reg} in modulating the response to *S. aureus* infection and how this may influence susceptibility.

5 Conclusion

Immune control of acute *S. aureus* infection is critically dependent on the innate immune system. However, adaptive immunity in the form of B cell and T cell responses may influence this control and is potentially of particular importance in determining the outcomes of chronic persistent infections. The search for a protective vaccine will depend on our ability to induce an effective adaptive immune

response. Recent studies suggest that induction of an antibody response alone may not be sufficient, and an appropriate vaccine-induced T cell response will be needed to confer protective immunity. The potential for eliciting deleterious adaptive immune responses has become apparent in both animal models and clinical vaccination trials. This highlights the need for further elucidation of the components of an effective immune response, a task complicated by the multiple virulence strategies and sites of infection employed by this bug that will each likely require targeting by unique strategies for effective prevention and therapy.

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References

Abdelnour A, Bremell T, Holmdahl R, Tarkowski A (1994) Role of T lymphocytes in experimental *Staphylococcus aureus* arthritis. Scand J Immunol 39(4):403–408

Adhikari RP, Ajao AO, Aman MJ, Karauzum H, Sarwar J, Lydecker AD, Johnson JK, Nguyen C, Chen WH, Roghmann MC (2012a) Lower antibody levels to *Staphylococcus aureus* exotoxins are associated with sepsis in hospitalized adults with invasive S. aureus infections. J Infect Dis 206(6):915–923. doi:10.1093/infdis/jis462

Adhikari RP, Karauzum H, Sarwar J, Abaandou L, Mahmoudieh M, Boroun AR, Vu H, Nguyen T, Devi VS, Shulenin S, Warfield KL, Aman MJ (2012b) Novel structurally designed vaccine for S. aureus alpha-hemolysin: protection against bacteremia and pneumonia. PLoS One 7(6):e38567. doi:10.1371/journal.pone.0038567

Allen JE, Sutherland TE (2014) Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin. Semin Immunol 26(4):329–340. doi:10.1016/j.smim.2014.06.003

Bagnoli F, Fontana MR, Soldaini E, Mishra RP, Fiaschi L, Cartocci E, Nardi-Dei V, Ruggiero P, Nosari S, De Falco MG, Lofano G, Marchi S, Galletti B, Mariotti P, Bacconi M, Torre A, Maccari S, Scarselli M, Rinaudo CD, Inoshima N, Savino S, Mori E, Rossi-Paccani S, Baudner B, Pallaoro M, Swennen E, Petracca R, Brettoni C, Liberatori S, Norais N, Monaci E, Bubeck Wardenburg J, Schneewind O, O'Hagan DT, Valiante NM, Bensi G, Bertholet S, De Gregorio E, Rappuoli R, Grandi G (2015) Vaccine composition formulated with a novel TLR7-dependent adjuvant induces high and broad protection against *Staphylococcus aureus*. Proc Natl Acad Sci USA 112(12):3680–3685. doi:10.1073/pnas.1424924112

Bavari S, Dyas B, Ulrich RG (1996) Superantigen vaccines: a comparative study of genetically attenuated receptor-binding mutants of staphylococcal enterotoxin A. J Infect Dis 174(2):338–345

Boguniewicz M, Leung DY (2011) Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev 242(1):233–246. doi:10.1111/j.1600-065X.2011.01027.x

Boles JW, Pitt ML, LeClaire RD, Gibbs PH, Torres E, Dyas B, Ulrich RG, Bavari S (2003) Generation of protective immunity by inactivated recombinant staphylococcal enterotoxin B vaccine in nonhuman primates and identification of correlates of immunity. Clin Immunol 108(1):51–59

Brauweiler AM, Goleva E, Leung DY (2014) Th2 cytokines increase *Staphylococcus aureus* alpha toxin-induced keratinocyte death through the signal transducer and activator of transcription 6 (STAT6). J Invest Dermatol 134(8):2114–2121. doi:10.1038/jid.2014.43

Bruton OC (1952) Agammaglobulinemia. Pediatrics 9(6):722–728

Bubeck Wardenburg J, Schneewind O (2008) Vaccine protection against *Staphylococcus aureus* pneumonia. J Exp Med 205(2):287–294. doi:10.1084/jem.20072208

Burbelo PD, Browne SK, Sampaio EP, Giaccone G, Zaman R, Kristosturyan E, Rajan A, Ding L, Ching KH, Berman A, Oliveira JB, Hsu AP, Klimavicz CM, Iadarola MJ, Holland SM (2010) Anti-cytokine autoantibodies are associated with opportunistic infection in patients with thymic neoplasia. Blood 116(23):4848–4858. doi:10.1182/blood-2010-05-286161

Chambers HF, Deleo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7(9):629–641. doi:10.1038/nrmicro2200

Chan LC, Chaili S, Filler SG, Barr K, Wang H, Kupferwasser D, Edwards JE Jr, Xiong YQ, Ibrahim AS, Miller LS, Schmidt CS, Hennessey JP Jr, Yeaman MR (2015) Non-redundant roles of IL-17A and IL-22 in murine host defense against cutaneous and hematogenous infection due to methicillin-resistant *Staphylococcus aureus*. Infect Immun. doi:10.1128/IAI.01061-15

Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, Magorien JE, Blauvelt A, Kolls JK, Cheung AL, Cheng G, Modlin RL, Miller LS (2010) IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. J Clin Invest 120(5):1762–1773. doi:10.1172/JCI40891

Choi SM, McAleer JP, Zheng M, Pociask DA, Kaplan MH, Qin S, Reinhart TA, Kolls JK (2013) Innate Stat3-mediated induction of the antimicrobial protein Reg3gamma is required for host defense against MRSA pneumonia. J Exp Med 210(3):551–561. doi:10.1084/jem.20120260

Choi SJ, Kim MH, Jeon J, Kim OY, Choi Y, Seo J, Hong SW, Lee WH, Jeon SG, Gho YS, Jee YK, Kim YK (2015) Active immunization with extracellular vesicles derived from *Staphylococcus aureus* effectively protects against staphylococcal lung infections, mainly via Th1 cell-mediated immunity. PLoS ONE 10(9):e0136021. doi:10.1371/journal.pone.0136021

Chung Y, Yang X, Chang SH, Ma L, Tian Q, Dong C (2006) Expression and regulation of IL-22 in the IL-17-producing CD4+ T lymphocytes. Cell Res 16(11):902–907. doi:10.1038/sj.cr.7310106

Conley ME, Howard VC (1993) X-linked agammaglobulinemia. In: Pagon RA, Adam MP, Ardinger HH et al (eds) GeneReviews. University of Washington, Seattle

Cook MC, Tangye SG (2009) Primary immune deficiencies affecting lymphocyte differentiation: lessons from the spectrum of resulting infections. Int Immunol 21(9):1003–1011. doi:10.1093/intimm/dxp076

Domanski PJ, Patel PR, Bayer AS, Zhang L, Hall AE, Syribeys PJ, Gorovits EL, Bryant D, Vernachio JH, Hutchins JT, Patti JM (2005) Characterization of a humanized monoclonal antibody recognizing clumping factor A expressed by *Staphylococcus aureus*. Infect Immun 73(8):5229–5232. doi:10.1128/IAI.73.8.5229-5232.2005

Etz H, Minh DB, Henics T, Dryla A, Winkler B, Triska C, Boyd AP, Sollner J, Schmidt W, von Ahsen U, Buschle M, Gill SR, Kolonay J, Khalak H, Fraser CM, von Gabain A, Nagy E, Meinke A (2002) Identification of in vivo expressed vaccine candidate antigens from *Staphylococcus aureus*. Proc Natl Acad Sci USA 99(10):6573–6578. doi:10.1073/pnas.092569199

Falugi F, Kim HK, Missiakas DM, Schneewind O (2013) Role of protein A in the evasion of host adaptive immune responses by *Staphylococcus aureus*. MBio 4(5):e00575–e00513. doi:10.1128/mBio.00575-13

Fattom A, Schneerson R, Szu SC, Vann WF, Shiloach J, Karakawa WW, Robbins JB (1990) Synthesis and immunologic properties in mice of vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides conjugated to Pseudomonas aeruginosa exotoxin A. Infect Immun 58(7):2367–2374

Fattom AI, Sarwar J, Ortiz A, Naso R (1996) A *Staphylococcus aureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. Infect Immun 64(5):1659–1665

Fattom A, Fuller S, Propst M, Winston S, Muenz L, He D, Naso R, Horwith G (2004) Safety and immunogenicity of a booster dose of *Staphylococcus aureus* types 5 and 8 capsular

polysaccharide conjugate vaccine (StaphVAX) in hemodialysis patients. Vaccine 23(5):656–663. doi:10.1016/j.vaccine.2004.06.043

Feldmann M, Brennan FM, Maini R (1998) Cytokines in autoimmune disorders. Int Rev Immunol 17(1–4):217–228

Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, Corey GR, Carmeli Y, Betts R, Hartzel JS, Chan IS, McNeely TB, Kartsonis NA, Guris D, Onorato MT, Smugar SS, DiNubile MJ, Sobanjo-ter Meulen A (2013) Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. JAMA 309 (13):1368–1378. doi:10.1001/jama.2013.3010

Frank KM, Zhou T, Moreno-Vinasco L, Hollett B, Garcia JG, Bubeck Wardenburg J (2012) Host response signature to *Staphylococcus aureus* alpha-hemolysin implicates pulmonary Th17 response. Infect Immun 80(9):3161–3169. doi:10.1128/IAI.00191-12

Fritz SA, Tiemann KM, Hogan PG, Epplin EK, Rodriguez M, Al-Zubeidi DN, Bubeck Wardenburg J, Hunstad DA (2013) A serologic correlate of protective immunity against community-onset *Staphylococcus aureus* infection. Clin Infect Dis 56(11):1554–1561. doi:10.1093/cid/cit123

Gaidamakova EK, Myles IA, McDaniel DP, Fowler CJ, Valdez PA, Naik S, Gayen M, Gupta P, Sharma A, Glass PJ, Maheshwari RK, Datta SK, Daly MJ (2012) Preserving immunogenicity of lethally irradiated viral and bacterial vaccine epitopes using a radio-protective Mn2+ - Peptide complex from Deinococcus. Cell Host Microbe 12(1):117–124. doi:10.1016/j.chom.2012.05.011

Geginat J, Paroni M, Facciotti F, Gruarin P, Kastirr I, Caprioli F, Pagani M, Abrignani S (2013) The CD4-centered universe of human T cell subsets. Semin Immunol 25(4):252–262. doi:10.1016/j.smim.2013.10.012

Gjertsson I, Hultgren OH, Stenson M, Holmdahl R, Tarkowski A (2000) Are B lymphocytes of importance in severe *Staphylococcus aureus* infections? Infect Immun 68(5):2431–2434

Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP (2000) Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. J Immunol 164(7):3713–3722

Guillen C, McInnes IB, Vaughan DM, Kommajosyula S, Van Berkel PH, Leung BP, Aguila A, Brock JH (2002) Enhanced Th1 response to *Staphylococcus aureus* infection in human lactoferrin-transgenic mice. J Immunol 168(8):3950–3957

Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nograles KE, Khatcherian A, Novitskaya I, Carucci JA, Bergman R, Krueger JG (2008) Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J Immunol 181(10):7420–7427

Hamid Q, Boguniewicz M, Leung DY (1994) Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. J Clin Invest 94(2):870–876. doi:10.1172/JCI117408

Harro C, Betts R, Orenstein W, Kwak EJ, Greenberg HE, Onorato MT, Hartzel J, Lipka J, DiNubile MJ, Kartsonis N (2010) Safety and immunogenicity of a novel *Staphylococcus aureus* vaccine: results from the first study of the vaccine dose range in humans. Clin Vaccine Immunol 17(12):1868–1874. doi:10.1128/CVI.00356-10

Harro CD, Betts RF, Hartzel JS, Onorato MT, Lipka J, Smugar SS, Kartsonis NA (2012) The immunogenicity and safety of different formulations of a novel *Staphylococcus aureus* vaccine (V710): results of two Phase I studies. Vaccine 30(9):1729–1736. doi:10.1016/j.vaccine.2011.12.045

Henningsson L, Jirholt P, Lindholm C, Eneljung T, Silverpil E, Iwakura Y, Linden A, Gjertsson I (2010) Interleukin-17A during local and systemic *Staphylococcus aureus*-induced arthritis in mice. Infect Immun 78(9):3783–3790. doi:10.1128/IAI.00385-10

Hidron AI, Kempker R, Moanna A, Rimland D (2010) Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. Infect Drug Resist 3:73–86

Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C,

Schaffer AA, Puck JM, Grimbacher B (2007) STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 357(16):1608–1619. doi:10.1056/NEJMoa073687

Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, Leung DY (2006) Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. Immunity 24(3):341–348. doi:10.1016/j.immuni.2006.02.006

Hu DL, Omoe K, Sasaki S, Sashinami H, Sakuraba H, Yokomizo Y, Shinagawa K, Nakane A (2003) Vaccination with nontoxic mutant toxic shock syndrome toxin 1 protects against *Staphylococcus aureus* infection. J Infect Dis 188(5):743–752. doi:10.1086/377308

Hultgren OH, Verdrengh M, Tarkowski A (2004) T-box transcription-factor-deficient mice display increased joint pathology and failure of infection control during staphylococcal arthritis. Microbes Infect 6(6):529–535. doi:10.1016/j.micinf.2004.02.005

Hume EB, Cole N, Khan S, Garthwaite LL, Aliwarga Y, Schubert TL, Willcox MD (2005) A *Staphylococcus aureus* mouse keratitis topical infection model: cytokine balance in different strains of mice. Immunol Cell Biol 83(3):294–300. doi:10.1111/j.1440-1711.2005.01326.x

Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, Fujikado N, Tanahashi Y, Akitsu A, Kotaki H, Sudo K, Nakae S, Sasakawa C, Iwakura Y (2009) Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 30(1):108–119. doi:10.1016/j.immuni.2008.11.009

Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR (2006) The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126(6):1121–1133. doi:10.1016/j.cell.2006.07.035

Joshi A, Pancari G, Cope L, Bowman EP, Cua D, Proctor RA, McNeely T (2012) Immunization with *Staphylococcus aureus* iron regulated surface determinant B (IsdB) confers protection via Th17/IL17 pathway in a murine sepsis model. Hum Vaccine Immunother 8(3):336–346. doi:10.4161/hv.18946

Karauzum H, Chen G, Abaandou L, Mahmoudieh M, Boroun AR, Shulenin S, Devi VS, Stavale E, Warfield KL, Zeitlin L, Roy CJ, Sidhu SS, Aman MJ (2012) Synthetic human monoclonal antibodies toward staphylococcal enterotoxin B (SEB) protective against toxic shock syndrome. J Biol Chem 287(30):25203–25215. doi:10.1074/jbc.M112.364075

Karauzum H, Adhikari RP, Sarwar J, Devi VS, Abaandou L, Haudenschild C, Mahmoudieh M, Boroun AR, Vu H, Nguyen T, Warfield KL, Shulenin S, Aman MJ (2013) Structurally designed attenuated subunit vaccines for S. aureus LukS-PV and LukF-PV confer protection in a mouse bacteremia model. PLoS ONE 8(6):e65384. doi:10.1371/journal.pone.0065384

Kennedy AD, Bubeck Wardenburg J, Gardner DJ, Long D, Whitney AR, Braughton KR, Schneewind O, DeLeo FR (2010) Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 202(7):1050–1058. doi:10.1086/656043

Kim HK, DeDent A, Cheng AG, McAdow M, Bagnoli F, Missiakas DM, Schneewind O (2010) IsdA and IsdB antibodies protect mice against *Staphylococcus aureus* abscess formation and lethal challenge. Vaccine 28(38):6382–6392. doi:10.1016/j.vaccine.2010.02.097

Kim HK, Kim HY, Schneewind O, Missiakas D (2011) Identifying protective antigens of *Staphylococcus aureus*, a pathogen that suppresses host immune responses. FASEB J 25(10):3605–3612. doi:10.1096/fj.11-187963

Kisand K, Boe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV, Ersvaer E, Perheentupa J, Erichsen MM, Bratanic N, Meloni A, Cetani F, Perniola R, Ergun-Longmire B, Maclaren N, Krohn KJ, Pura M, Schalke B, Strobel P, Leite MI, Battelino T, Husebye ES, Peterson P, Willcox N, Meager A (2010) Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 207(2):299–308. doi:10.1084/jem.20091669

Kisich KO, Carspecken CW, Fieve S, Boguniewicz M, Leung DY (2008) Defective killing of *Staphylococcus aureus* in atopic dermatitis is associated with reduced mobilization of human beta-defensin-3. J Allergy Clin Immunol 122(1):62–68. doi:10.1016/j.jaci.2008.04.022

Kobayashi SD, DeLeo FR (2013) *Staphylococcus aureus* protein A promotes immune suppression. MBio 4(5):e00764–e00713. doi:10.1128/mBio.00764-13

Kolata JB, Kuhbandner I, Link C, Normann N, Vu CH, Steil L, Weidenmaier C, Broker BM (2015) The fall of a dogma? Unexpected high T-cell memory response to *Staphylococcus aureus* in humans. J Infect Dis 212(5):830–838. doi:10.1093/infdis/jiv128

Kudva A, Scheller EV, Robinson KM, Crowe CR, Choi SM, Slight SR, Khader SA, Dubin PJ, Enelow RI, Kolls JK, Alcorn JF (2011) Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. J Immunol 186(3):1666–1674. doi:10.4049/jimmunol.1002194

Kuklin NA, Clark DJ, Secore S, Cook J, Cope LD, McNeely T, Noble L, Brown MJ, Zorman JK, Wang XM, Pancari G, Fan H, Isett K, Burgess B, Bryan J, Brownlow M, George H, Meinz M, Liddell ME, Kelly R, Schultz L, Montgomery D, Onishi J, Losada M, Martin M, Ebert T, Tan CY, Schofield TL, Nagy E, Meineke A, Joyce JG, Kurtz MB, Caulfield MJ, Jansen KU, McClements W, Anderson AS (2006) A novel *Staphylococcus aureus* vaccine: iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine S. aureus sepsis model. Infect Immun 74(4):2215–2223. doi:10.1128/IAI.74.4.2215-2223.2006

Larkin EA, Stiles BG, Ulrich RG (2010) Inhibition of toxic shock by human monoclonal antibodies against staphylococcal enterotoxin B. PLoS ONE 5(10):e13253. doi:10.1371/journal.pone.0013253

Lazarevic V, Glimcher LH, Lord GM (2013) T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol 13(11):777–789. doi:10.1038/nri3536

Leijh PC, van den Barselaar MT, Daha MR, van Furth R (1981) Participation of immunoglobulins and complement components in the intracellular killing of *Staphylococcus aureus* and Escherichia coli by human granulocytes. Infect Immun 33(3):714–724

Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 203(10):2271–2279. doi:10.1084/jem.20061308

Lin L, Ibrahim AS, Xu X, Farber JM, Avanesian V, Baquir B, Fu Y, French SW, Edwards JE Jr, Spellberg B (2009) Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and Candida albicans infection in mice. PLoS Pathog 5(12):e1000703. doi:10.1371/journal.ppat.1000703

Lowell GH, Colleton C, Frost D, Kaminski RW, Hughes M, Hatch J, Hooper C, Estep J, Pitt L, Topper M, Hunt RE, Baker W, Baze WB (1996) Immunogenicity and efficacy against lethal aerosol staphylococcal enterotoxin B challenge in monkeys by intramuscular and respiratory delivery of proteosome-toxoid vaccines. Infect Immun 64(11):4686–4693

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339(8):520–532. doi:10.1056/NEJM199808203390806

Matsui K, Nishikawa A (2002) Lipoteichoic acid from *Staphylococcus aureus* induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. Clin Exp Allergy 32(5):783–788

Matsui K, Nishikawa A (2012) Peptidoglycan from *Staphylococcus aureus* induces T(H)2 immune response in mice. J Investig Allergol Clin Immunol 22(2):80–86

McLoughlin RM, Solinga RM, Rich J, Zaleski KJ, Cocchiaro JL, Risley A, Tzianabos AO, Lee JC (2006) CD4+ T cells and CXC chemokines modulate the pathogenesis of *Staphylococcus aureus* wound infections. Proc Natl Acad Sci USA 103(27):10408–10413. doi:10.1073/pnas.0508961103

McLoughlin RM, Lee JC, Kasper DL, Tzianabos AO (2008) IFN-gamma regulated chemokine production determines the outcome of *Staphylococcus aureus* infection. J Immunol 181(2):1323–1332

McNeely TB, Shah NA, Fridman A, Joshi A, Hartzel JS, Keshari RS, Lupu F, DiNubile MJ (2014) Mortality among recipients of the Merck V710 *Staphylococcus aureus* vaccine after postoperative S. aureus infections: an analysis of possible contributing host factors. Hum Vaccine Immunother 10(12):3513–3516. doi:10.4161/hv.34407

Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, Davis J, Hsu A, Asher AI, O'Shea J, Holland SM, Paul WE, Douek DC (2008) Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452(7188):773–776. doi:10.1038/nature06764

Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, Pasic S, Stojkovic O, Metin A, Karasuyama H (2007) Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 448(7157):1058–1062. doi:10.1038/nature06096

Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, Karasuyama H (2009) Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. J Exp Med 206(6):1291–1301. doi:10.1084/jem.20082767

Mocca CP, Brady RA, Burns DL (2014) Role of antibodies in protection elicited by active vaccination with genetically inactivated alpha hemolysin in a mouse model of *Staphylococcus aureus* skin and soft tissue infections. Clin Vaccine Immunol 21(5):622–627. doi:10.1128/CVI.00051-14

Montgomery CP, Daniels M, Zhao F, Alegre ML, Chong AS, Daum RS (2014) Protective immunity against recurrent *Staphylococcus aureus* skin infection requires antibody and interleukin-17A. Infect Immun 82(5):2125–2134. doi:10.1128/IAI.01491-14

Murphy AG, O'Keeffe KM, Lalor SJ, Maher BM, Mills KH, McLoughlin RM (2014) *Staphylococcus aureus* infection of mice expands a population of memory gammadelta T cells that are protective against subsequent infection. J Immunol 192(8):3697–3708. doi:10.4049/jimmunol.1303420

Myles IA, Fontecilla NM, Valdez PA, Vithayathil PJ, Naik S, Belkaid Y, Ouyang W, Datta SK (2013) Signaling via the IL-20 receptor inhibits cutaneous production of IL-1beta and IL-17A to promote infection with methicillin-resistant *Staphylococcus aureus*. Nat Immunol 14(8):804–811. doi:10.1038/ni.2637

Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Munoz-Planillo R, Hasegawa M, Villaruz AE, Cheung GY, McGavin MJ, Travers JB, Otto M, Inohara N, Nunez G (2013) Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. Nature 503(7476):397–401. doi:10.1038/nature12655

Narita K, Hu DL, Mori F, Wakabayashi K, Iwakura Y, Nakane A (2010) Role of interleukin-17A in cell-mediated protection against *Staphylococcus aureus* infection in mice immunized with the fibrinogen-binding domain of clumping factor A. Infect Immun 78(10):4234–4242. doi:10.1128/IAI.00447-10

Nilsson IM, Verdrengh M, Ulrich RG, Bavari S, Tarkowski A (1999) Protection against *Staphylococcus aureus* sepsis by vaccination with recombinant staphylococcal enterotoxin A devoid of superantigenicity. J Infect Dis 180(4):1370–1373. doi:10.1086/315023

Nippe N, Varga G, Holzinger D, Loffler B, Medina E, Becker K, Roth J, Ehrchen JM, Sunderkotter C (2011) Subcutaneous infection with S. aureus in mice reveals association of resistance with influx of neutrophils and Th2 response. J Invest Dermatol 131(1):125–132. doi:10.1038/jid.2010.282

Nizet V (2007) Understanding how leading bacterial pathogens subvert innate immunity to reveal novel therapeutic targets. J Allergy Clin Immunol 120(1):13–22. doi:10.1016/j.jaci.2007.06.005

Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY (2003) Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. J Allergy Clin Immunol 112(6):1195–1202. doi:10.1016/j.jaci.2003.08.049

Ohlsen K, Lorenz U (2010) Immunotherapeutic strategies to combat staphylococcal infections. Int J Med Microbiol 300(6):402–410. doi:10.1016/j.ijmm.2010.04.015

O'Shea JJ, Paul WE (2010) Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 327(5969):1098–1102. doi:10.1126/science.1178334

Ou LS, Goleva E, Hall C, Leung DY (2004) T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J Allergy Clin Immunol 113(4):756–763. doi:10.1016/j.jaci.2004.01.772

Ouyang W, Kolls JK, Zheng Y (2008) The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity 28(4):454–467. doi:10.1016/j.immuni.2008.03.004

Parker D, Ryan CL, Alonzo F 3rd, Torres VJ, Planet PJ, Prince AS (2015) CD4+ T cells promote the pathogenesis of *Staphylococcus aureus* pneumonia. J Infect Dis 211(5):835–845. doi:10.1093/infdis/jiu525

Pauli NT, Kim HK, Falugi F, Huang M, Dulac J, Henry Dunand C, Zheng NY, Kaur K, Andrews SF, Huang Y, DeDent A, Frank KM, Charnot-Katsikas A, Schneewind O, Wilson PC (2014) *Staphylococcus aureus* infection induces protein A-mediated immune evasion in humans. J Exp Med 211(12):2331–2339. doi:10.1084/jem.20141404

Pozzi C, Wilk K, Lee JC, Gening M, Nifantiev N, Pier GB (2012) Opsonic and protective properties of antibodies raised to conjugate vaccines targeting six *Staphylococcus aureus* antigens. PLoS ONE 7(10):e46648. doi:10.1371/journal.pone.0046648

Prabhakara R, Harro JM, Leid JG, Keegan AD, Prior ML, Shirtliff ME (2011) Suppression of the inflammatory immune response prevents the development of chronic biofilm infection due to methicillin-resistant *Staphylococcus aureus*. Infect Immun 79(12):5010–5018. doi:10.1128/IAI.05571-11

Prendergast A, Prado JG, Kang YH, Chen F, Riddell LA, Luzzi G, Goulder P, Klenerman P (2010) HIV-1 infection is characterized by profound depletion of CD161+ Th17 cells and gradual decline in regulatory T cells. AIDS 24(4):491–502. doi:10.1097/QAD.0b013e3283344895

Proctor RA, van Langevelde P, Kristjansson M, Maslow JN, Arbeit RD (1995) Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. Clin Infect Dis 20(1):95–102

Puel A, Doffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, Cobat A, Ouachee-Chardin M, Toulon A, Bustamante J, Al-Muhsen S, Al-Owain M, Arkwright PD, Costigan C, McConnell V, Cant AJ, Abinun M, Polak M, Bougneres PF, Kumararatne D, Marodi L, Nahum A, Roifman C, Blanche S, Fischer A, Bodemer C, Abel L, Lilic D, Casanova JL (2010) Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med 207(2):291–297. doi:10.1084/jem.20091983

Puel A, Cypowyj S, Bustamante J, Wright JF, Liu L, Lim HK, Migaud M, Israel L, Chrabieh M, Audry M, Gumbleton M, Toulon A, Bodemer C, El-Baghdadi J, Whitters M, Paradis T, Brooks J, Collins M, Wolfman NM, Al-Muhsen S, Galicchio M, Abel L, Picard C, Casanova JL (2011) Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science 332(6025):65–68. doi:10.1126/science.1200439

Ragle BE, Bubeck Wardenburg J (2009) Anti-alpha-hemolysin monoclonal antibodies mediate protection against *Staphylococcus aureus* pneumonia. Infect Immun 77(7):2712–2718. doi:10.1128/IAI.00115-09

Raphael I, Nalawade S, Eagar TN, Forsthuber TG (2014) T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine. doi:10.1016/j.cyto.2014.09.011

Rooijakkers SH, van Kessel KP, van Strijp JA (2005) Staphylococcal innate immune evasion. Trends Microbiol 13(12):596–601. doi:10.1016/j.tim.2005.10.002

Rosenzweig SD, Holland SM (2005) Defects in the interferon-gamma and interleukin-12 pathways. Immunol Rev 203:38–47. doi:10.1111/j.0105-2896.2005.00227.x

Schaffer AC, Lee JC (2008) Vaccination and passive immunisation against *Staphylococcus aureus*. Int J Antimicrob Agents 32(Suppl 1):S71–S78. doi:10.1016/j.ijantimicag.2008.06.009

Schlievert PM, Strandberg KL, Lin YC, Peterson ML, Leung DY (2010) Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, and its relevance to atopic dermatitis. J Allergy Clin Immunol 125(1):39–49. doi:10.1016/j.jaci.2009.10.039

Schmaler M, Jann NJ, Ferracin F, Landmann R (2011) T and B cells are not required for clearing *Staphylococcus aureus* in systemic infection despite a strong TLR2-MyD88-dependent T cell activation. J Immunol 186(1):443–452. doi:10.4049/jimmunol.1001407

Schmitt N, Ueno H (2015) Regulation of human helper T cell subset differentiation by cytokines. Curr Opin Immunol 34:130–136. doi:10.1016/j.coi.2015.03.007

Shinefield H, Black S, Fattom A, Horwith G, Rasgon S, Ordonez J, Yeoh H, Law D, Robbins JB, Schneerson R, Muenz L, Fuller S, Johnson J, Fireman B, Alcorn H, Naso R (2002) Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. N Engl J Med 346(7):491–496. doi:10.1056/NEJMoa011297

Skurnik D, Merighi M, Grout M, Gadjeva M, Maira-Litran T, Ericsson M, Goldmann DA, Huang SS, Datta R, Lee JC, Pier GB (2010) Animal and human antibodies to distinct *Staphylococcus aureus* antigens mutually neutralize opsonic killing and protection in mice. J Clin Invest 120(9):3220–3233. doi:10.1172/JCI42748

Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ (2011) The Sbi protein is a multifunctional immune evasion factor of *Staphylococcus aureus*. Infect Immun 79(9):3801–3809. doi:10.1128/IAI.05075-11

Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DY, Schlievert PM (2013) Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 26(3):422–447. doi:10.1128/CMR.00104-12

Spaulding AR, Salgado-Pabon W, Merriman JA, Stach CS, Ji Y, Gillman AN, Peterson ML, Schlievert PM (2014) Vaccination against *Staphylococcus aureus* pneumonia. J Infect Dis 209 (12):1955–1962. doi:10.1093/infdis/jit823

Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, De Saint-Basile G, Durandy A, Griscelli C, Fischer A (1993) Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. J Pediatr 123(4):564–572

Stiles BG, Krakauer T, Bonventre PF (1995) Biological activity of toxic shock syndrome toxin 1 and a site-directed mutant, H135A, in a lipopolysaccharide-potentiated mouse lethality model. Infect Immun 63(4):1229–1234

Stranger-Jones YK, Bae T, Schneewind O (2006) Vaccine assembly from surface proteins of *Staphylococcus aureus*. Proc Natl Acad Sci USA 103(45):16942–16947. doi:10.1073/pnas.0606863103

Tebartz C, Horst SA, Sparwasser T, Huehn J, Beineke A, Peters G, Medina E (2015) A major role for myeloid-derived suppressor cells and a minor role for regulatory T cells in immunosuppression during *Staphylococcus aureus* infection. J Immunol 194(3):1100–1111. doi:10.4049/jimmunol.1400196

Tkaczyk C, Hua L, Varkey R, Shi Y, Dettinger L, Woods R, Barnes A, MacGill RS, Wilson S, Chowdhury P, Stover CK, Sellman BR (2012) Identification of anti-alpha toxin monoclonal antibodies that reduce the severity of *Staphylococcus aureus* dermonecrosis and exhibit a correlation between affinity and potency. Clin Vaccine Immunol 19(3):377–385. doi:10.1128/CVI.05589-11

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28(3):603–661. doi:10.1128/CMR.00134-14

Tuchscherr L, Medina E, Hussain M, Volker W, Heitmann V, Niemann S, Holzinger D, Roth J, Proctor RA, Becker K, Peters G, Loffler B (2011) *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med 3(3):129–141. doi:10.1002/emmm.201000115

van Kessel KP, Bestebroer J, van Strijp JA (2014) Neutrophil-mediated phagocytosis of *Staphylococcus aureus*. Front Immunol 5:467. doi:10.3389/fimmu.2014.00467

Varshney AK, Wang X, MacIntyre J, Zollner RS, Kelleher K, Kovalenko OV, Pechuan X, Byrne FR, Fries BC (2014) Humanized staphylococcal enterotoxin B (SEB)-specific monoclonal antibodies protect from SEB intoxication and *Staphylococcus aureus* infections alone or as adjunctive therapy with vancomycin. J Infect Dis 210(6):973–981. doi:10.1093/infdis/jiu198

Verbrugh HA, Peterson PK, Nguyen BY, Sisson SP, Kim Y (1982) Opsonization of encapsulated *Staphylococcus aureus*: the role of specific antibody and complement. J Immunol 129(4):1681–1687

Verhoeven PO, Gagnaire J, Botelho-Nevers E, Grattard F, Carricajo A, Lucht F, Pozzetto B, Berthelot P (2014) Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: an update. Expert Rev Anti Infect Ther 12(1):75–89. doi:10.1586/14787210.2014.859985

von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001a) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med 344(1):11–16. doi:10.1056/NEJM200101043440102

von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G (2001b) Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease. Clin Infect Dis 32(11):1643–1647. doi:10.1086/320519

Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, Musser JM, DeLeo FR (2005) Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. J Immunol 175(6):3907–3919

Weisman LE, Fischer GW, Thackray HM, Johnson KE, Schuman RF, Mandy GT, Stratton BE, Adams KM, Kramer WG, Mond JJ (2009) Safety and pharmacokinetics of a chimerized anti-lipoteichoic acid monoclonal antibody in healthy adults. Int Immunopharmacol 9(5):639–644. doi:10.1016/j.intimp.2009.02.008

Weisman LE, Thackray HM, Steinhorn RH, Walsh WF, Lassiter HA, Dhanireddy R, Brozanski BS, Palmer KG, Trautman MS, Escobedo M, Meissner HC, Sasidharan P, Fretz J, Kokai-Kun JF, Kramer WG, Fischer GW, Mond JJ (2011) A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. Pediatrics 128(2):271–279. doi:10.1542/peds.2010-3081

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5(12):751–762. doi:10.1016/S1473-3099(05)70295-4

Yeaman MR, Filler SG, Chaili S, Barr K, Wang H, Kupferwasser D, Hennessey JP Jr, Fu Y, Schmidt CS, Edwards JE Jr, Xiong YQ, Ibrahim AS (2014) Mechanisms of NDV-3 vaccine efficacy in MRSA skin versus invasive infection. Proc Natl Acad Sci USA 111(51):E5555–E5563. doi:10.1073/pnas.1415610111

Yoong P, Pier GB (2010) Antibody-mediated enhancement of community-acquired methicillin-resistant *Staphylococcus aureus* infection. Proc Natl Acad Sci USA 107(5):2241–2246. doi:10.1073/pnas.0910344107

Yoong P, Torres VJ (2015) Counter inhibition between leukotoxins attenuates *Staphylococcus aureus* virulence. Nat Commun 6:8125. doi:10.1038/ncomms9125

Zhang L, Jacobsson K, Vasi J, Lindberg M, Frykberg L (1998) A second IgG-binding protein in *Staphylococcus aureus*. Microbiology 144(Pt 4):985–991

Zhang L, Rosander A, Jacobsson K, Lindberg M, Frykberg L (2000) Expression of staphylococcal protein Sbi is induced by human IgG. FEMS Immunol Med Microbiol 28(3):211–218

Ziegler C, Goldmann O, Hobeika E, Geffers R, Peters G, Medina E (2011) The dynamics of T cells during persistent *Staphylococcus aureus* infection: from antigen-reactivity to in vivo anergy. EMBO Mol Med 3(11):652–666. doi:10.1002/emmm.201100173

Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, Monticelli S, Lanzavecchia A, Sallusto F (2012) Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature 484(7395):514–518. doi:10.1038/nature10957

Staphylococcal Immune Evasion Proteins: Structure, Function, and Host Adaptation

Kirsten J. Koymans, Manouk Vrieling, Ronald D. Gorham Jr. and Jos A.G. van Strijp

Abstract *Staphylococcus aureus* is a successful human and animal pathogen. Its pathogenicity is linked to its ability to secrete a large amount of virulence factors. These secreted proteins interfere with many critical components of the immune system, both innate and adaptive, and hamper proper immune functioning. In recent years, numerous studies have been conducted in order to understand the molecular mechanism underlying the interaction of evasion molecules with the host immune system. Structural studies have fundamentally contributed to our understanding of the mechanisms of action of the individual factors. Furthermore, such studies revealed one of the most striking characteristics of the secreted immune evasion molecules: their conserved structure. Despite high-sequence variability, most immune evasion molecules belong to a small number of structural categories. Another remarkable characteristic is that *S. aureus* carries most of these virulence factors on mobile genetic elements (MGE) or ex-MGE in its accessory genome. Coevolution of pathogen and host has resulted in immune evasion molecules with a highly host-specific function and prevalence. In this review, we explore how these shared structures and genomic locations relate to function and host specificity. This is discussed in the context of therapeutic options for these immune evasion molecules in infectious as well as in inflammatory diseases.

List of Abbreviations

Abbreviation (*gene name*, if applicable)

AMP	Antimicrobial peptide
APC	Antigen-presenting cell
Aur	Aureolysin
Bov	Bovine
CA-MRSA	Community-acquired MRSA
CHIPS (*chp*)	Chemotaxis inhibitory protein of *S. aureus*

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DARC	Duffy antigen receptor for chemokines
EAP (*eap*)	Extracellular adherence protein
Ecb (e*cb*)	Extracellular complement-binding protein
Efb (*efb*)	Extracellular fibrinogen-binding protein
Egc (*egc*)	Enterotoxin gene cluster
Eq	Equine
FLIPr (*flipr*)	FPR2 inhibitory protein
FPR	Formyl peptide receptor
GPCR	G-protein-coupled receptor
HLA	Human leukocyte antigen
Hla (*hla*)	α-hemolysin
Hlb (*hlb*)	β-hemolysin
Hlg (*hlg*)	γ-hemolysin
IEC	Immune evasion cluster
Ig	Immunoglobulin
Luk (*luk*)	Leukocidin
mAb	Monoclonal antibody
MAC	Membrane attack complex
MGE	Mobile genetic element
MHC-II	Major histocompatibility complex II
MMP-9	Matrix metalloproteinase 9
MRSA	Methicillin-resistant *Staphylococcus aureus*
NET	Neutrophil extracellular trap
NMR	Nuclear magnetic resonance
NSP	Neutrophil serine protease
OB	Oligomer-binding
Ov	Ovine
PSGL-1	P-selection glycoprotein ligand-1
PSM (*psm*)	Phenol-soluble modulin
PVL (*pvl*)	Panton–Valentine leukocidin
SAg	Superantigen
SAK (*sak*)	Staphylokinase
SaPI	Staphylococcal pathogenicity island
Sbi (*sbi*)	Second immunoglobulin-binding protein
SC (*coa*)	Staphylocoagulase
SCC	Staphylococcal cassette chromosome
SCIN (*scn*)	Staphylococcal complement inhibitor
SE (*se*)	Staphylococcal enterotoxin
SEl (*sel*)	Staphylococcal enterotoxin-like
SNase (*nuc*)	Staphylococcal nuclease
SpA (*spa*)	Staphylococcal protein A
SSL (*ssl*)	Superantigen-like protein
TCR	T cell receptor

TLR	Toll-like receptor
TSST-1 (*tst*)	Toxic shock syndrome toxin-1
vWbp (*vwb*)	Von Willebrand factor-binding protein

Contents

1 Introduction

Staphylococcus aureus is a successful human and animal pathogen and is rising as a major health threat worldwide. It is a commensal in both human and animals, but can cause severe diseases in most mammalian species when it becomes invasive (Lowy 1998; Peton and Le Loir 2014). *S. aureus* disease in livestock is common and ranges from mastitis in dairy cattle to dermatitis in poultry and rabbits (Peton and Le Loir 2014). In humans, invasive disease is mostly found in patients with immunological or barrier defects; however, more pathogenic strains have recently emerged with the ability to cause disease in otherwise healthy people with functional immune systems (Bassetti et al. 2009; Okumura and Nizet 2014). This ability is related to the large number of virulence factors, including immune evasion molecules, that interfere with proper immune functioning. These evasion molecules are generally secreted proteins that specifically interact with components of, mainly, the innate immune system and play a role in causing disease and successful colonization. So far over 35 staphylococcal evasion molecules have been described, which is more than what is known for any other bacterium, making staphylococci the textbook example for immune evasion. A closer look at the genome of *S. aureus*

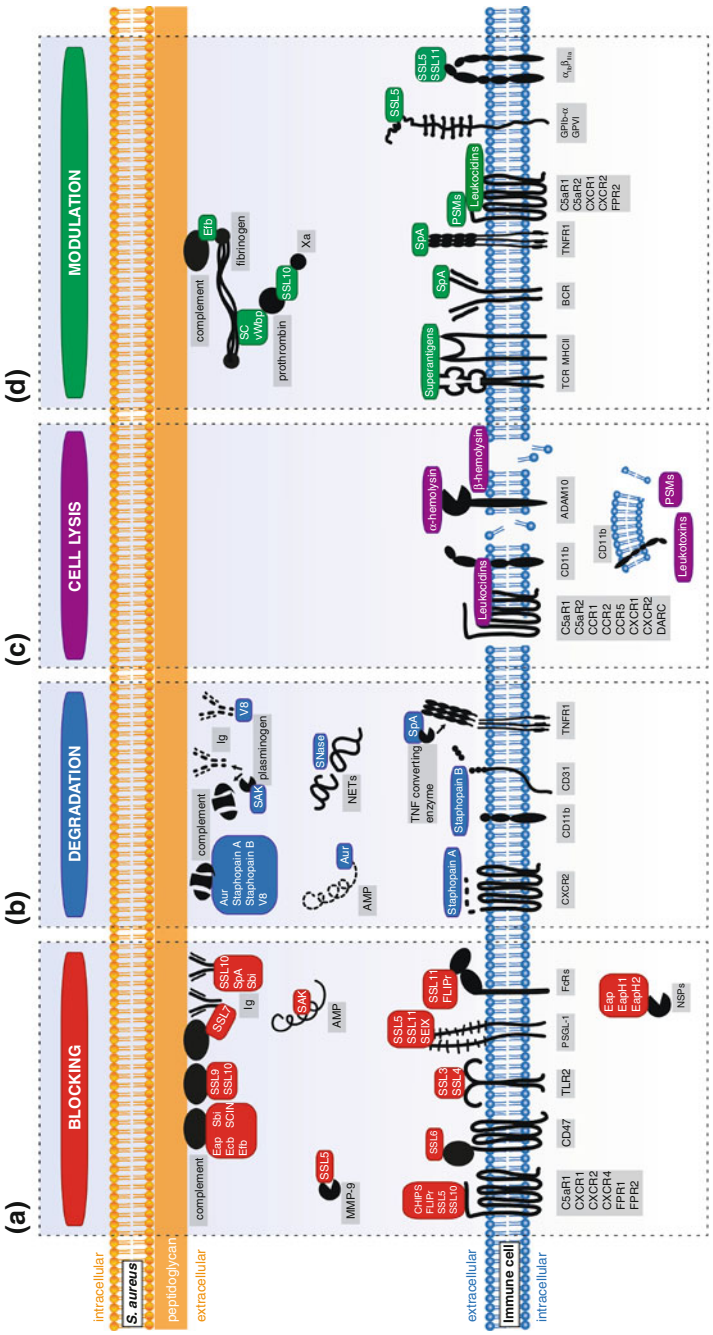
reveals that roughly 10 % of all staphylococcal proteins are secreted (Economou 2002), meaning up to 270 proteins could be secreted. Indeed, the list of evasion molecules is still growing.

In this paper, we discuss the principles of staphylococcal immune evasion and respective immune targets. *S. aureus* has developed ways to interfere with different immunological processes involving host receptors and proteins that share little similarity; however, the immunomodulatory staphylococcal proteins do show remarkable resemblances: They have very conserved structural properties, are often small, varying in size between 8.9- and 35-kDa, and have extreme isoelectric points (above 9 or below 5). Because of the multitude of evasion molecules and their redundancy, we grouped them based on their shared structural characteristics and evaluate the structure–function relationship in Chap. 3. Another common property is that they are located on genomic clusters with other virulence factors. In general, these genomic clusters are mobile or display a high degree of genetic variability. Moreover, different strains and lineages of *S. aureus* carry and express different evasion molecules. This enables *S. aureus* to adapt to its environment and host; possibly leading to the development of immune evasion genes with species–specific functions. This is reviewed in Chap. 4. Finally, we will briefly deliberate on how targeting these evasion molecules might pave the way for new therapeutic strategies.

2 The Mechanisms of Immune Evasion

S. aureus has evolved ways to target diverse branches of the immune system. Since the innate immune system is key in the clearance of staphylococci, most evasion molecules interfere at this stage. They can hinder initial bacterial recognition, manipulate coagulation processes, and inhibit the function of antimicrobial compounds, complement, and phagocytes. Other staphylococcal virulence factors interfere with adaptive immunity and the generation of successful memory responses. There are some general strategies that staphylococci use to prevent recognition by the immune system, which usually involve shielding. For example, *S. aureus* can surround itself by an extracellular polysaccharide capsule or induce the formation of fibrin clots at the bacterial surface, both of which inhibit phagocytosis (Berends et al. 2014). Other strategies include the formation of biofilms, where the bacteria provide each other with a protective milieu (Thurlow et al. 2011), or the adaption to an intracellular lifestyle (Tuchscherr et al. 2010). Most staphylococcal evasion strategies, however, involve manipulation of host functions through direct molecular interaction between secreted bacterial proteins and immune targets, which is the focus of this review.

The main molecular evasion mechanism employed by the secreted proteins of *S. aureus* is the blocking of host receptors or host enzymes, by interfering with ligand–receptor interactions or by occupying the catalytic sites of enzymes (Fig. 1a). Chemokine, Fc, and pattern recognition receptors are affected, thereby preventing bacterial recognition and influx of immune cells to the site of infection.


(a)
BLOCKING
(b)
DEGRADATION
(c)
CELL LYSIS
(d)
MODULATION
intracellular
S. aureus
peptidoglycan
extracellular
complement
Eap
Sbi
Ecb
Efb
SSL9
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SpA
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Ig
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SEIX
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FLIPr
C5aR1
CXCR1
CXCR2
CXCR4
FPR1
FPR2
CD47
TLR2
PSGL-1
FcRs
Eap
EapH1
EapH2
NSPs
extracellular
Immune cell
intracellular
complement
Aur
Staphopain A
Staphopain B
V8
Ig
V8
plasminogen
SAK
SNase
NETs
Aur
AMP
Staphopain A
TNF converting enzyme
SpA
Staphopain B
CXCR2
CD11b
CD31
TNFR1
α-hemolysin
β1-hemolysin
Leukocidins
C5aR1
C5aR2
CCR1
CCR2
CCR5
CXCR1
CXCR2
DARC
CD11b
ADAM10
PSMs
Leukotoxins
complement
Efb
fibrinogen
SC
vWbp
prothrombin
SSL10
Xa
Superantigens
SpA
SpA
PSMs
Leukocidins
SSL5
SSL5
SSL11
TCR MHCII
BCR
TNFR1
C5aR1
C5aR2
CXCR1
CXCR2
FPR2
GPIb-α
GPVI
$\alpha_{IIb}\beta_{IIIa}$

◀ **Fig. 1** Evasion mechanisms of secreted proteins of *S. aureus*. The immune evasion mechanisms of *S. aureus* secreted proteins can be grouped into four categories: panel **a** (*red*), blocking of host components through blocking of immune receptors (e.g., TLR2), host enzymes (e.g., MMP-9), antimicrobial peptides (e.g., LL-37), or host factors deposited on the bacterial cell membrane (e.g., complement); panel **b** (*blue*), degradation of host factors, such as immune receptors (e.g., CXCR2), DNA (e.g., NETs), antimicrobial peptides, or factors deposited on the bacterial cell membrane (e.g., complement); panel **c** (*purple*), cell lysis, which can either be receptor dependent (e.g., C5aR1) or independent and extra- or intracellular; panel **d** (*green*): modulation or (non-specific) activation of host processes that affect host receptors (e.g., TCR) and coagulation processes. The evasion molecules discussed in this review are added in colored boxes depending on the category they belong to. All host targets are drawn schematically in black and the names of the affected host components are indicated in gray. Receptors are represented on the membrane of a "general" immune cell for simplicity reasons. Abbreviations: *AMP* (antimicrobial peptide), *Aur* (aureolysin), *BCR* (B cell receptor), *CHIPS* (chemotaxis inhibitory protein of *S. aureus*), *DARC* (Duffy antigen receptor for chemokines), *Eap* (extracellular adherence protein), *Ecb* (extracellular complement-binding protein), *Efb* (extracellular fibrinogen-binding protein), *FLIPr* (FPR2 inhibitory protein), *FPR* (formyl peptide receptor), *Ig* (immunoglobulin), *MMP-9* (matrix metalloproteinase 9), *NET* (neutrophil extracellular trap), *NSP* (neutrophil serine protease), *PSGL-1* (P-selection glycoprotein ligand-1), *PSM* (phenol-soluble modulin), *SAK* (staphylokinase), *Sbi* (second immunoglobulin-binding protein), *SC* (staphylocoagulase), *SCIN* (staphylococcal complement inhibitor), *SElX* (staphylococcal enterotoxin-like X), *SNase* (staphylococcal nuclease), *SpA* (staphylococcal protein A), *SSL* (staphylococcal superantigen-like protein), *TCR* (T cell receptor), and *vWbp* (von Willebrand factor-binding protein)

S. aureus has also developed ways to interact with factors that have been deposited on the bacterial membrane; it prevents opsonization by inhibiting complement activation and antibody binding. Furthermore, there are staphylococcal proteins that bind to soluble factors such as antimicrobial peptides. Usually, the secreted molecules act extracellularly, but there are also examples of proteins that inhibit intracellular host enzymes.

An alternative strategy of *S. aureus* is to secrete enzymes that cause cleavage of host components and degrade important parts of the immune system (Fig. 1b). This can be done directly by secreted proteases or indirectly through molecules that activate host enzymes. At the bacterial surface, both complement components and antibodies are cleaved, thereby limiting opsonization. Furthermore, the extracellular domains of host receptors can be cleaved, which results in truncated non-functional receptors. *S. aureus* also secretes enzymes that can degrade DNA [i.e., neutrophil extracellular traps (NETs)].

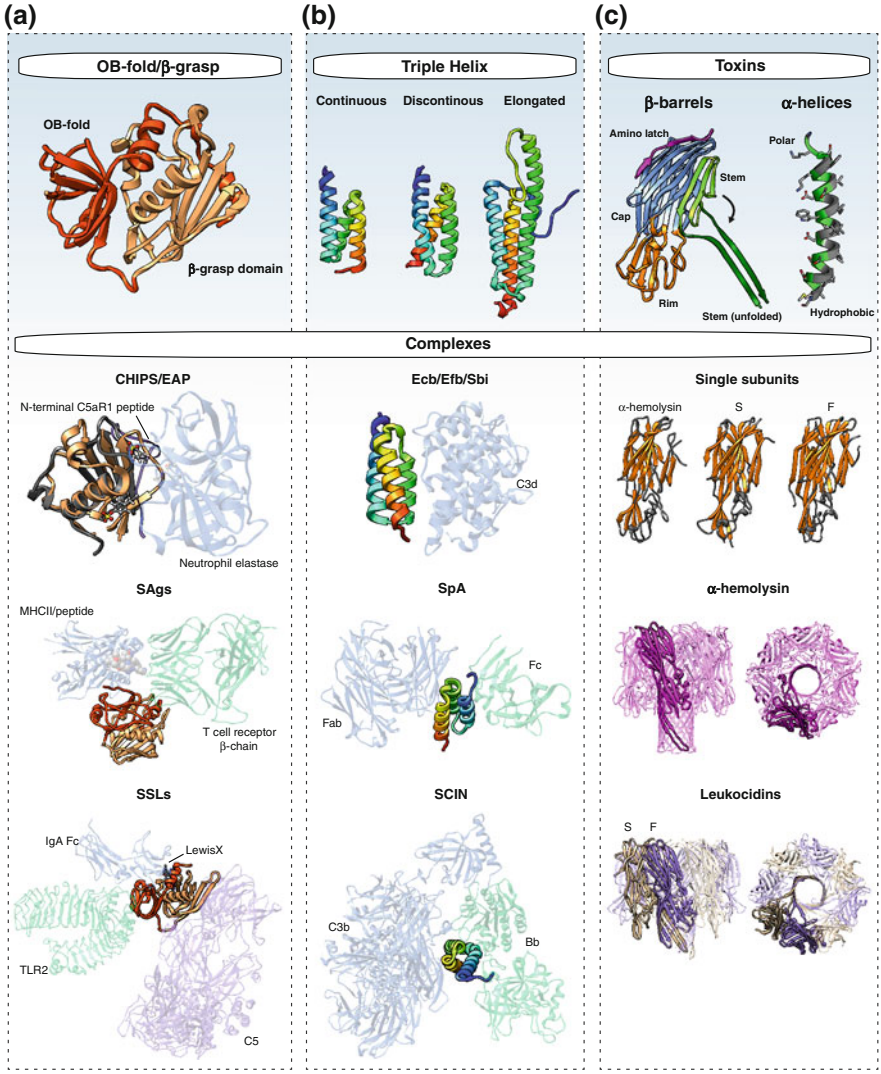
Another, very direct, strategy employed by staphylococci is to induce immune cell death through toxins (Fig. 1c). Some staphylococcal proteins disrupt membrane integrity by acting as detergents, while others target specific, usually chemokine, receptors on the host cell membrane and induce the formation of pores. In this way, both cells from the innate and the adaptive immune system can be targeted, depending on the receptor repertoire present on the cells and the specificity of the toxins for the chemokine receptors. These processes can be employed extracellularly or intracellularly, after the bacterium has been phagocytozed as a way to escape from the phagosome and avoid death.

Aside the above-described inhibitory functions, *S. aureus* is also capable of modulating or activating parts of the immune system, which specially affects components of the adaptive immune system (Fig. 1d). Secreted staphylococcal proteins can activate both B and T cells, which results in malfunctioning responses and overactivation. In time, this can inhibit a proper immune response. Furthermore, several secreted proteins can activate glycoproteins present on platelets and manipulate the coagulation system by promoting clotting or degrading existing fibrin clots, depending on the situation. Some inflammatory receptors can also be activated by evasion molecules; however, it is debatable whether these are side effects or if they actually contribute to pathogenesis.

Which host cells are affected by all these different strategies depends mainly on the receptor repertoire on the cells, since most functions are mediated through staphylococcal protein–host receptor interactions. The effects are also not limited to immune cells since, for example, epithelial cells can be targeted as well. These often are the first cells to come in contact with bacteria and are thus important in initiating primary danger signals. Altogether, *S. aureus* has developed ways to interfere with all important defense mechanisms and can target basically any immune cell. Figure 1 summarizes these mechanisms and gives an overview of all currently known secreted evasion molecules and their targets within the immune system. In the next chapter, we take a closer look at these molecules, their conserved structures, host counterparts, and functions.

3 Conserved Structural Properties of Evasion Molecules: A Structure–Function Analysis

The secreted evasion molecules can be subdivided into two main distinct structural clusters and the group of membrane-damaging toxins (Fig. 2). The largest structural group consists of proteins with a typical OB-fold and/or a β-grasp domain. The second group contains triple α-helix N-shaped bundle(s). The toxins can be divided into two further structurally distinct groups: the multiple β-barrel pore-forming toxins and the α-helices. These general structures are evolutionary conserved and found in all three domains of life. Thus, rather than being specific folds evolved in staphylococcal virulence, it is more likely the bacteria benefits from their extremely stable and energetically favorable conformation. However, it is interesting to look at how these basic structural elements can give rise to a wide variety of immune evasive functions. The stability of these folds permit a large variety of different sequences to be incorporated which allows for broad divergence and rapid adaptation (Murzin 1993; Arcus 2002). Using these basic structures, *S. aureus* has evolved molecules to target almost every branch of the immune system.


(a)
OB-fold/β-grasp
OB-fold
β-grasp domain
(b)
Triple Helix
Continuous
Discontinous
Elongated
(c)
Toxins
β-barrels
α-helices
Amino latch
Polar
Stem
Cap
Rim
Stem (unfolded)
Hydrophobic
Complexes
CHIPS/EAP
N-terminal C5aR1 peptide
Neutrophil elastase
Ecb/Efb/Sbi
C3d
Single subunits
α-hemolysin
S
F
SAgs
MHCII/peptide
T cell receptor β-chain
SpA
Fc
Fab
α-hemolysin
SSLs
IgA Fc
LewisX
TLR2
C5
SCIN
C3b
Bb
Leukocidins
S
F

◀ **Fig. 2** Structural comparison of immune evasion molecules and their targets. Panel **a** shows members of the OB-/β-grasp fold family. At the *top*, a representative structure of this fold type is shown (PDB 1ESF) (Schad et al. 1995) with the OB-domain in *dark orange*, and the β-grasp domain in *light orange*. *Below* are cocrystal structures of EapH1 (*light orange*) with neutrophil elastase (*light blue*) (PDB 4NZL, Stapels et al. 2014) and CHIPS (*gray*) with C5aR1 *N*-terminus (*purple*, sulfated tyrosine residues are indicated in *ball and stick*) (PDB 2K3U, Ippel et al. 2009). Superantigen SEB (*orange*) with MHC-II (*light blue*), hemagglutinin peptide (*spheres*), and T cell receptor (*light green*) (PDB 4C56, Rödström et al. 2014), and SSL3, 7, and 11 (*orange*) in complex with TLR2 (*light green*) (PDB 5D3I, Koymans et al. 2015), IgA Fc (*light blue*) (PDB 2QEJ, Ramsland et al. 2007) and C5 (*light purple*) (PDB 3KLS, Laursen et al. 2010), and sialyl Lewis X (*ball and stick*) (PDB 2RDG, Chung et al. 2007), respectively. Panel **b** shows members of the triple-helix family, with representative images of three different subgroups. The continuous N-shaped fold is on the *left* (Ecb/Efb/Sbi) (PDB 2GOX, Hammel et al. 2007), discontinuous N-shaped fold in the *center* (SCIN/SpA) (PDB 2QFF, Rooijakkers et al. 2007), and the extended staphylocoagulase (SC) domain on the *right* (PDB 1NU7, Friedrich et al. 2003). These are colored rainbow from *N*- to *C*-terminus (*blue to red*). *Below* are cocrystal structures of Efb-C (*rainbow*) with C3d (*light blue*) (PDB 2GOX, Hammel et al. 2007), SpA (*rainbow*) with IgG Fc (*light green*) (PDB 4WWI, Deis et al. 2015) and IgM Fab (*light blue*) (PDB 1DEE, Graille et al. 2000), and SCIN-A (*rainbow*) with C3b (*light blue*) and Bb (*light green*) (PDB 2WIN, Rooijakkers et al. 2009). Panel **c** shows members of the pore-forming toxin family. The *top image* shows the β-barrel toxin subunit (*left*), with the *N*-terminal latch in *magenta*, the cap in *blue*, the rim in *orange*, and the (folded) stem in *light green* (monomeric conformation, PDB 1LKF, Olson et al. 1999). The stem in its unfolded conformation is shown in the same image in *dark green* (oligomeric conformation, PDB 4P1Y, Yamashita et al. 2014). The *top right image* shows an amphipathic α-helical toxin (hydrophobic residues in *gray*, polar residues in *green*, PDB 2KAM, Loureiro-Ferreira et al. to be published). *Below* are representative structures of monomeric β-barrel toxin subunits, with α-hemolysin on the *left* (PDB 4IDJ, Foletti et al. 2013), LukS-PV in the *center* (PDB 1T5R, Guillet et al. 2004), and LukF-PV on the *right* (PDB 1PVL, Pédelacq et al. 1999), and the side and top views of complete hemolytic pores formed by α-hemolysin (*magenta*, PDB 7AHL, Song et al. 1996) and LukAB (LukA in tan, LukB in *purple*, PDB 4TW1, Badarau et al. 2015)

3.1 Proteins Consisting of an OB-Fold and/or β-Grasp Domain

Most staphylococcal evasion proteins belong to the category of the OB-fold/β-grasp domain, two highly conserved regions that together form an extremely stable protein structure (Fig. 2a). The *N*-terminal oligomer-binding (OB) domain is a mixed β-barrel with a Greek key motif (Murzin 1993). It was first described by Murzin in 1993 and named OB because the first described proteins that belong to this group bind oligonucleotides and oligosaccharides. The OB-fold is not at all limited to staphylococcal virulence factors; many other pathogenic bacteria also secrete proteins containing this domain (Murzin 1993). The ligand binding sites of this domain are often more fold than sequence related allowing a large degree of sequence variability. The *C*-terminal β-grasp domain typically consists of a five-stranded β-sheet that is diagonally twisted against a conserved α-helix (the β-sheet appears to grasp the helical segment, hence the name) and is a widespread fold with highly versatile functions (Burroughs et al. 2007). Some evasion proteins contain both domains, while others contain either the OB-fold or the β-grasp. Below we will discuss the evasion molecules belonging to these structural groups: the

superantigens (SAgs), the superantigen-like proteins (SSLs), the extracellular adherence proteins (EAPs), chemotaxis inhibitory protein of *S. aureus* (CHIPS), FPR2 inhibitory protein (FLIPr), staphylokinase (SAK), and staphylococcal nuclease (SNase).

3.1.1 The Superantigens

The group of SAgs is the largest and first described OB-fold-/β-grasp-containing family and is a good example of the adaptability of these conserved structural properties. The SAgs are best known for their capacity to induce, the often fatal, toxic shock syndrome (Spaulding et al. 2013). Initially, they were described as enterotoxins because of their ability to cause food poisoning, but it is now known they have a much broader function. Superantigens massively activate T cells and antigen-presenting cells (APCs), thereby interfering with T cell activation and the buildup of a proper adaptive immune response, hence their importance in immune evasion. SAgs can activate up to 30–40 % of all T cells (as compared to 0.0001–0.01 % during a normal T cell response induced by a regular antigen) by interacting with a set of Vβ-T cell receptors (TCRs) and cross-linking these with major histocompatibility complex II (MHC-II) (Choi et al. 1990). *S. aureus* secretes up to 25 different SAgs that all range between 20- and 28-kDa: toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEA-E, G-J, and S-T), and staphylococcal enterotoxin-like (SElK-R, U and U2, V, and X-Y) (Langley et al. 2010; Wilson et al. 2011; Ono et al. 2015). Overall, the groove in between the OB-fold and β-grasp domain appears to be crucial for their superantigenic activity (Fig. 2a). This groove contains a variable region that determines specificity for the TCR, and most SAgs therefore interact with a unique set of TCRs (Sundberg et al. 2007). Some SAgs are more specific than others, and this is dependent on the amount of TCR variable loops the SAg interacts with, and whether specific structural elements or uncommon residues are required at the interface. The OB-fold of the SAgs is involved in a low-affinity interaction with MHC-II. As with TCRs, there are differences in preferences for MHC-II alleles. Furthermore, some of the SAgs require peptide-bound MHC-II for their interactions and others contain an additional high-affinity, zinc-dependent, MHC-II binding site in their β-grasp domain (Sundberg et al. 2007). These basic differences in mechanisms have mainly been resolved through crystallographic studies as reviewed extensively by Sundberg et al. Besides their T cell stimulating capacities, SAgs have also been shown to down-regulate chemokine receptors on monocytes (Rahimpour et al. 1999). By interacting with MHC-II, they activate cellular tyrosine protein kinases that induce chemokine receptor down-modulation. Overall, the SAgs are a great example of proteins in which their general structure determines their overall function, but sequence differences result in remarkable diversity. This is required to stimulate a broad spectrum of T cells considering the variability of the Vβ elements of the TCRs and the different MHC-II alleles present. The superantigens nicely illustrate the evolution of the OB-fold.

3.1.2 Superantigen-like Proteins

In 2000, a new gene cluster was defined that contained proteins structurally similar to the SAgs (Williams et al. 2000). These proteins were part of a larger group of 14 that were first annotated as SET proteins for "staphylococcal enterotoxin-like," but later renamed to staphylococcal superantigen-like (SSL, 1–14) proteins when it became clear that they did not have enterotoxic activity (Lina et al. 2004). Even though the SSLs are highly conserved and involved in innate immune evasion, they have distinct functions. The SSLs are approximately 25-kDa, with the exception of SSL3 and SSL4 that both contain an additional *N*-terminal domain.

Functions (often multiple) have been described for SSL3, SSL4, SSL5, SSL6, SSL7, SSL8, SSL10, and SSL11. Both SSL3 and SSL4 are Toll-like receptor 2 (TLR2) inhibitors (SSL3 is far more potent than SSL4), which inhibit bacterial lipopeptide binding, as well as TLR2 hetero-dimerization with TLR1 and TLR6 (Bardoel et al. 2012; Yokoyama et al. 2012; Koymans et al. 2015). SSL5 binds P-selectin glycoprotein ligand-1 (PSGL-1) and thereby inhibits neutrophil extravasation (Bestebroer et al. 2007; Walenkamp et al. 2010). Moreover, it binds directly to the *N*-terminus of a wide variety of chemokine and anaphylatoxin receptors, thereby preventing leukocyte activation and migration (Bestebroer et al. 2009) and it further interferes with neutrophil migration by binding to and inhibiting the enzymatic activity of matrix metalloproteinase 9 (MMP-9) (Itoh et al. 2010a). SSL5 can also bind and activate platelets by interacting with the platelet glycoprotein receptors GPIb-alpha and GPVI and the integrins $\alpha_{IIb}\beta_3$, causing platelet aggregation, which is beneficial for staphylococcal colonization (de Haas et al. 2009; Hu et al. 2011). SSL6 was found to interact with CD47, a cell surface protein ubiquitously expressed and involved in a range of cellular processes (Fevre et al. 2014). SSL7 has a dual function by which it inhibits opsonization; with its *N*-terminal OB-domain it binds to the Fc region of IgA and thereby interferes with antibody recognition, and with its *C*-terminal β-grasp domain, it binds C5 and inhibits terminal complement activation (Laursen et al. 2010; Bestebroer et al. 2010). SSL8 binds to the extracellular matrix protein tenascin C and inhibits its interaction with fibronectin, which might lead to attenuated motility of keratinocytes (Itoh et al. 2013a). SSL10 targets IgG1 through its *N*-terminal tail and C4 through its *C*-terminal domain (Patel et al. 2010; Itoh et al. 2010b). Furthermore, it targets prothrombin and factor Xa to inhibit blood coagulation (Itoh et al. 2013b) and binds and inhibits CXCR4 (Walenkamp et al. 2009). SSL11 binds glycoproteins present on neutrophils and prevents attachment to P-selectin. In addition, it binds FcαRI (Chung et al. 2007) and possibly also targets GPIIb/IIIa on platelets; however, no experimental data have been published for this as of yet (Langley et al. 2010). SElX, apart from being a superantigen, has recently been found to bind to PSGL-1 and block neutrophil recruitment. It is the first superantigen described to have a further immune evasive function through direct interaction with an additional receptor (Fevre et al. 2014). This is likely due to the fact that SElX shows higher sequence identity to SSLs than to superantigens (Langley and Fraser 2013).

Although there is a large diversity in the target proteins and in the mechanism of action of the SSLs, there are some shared principles. Most knowledge on the functional mechanisms is derived from (co)crystal structures (Fig. 2a). For example, SSL2-6, 11, and SElX all contain a glycan-binding motif through which they bind glycoproteins present on cell surfaces (Chung et al. 2007; Baker et al. 2007; Hermans et al. 2012; Koymans et al. 2015). This motif (TxExxKxxQx[D/H/N]RxxD) is located in the *C*-terminal β-grasp domain of the proteins and binds to sialyl Lewis X (sLe^x), a tetrasaccharide present on immune cells. Crystal structures of SSL4, SSL5, and SSL11 in complex with sLe^x have been solved, and for SSL5 and SSL11, the sLe^x interaction is required for their inhibitory functions (Chung et al. 2007; Baker et al. 2007; Hermans et al. 2012). Interestingly, their functions still appear to be distinct, which might in part be explained by the cationic properties of SSL5 that could result in additional interactions with the cell surface (Langley et al. 2010). For SSL6, removal of the sLe^x residues also inhibited its interaction with CD47 (Fevre et al. 2014). For SSL3 on the other hand, which has a fully conserved glycan-binding motif, this motif is not involved in TLR2 binding and the interaction is based purely on protein–protein interactions (Koymans et al. 2015). Sugar binding might still be a functionally important mechanism, however, as it is described to be important in immune cell binding (Bardoel et al. 2012; Yokoyama et al. 2012), and could increase local cell surface SSL3 concentration. In fact, the glycan-binding motif may be a more general immune evasion mechanism employed by many of the SSLs and is likely partly responsible for the multitude of functions the SSLs have.

The SSL3-TLR2 cocrystal revealed that SSL3 inhibits both lipopeptide binding and also subsequent receptor dimerization. Modeling of SSL4 in this complex showed that SSL4 inhibits TLR2 through the same mechanism, and mutagenesis studies showed that only a few amino acid differences between the two proteins, located in the binding interface with TLR2, result in the 100-fold difference in inhibitory capacity (Koymans et al. 2015). As mentioned earlier SSL3 and SSL4 contain an *N*-terminus, however, for crystallization of both proteins, truncation of this domain was essential, indicating these *N*-termini render the proteins unstable and the domains also do not play a role in their known functional activity (Hermans et al. 2012; Koymans et al. 2015).

The crystal structures of SSL7 in complex with IgA and in complex with C5 revealed that SSL7 uses distinct parts of the protein for these interactions and that it can interact simultaneously with both targets (Ramsland et al. 2007; Laursen et al. 2010). SSL7 prevents IgA binding to its receptor FcαRI with its OB-domain, while it prevents cleavage of C5 through its β-grasp domain by interfering with C5 to C5 convertase binding, thereby limiting generation of the chemoattractant C5a (Ramsland et al. 2007; Bestebroer et al. 2010). It was found that not only SSL7 is capable of binding C5 and IgA simultaneously, but that this is required to reach full inhibitory function; complete inhibition of C5 cleavage and C5a generation is dependent on simultaneous IgA and C5 binding. IgA binding probably contributes to the inhibition by enhancing steric hindrance between C5 and its convertase (Laursen et al. 2010; Bestebroer et al. 2010). Furthermore, SSL7 can inhibit

formation of the membrane attack complex (MAC) by binding to preformed C5b and inhibit complement-mediated cell lysis of erythrocytes and *Escherichia coli* (Langley et al. 2005). In this case, IgA binding was found to be required for inhibition of bacteriolysis, but not for hemolysis (Laursen et al. 2010).

3.1.3 The EAP Domain Proteins

The extracellular adherence protein (EAP) family consists of three proteins: Eap, EapHI, and EapH2, which contain one or more highly similar EAP domains of about 11-kDa (Stapels et al. 2014). Crystallography revealed that these domains adopt a typical β-grasp fold (Geisbrecht et al. 2005). Eap consists of several EAP domains which are joined by short linker regions with a flexible structure. Because of these flexible linkers, crystallization of the multidomain protein (Eap) has been unsuccessful (Hammel et al. 2007). Fortunately, structural studies in solution have shown that the domains convey a linear confirmation suggesting that, rather than stacking on top of each other, they interact with several ligands simultaneously (Hammel et al. 2007). EapH1 and EapH2 are two small homologs of Eap and, in contrast to Eap, are comprised of only one EAP domain.

A lot of different ligands and functions have been described for Eap. Initially, Eap was reported to bind multiple extracellular host proteins, resulting in bacterial agglutination, adherence to host tissues and cells, and inhibition of neutrophil extravasation. Recently, Eap was found to bind C4 and thereby inhibit complement activation (Woehl et al. 2014). Furthermore, Eap inhibits the activity of neutrophil serine proteases (NSPs: neutrophil elastase, cathepsin G, and proteinase 3) (Stapels et al. 2014). It was shown that by inhibiting these NSPs, *S. aureus* protects its own immune evasion proteins from cleavage and degradation (Stapels et al. 2015). Multiple EAP domains are required for most described functions (Hussain et al. 2008; Woehl et al. 2014), whereas the inhibition of NSPs only depends on a single EAP domain (Stapels et al. 2014). Therefore, it is not surprising that the single-domain homologs EapH1 and EapH2 can also inhibit NSP activity. The inhibition of NSPs is also the only function of EAP domains in which the structure–function relationship has clearly been defined. A cocrystal structure of EapH1 in complex with the NSP elastase revealed that EAP domains inhibit these proteases by occluding their catalytic site through non-covalent interactions (Stapels et al. 2014) (Fig. 2a).

3.1.4 Chemotaxis Inhibitory Protein of *S. aureus*

CHIPS is a 14.1-kDa protein that impairs the response of neutrophils and monocytes to the chemoattractants C5a and the formylated peptide fMLP (de Haas et al. 2004). It does so by specifically binding and blocking the GPCRs C5aR1 and the high-affinity formyl peptide receptor (FPR1) (de Haas et al. 2004; Postma et al. 2004). While the structure of the *N*-terminal domain of CHIPS is unknown, the *C*-

terminal domain of CHIPS is highly similar to the superantigens and superantigen-like proteins and contains the structured β-grasp domain (Haas et al. 2005). The two antagonistic functions of CHIPS are located in different domains of the protein. With its *N*-terminus CHIPS blocks FPR1 and its activity is attributed to two phenylalanine residues located in the *N*-terminus. *N*-terminal CHIPS-based peptides can inhibit FPR1, but do not block C5aR1 (Haas et al. 2004). Rather, CHIPS targets the C5aR1 through its *C*-terminal domain and interferes with the initial interaction of C5a with the *N*-terminus of C5aR1. It thereby prevents C5a binding to the activation domain of the C5aR1 and further signaling. NMR spectroscopy with an *N*-terminal C5aR peptide revealed the exact residues involved in the interaction. In particular, C5aR residues 10–24, and specifically three aspartic acid and two tyrosine residues, were found to be important (Haas et al. 2005; Postma et al. 2005; Ippel et al. 2009). Sulfation of these two tyrosine residues was found to be critical for high-affinity C5aR–CHIPS binding (Ippel et al. 2009; Liu et al. 2011). There are multiple parts of CHIPS that make contact with C5aR1, wherein several positively charged CHIPS residues are important. Another key determinant in CHIPS is the loop located between the α-helix and the first β-strand that is in contact with one of the sulfated tyrosines (Ippel et al. 2009) (Fig. 2a).

3.1.5 FPR2 Inhibitory Proteins

FLIPr and FLIPr-like were discovered as two individual proteins that were highly homologous and structurally related to CHIPS (Prat et al. 2006, 2009). It was later recognized that FLIPr and FLIPr-like, rather than being two separate proteins, are two allelic variants of the same gene with 73 % homology on the amino acid level (Prat et al. 2009; McCarthy and Lindsay 2013). The crystal structure of FLIPr has not been published yet, but, like CHIPS, it contains a β-grasp domain. Both variants also inhibit formyl peptide receptors. FLIPr binds to and blocks FPR2, a homolog of FPR1 (Prat et al. 2006), that like FPR1 recognizes formyl peptides but with lower affinity. FLIPr-like inhibits both FPR1 and FPR2 (Prat et al. 2009). The enhanced activity of FLIPr-like as compared to FLIPr toward FPR1 is explained by an *N*-terminal phenylalanine only present in FLIPr-like. This shows an interesting parallel with the phenylalanines in the *N*-terminal domain of CHIPS, which were found crucial for FPR1 inhibition. More recently, both FLIPr and FLIPr-like were shown to inhibit FcγR by competitively blocking IgG binding (Stemerding et al. 2013). FLIPr specifically binds to and inhibits FcγRIIa, while FLIPr-like shows broader activity and binds to most FcγRs.

3.1.6 Staphylokinase

SAK is structurally most similar to CHIPS and contains a β-grasp-like fold (Rabijns et al. 1997). SAK is a plasminogen activator and is not an enzyme by itself, but forms a complex with human plasminogen, a potent serine protease, and activates it

to cleave fibrin and extracellular matrix proteins (Parry et al. 1998). Additionally, at the bacterial surface, it activates plasminogen to cleave bound IgG and C3b, thereby indirectly blocking opsonization and complement activation, which consequently interferes with neutrophil phagocytosis (Rooijakkers et al. 2005b). SAK also directly binds to human α-defensin and inhibits its antimicrobial potency (Jin et al. 2004).

3.1.7 Staphylococcal Nuclease

SNase is an enzyme that contains an OB-fold that cleaves DNA and RNA and binds to the DNA through the conserved oligonucleotide binding site common to the OB-folds (Watson et al. 2007). SNase is important in the breakdown of neutrophil extracellular traps (NETs) and staphylococci can thereby escape from their entanglement and prevent getting killing by antimicrobial peptides and proteases that are entwined in the NETs (Berends et al. 2010). Apart from releasing bacteria from their entrapment, SNase has a role in promoting immune cell death. It was shown to act together with the staphylococcal enzyme adenosine synthase to convert NET DNA into deoxyadenosine, which induces caspase-3-mediated apoptosis in macrophages (Thammavongsa et al. 2013).

3.2 Proteins Consisting of a Triple Alpha Helix

The second most common fold found in evasion proteins is the triple alpha helix, also called the trihelical bundle, which resembles the form of an "N". Although structurally similar, there are three distinct subgroups of triple helices, including the continuous N-shape, the discontinuous N-shape, and the elongated bundle (Fig. 2B). As with the OB- and β-grasp fold, there is no general sequence motif, meaning they cannot be predicted from the sequence alone. The immunoglobulin-binding proteins (SpA and Sbi), the staphylococcal complement inhibitor (SCIN), the extracellular fibrinogen-binding proteins (Ecb and Efb), and the staphylococcal coagulases (SC and vWbp) all belong to this category.

3.2.1 The Immunoglobulin-Binding Proteins

Staphylococcal protein A (SpA) is one of the best studied evasion molecules and it consists of five homologous domains, of approximately 6-kDa, with high-sequence identities that all have the topology of the triple alpha helix (Deis et al. 2014). After secretion, SpA is initially cell wall bound through its *C*-terminal domain, but it can be released upon hydrolysis (Schneewind et al. 1992; Becker et al. 2014b). SpA can bind to both the constant (Fc) and variable (Fab) regions of immunoglobulins with each of its five repetitive triple-helix domains (Fig. 2b). It binds to the Fc domain of IgG and

disrupts opsonization and phagocytosis. The first two helices of each SpA domain are involved in Fc binding (Goodyear and Silverman 2003). Binding to the Fab domain on the other hand involves SpA helices 2 and 3 and through this interaction it acts as a B cell superantigen by cross-linking the V_H3 B cell receptor (surface IgM). This can result in both B cell proliferation and subsequent B cell apoptosis and also in the induction of class-switching, resulting in the secretion of non-specific antibodies. SpA thereby interferes with the ability to develop a good adaptive response (Falugi et al. 2013). SpA has also been demonstrated to bind to TNFR1 and can both activate the receptor, which contributes to pathogenesis, and induce shedding by activating an enzyme complex. This complex has anti-inflammatory consequences because it inhibits further signaling (Gómez et al. 2004, 2006). Furthermore, SpA binds von Willebrand factor (Hartleib et al. 2000) and complement receptor gC1qR, which is often found on platelets and endothelial cells (Nguyen et al. 2000), albeit not much is known about the consequences of these interactions.

The second immunoglobulin-binding protein (Sbi), identified through phage display screens, resembles the IgG-binding properties of SpA (Zhang et al. 1995, 1999). As with SpA, it exists in both cell-bound and secreted forms (Smith et al. 2011). In contrast to SpA, Sbi has only two domains with which it binds to IgG and it can only interact with the IgG Fc domain (Atkins et al. 2008; Burman et al. 2008). Besides the two IgG-binding domains, Sbi has two further independently folded domains that also resemble the triple alpha helix but instead bind to C3 and factor H to inhibit complement activation (Burman et al. 2008; Haupt et al. 2008).

3.2.2 The Staphylococcal Complement Inhibitor Family

The SCIN family consists of three members with approximately 50 % sequence similarity, SCIN, SCIN-B, and SCIN-C (Rooijakkers et al. 2005a, 2007). However, SCIN-B and SCIN-C are allelic variants rather than separate proteins since the presence of the genes is mutually exclusive (McCarthy and Lindsay 2010). The SCIN proteins are small (10-kDa) potent complement inhibitors. They bind to and inhibit C3 convertase activity and thereby limit the release of the chemoattractant C5a and C3b deposition, which results in inhibited phagocytosis. Structural studies with SCIN in complex with the C3 convertase revealed that SCIN can bind two C3b molecules and a single Bb molecule simultaneously (Fig. 2b); however, SCIN also binds directly to a single C3b molecule (Rooijakkers et al. 2009; Garcia et al. 2010). This has also been confirmed for SCIN-B (Garcia et al. 2012b). By binding to C3b, they impair the rate of convertase assembly and also render the convertase inactive but in a stable state, by occluding the binding site for important host complement cofactors (Ricklin et al. 2009; Rooijakkers et al. 2009). Furthermore, SCIN induces convertase dimerization which inhibits opsonophagocytosis because it modulates recognition of complement by phagocytic receptors (Rooijakkers et al. 2009; Jongerius et al. 2010). Both the second alpha helix in the structure of SCIN and the *N*-terminus have shown to be important for the interaction with C3b (Rooijakkers et al. 2009; Garcia et al. 2013).

3.2.3 The Extracellular Fibrinogen-Binding Protein Family

Efb, extracellular fibrinogen-binding protein, is a 15.8-kDa protein with two functionally distinct domains: Its *N*-terminus is involved in fibrinogen binding, while its *C*-terminus is involved in complement inhibition by binding directly to the C3d domain of C3 (Fig. 2b), thereby blocking active convertase formation. The crystal structure of Efb in complex with C3d, in conjunction with biophysical studies, revealed that Efb binding (when bound to C3b) forces C3b in an altered, but stable, conformation (Garcia et al. 2012a). Efb links its *N*-terminal fibrinogen-binding and *C*-terminal complement-binding domains to form a fibrinogen shield around itself. The thick layer of fibrinogen resembles a capsule and shields surface-bound complement and antibodies from recognition by phagocytes (Ko et al. 2013).

Ecb, extracellular complement-binding protein, is a homolog of Efb and has similar complement inhibitory activity, but lacks the fibrinogen-binding domain. Interaction of Ecb with C3 is nearly identical to that of Efb as revealed by crystallographic studies (Garcia et al. 2012a); however, mutagenesis studies revealed that Ecb contains a second, lower affinity, C3 binding site in its first alpha helix. This extra binding site results in enhanced complement inhibitory potency of Ecb as compared to Efb (Garcia et al. 2012a). Both Efb and Ecb can also interfere with adaptive immune responses by inhibiting C3d to complement receptor 2 binding on B cells, which is important for B cell activation and maturation (Henson et al. 2001; Ricklin et al. 2008).

3.2.4 Staphylococcal Coagulases

S. aureus secretes two coagulases, staphylocoagulase (SC), and von Willebrand factor-binding protein (vWbp) that are highly homologous, especially in their *N*-termini, which contain two domains with triple alpha helices (Friedrich et al. 2003). This region is also crucial for their shared function. They both associate with prothrombin to create staphylothrombin, an enzyme complex that can cleave fibrinogen without activating blood clotting, creating a fibrin mesh which protects *S. aureus* from phagocytosis (Friedrich et al. 2003; Panizzi et al. 2006). The *C*-terminal domains of these proteins are more distinct. The *C*-terminus of SC is similar to the *N*-terminal domain of Efb, which is involved in fibrinogen binding (Palma et al. 2001) and indeed provides SC with a fibrinogen-binding site. For vWbp, the *C*-terminal region is involved in binding to the von Willebrand factor, but the function of this interaction is not yet known (Bjerketorp et al. 2002). Apart from prothrombin and fibrinogen, both proteins also bind to fibronectin, and vWbp also binds FXIII (Thomer et al. 2013).

3.3 The Staphylococcal Toxins: β-Barrel Pore-Formers and α-Helices

S. aureus secretes a wide variety of toxins that directly kill (immune) cells by disrupting the cell membrane. Structurally speaking, these toxins can be classified into two groups (Fig. 2c), the β-barrel pore-forming toxins (α-hemolysin and the leukocidins), that target cells expressing specific receptors, and the small α-helical peptides that have detergent-like properties (the phenol-soluble modulins (PSMs)).

3.3.1 The β-Barrel Pore-Forming Toxins

Transmembrane proteins often take on the conformation of a β-barrel to insert themselves in the membrane. *S. aureus* uses this principle by secreting toxins that are composed of β-strand domains to create 1–2 nm cytolytic pores in the host cellular membrane by spanning the phospholipid bilayer. The proteins are expressed in monomeric form and assemble on the target cell membrane through interaction with specific host receptors, inducing leakage and subsequent cell death. α-hemolysin (also known as α-toxin and Hla), a 33-kDa protein, was the first characterized member of this group, and it assembles into a homo-heptameric pore in the target membrane through interaction with its receptor, the zinc-dependent metalloprotease ADAM-10 (Wilke and Bubeck Wardenburg 2010), mainly found on host epithelium and endothelium. Apart from inducing cell lysis it induces ADAM-10 up-regulation, which results in epithelial and endothelial barrier disruption through ADAM-10's metalloprotease activity (Inoshima et al. 2012; Powers et al. 2012). α-hemolysin is also cytotoxic for monocytes, B cells, and T cells. Neutrophils, however, are fairly resistant to α-hemolysin-mediated killing, which is due to the low expression levels of ADAM-10 on these cells (Nygaard et al. 2012). α-hemolysin can, however, affect downstream signaling in several immune cells, including neutrophils, thereby altering cytokine responses and immune function of these cells (Becker et al. 2014a).

Apart from α-hemolysin, *S. aureus* secretes bicomponent pore-forming toxins, also termed leukocidins. The main difference between these and α-hemolysin is that they consist of two different, independently secreted, 33-kDa components that form hetero-multimeric pores. They are designated the S (slow) and F (fast) component, based on their chromatography elution profiles. The cell specificity is generally determined by the S-component that binds to a specific receptor on the cell surface. The final hetero-octameric pore consists of eight alternating S- and F-components. So far six pairs of bicomponent pore-forming have been discovered: LukAB (also named LukGH), LukED, LukMF', LukSF (PVL), and two γ-hemolysins (HlgAB and HlgCB) (Alonzo and Torres 2014). In recent years, it was revealed that the leukocidins exert their lytic ability through association of their S-component with chemokine receptors. These GPCRs are found on a wide variety of immune cells, ranging from innate to adaptive. Some leukocidins have the ability to lyse

erythrocytes, which provides *S. aureus* with a source of iron (Spaan et al. 2015a). It was recently shown that *S. aureus* can use leukocidins to form a protective zone around its colonies killing neutrophils at a distance, thereby protecting them from phagocytosis and NETosis (Vrieling et al. 2015). Apart from killing cells, leukocidins have shown to induce proinflammatory signals at sublytic concentrations, resulting in inflammasome activation, and induction of apoptosis (reviewed by Alonzo and Torres 2014). Inhibition of signaling of chemokine receptors through the S-components has also been described (Spaan et al. 2014).

A lot of structural studies have been performed to understand the mechanism behind the pore-formation. Crystal structures of the monomeric forms have been available for many years already. These revealed that the monomeric α-hemolysin and leukocidins are highly similar. They consist of three domains, the cap, the rim, and the stem (Fig. 2c). The cap domain lies on top and consists of β-sandwiches. The rim domain is located below the cap and is buildup of four β-strands. Lastly, there is the stem domain, which is highly hydrophobic and will later form the antiparallel β-barrel in the membrane. In the monomers, the stem region is packed within the cap domain. The heptameric pore of α-hemolysin and the octameric pore of γ-hemolysin have been structurally determined, which revealed that pore-formation is largely dependent on conformational changes (Song et al. 1996; Galdiero and Gouaux 2004; Tanaka et al. 2011; Yamashita et al. 2011). After secretion of the soluble monomers, which are cotranscribed from a single promoter, dimerization of two components occurs at the host cell membrane. This induces a conformational change in an *N*-terminal region called the amino latch that results in the extension and unfolding of the stem domain, releasing it from the cap and positioning it into the host membrane. Subsequently there is formation of the ring-shaped prepore, after which the transmembrane half of the β-barrel is inserted into the membrane, completing the formation of the pore (Fig. 2c) (Yamashita et al. 2011, 2014).

The general mechanism of action of all these β-barrel pore-forming toxins is highly similar. Overall, they have remarkable sequence similarity, especially within the groups of S- or F-components. Nonetheless, there are also differences and this is reflected by the different receptors targeted. LukED binds to CXCR1, CXCR2, and CCR5 on immune cells and targets the Duffy antigen receptor for chemokines (DARC) to lyse erythrocytes (Reyes-Robles et al. 2013; Alonzo et al. 2013; Spaan et al. 2015a). LukMF', a toxin found in isolates from ruminants, binds to CCR1, CCR2, and CCR5 (Vrieling et al. 2015), while LukSF targets the C5aR1 and C5aR2 (formerly C5L2) (Spaan et al. 2013). HlgAB targets CXCR1, CXCR2, CCR2, and DARC, and HlgCB has affinity for the same receptors as LukSF, namely the C5a receptors (Spaan et al. 2014). LukAB is the only exception as it does not bind to a GPCR, but to the integrin CD11b (DuMont et al. 2013). This can be explained by the fact that LukAB is the most distantly related member of the leukocidin family. Through specific interactions with their receptors, the leukocidins target distinct cell populations. Receptor expression and conservation results in differential interaction of certain leukocidin pairs with immune cells from different species (Spaan et al. 2015a; Vrieling et al. 2015). Studies have been

performed to localize the receptor-binding parts in the toxins. Amino acid sequence alignment of leukocidins revealed specific regions in the rim domain of the S-components that are highly divergent and termed divergence regions 1–5 (DR1-5) (Reyes-Robles et al. 2013). For both LukED and LukMF', the DR4 region, located at the bottom of the rim domain, showed to be important in specific receptor recognition (Reyes-Robles et al. 2013; Vrieling et al. 2015). Also on the receptor side, there are differences in regions that are targeted and involved. For example, PVL and HlgCB may target the same receptors, but do so differently as distinct parts of the C5a receptors are involved in the initial binding, but also in the subsequent pore-formation (Spaan et al. 2015b). More recently, the structure of the LukAB octamer was solved. LukAB is distinct from the other leukocidins not only because it targets an integrin, but also because it forms a dimer even in solution, before contacting its target cell. Furthermore, the S-component is mainly involved in receptor binding, but the F-component of LukAB showed to strengthen the interaction. Therefore, dimerization before targeting the cell is a prerequisite for this toxin to gain sufficient binding affinity (Badarau et al. 2015).

Overall, the β-barrel pore-forming toxins share their mechanism of action as a result of their highly conserved structure, and their main differences lie in receptor recognition through divergent sequence regions. This also results in different target cell populations and species specificity.

3.3.2 The Phenol-Soluble Modulins

The PSMs are a family of small amphipathic peptides with similar α-helical structures and membrane damaging potential (Fig. 2C). Although their structures are similar, their amino acid sequences are barely conserved. The toxic activity of PSMs is receptor independent and related to their detergent-like properties caused by their strong α-helical structure that results in a high affinity for lipids (Wang et al. 2007). PSMs can be divided into two families based on their length. There is the shorter PSMα family that contains 6 family members (all between 20 and 26 amino acids), which includes PSMα1-4, δ-toxin, and PSM-*mec,* and the longer PSMβ family containing PSMβ1-2 of both 44 amino acids. The PSMα-peptides are the most cytolytic, with PSMα3 being the most potent (Wang et al. 2007). Their lytic function is effective intracellularly rather than extracellularly, in contrast to most leukocidins, because PSM function is neutralized by serum lipoproteins (Surewaard et al. 2012). Furthermore, in small intracellular compartments the high PSM concentration threshold required for lysis is more easily reached. By lysing phagocytes from the inside, *S. aureus* can escape phagocytic killing (Surewaard et al. 2013). PSMs also have receptor-mediated actions. Through interacting with FPR2, they activate cells and stimulate chemotaxis at low PSM concentrations (Kretschmer et al. 2010). It is not known whether this is a strategy aiding the bacterium or rather a host-defense mechanism. PSMs have also been shown to affect the adaptive immune response by impairing the function of dendritic cells and inducing IL-10 secretion and T regulatory cells (Schreiner et al. 2013). They are also involved in

biofilm formation and structuration (Le et al. 2014). Structure–function studies using an alanine substitution library on PSMα3 revealed that the different activities of PSMs are linked to different parts of the peptides (Cheung et al. 2014b). The hydrophilic side, containing a lot of positively charged residues, is highly important for the initiation of their cytolytic activities (for full pore-formation, the amphipathic properties are required), and for the pro-inflammatory properties. The hydrophobic side seems more involved in affecting biofilm formation and also to prevent antimicrobial activity while retaining cytolytic abilities (Cheung et al. 2014b).

3.4 Additional Secreted Enzymes

3.4.1 Staphylococcal Proteases

The staphylococcal proteases do not belong to any of the discussed structural categories, but are nonetheless potent secreted evasion molecules. Aureolysin (Aur), staphopain A, staphopain B, and V8 are all proteases that cleave host factors and are especially involved in the shutdown of complement (Jusko et al. 2014). Aur is a zinc-dependent metalloprotease that cleaves C3, which is then further degraded by host factors (Laarman et al. 2011). Furthermore, Aur cleaves and inactivates antimicrobial peptides (Sieprawska-lupa et al. 2004). Staphopain A and staphopain B are cysteine proteases that cleave complement components and interfere with neutrophil receptors. Staphopain A cleaves the *N*-terminus of the chemokine receptor CXCR2 (Laarman et al. 2012), thereby interfering with neutrophil migration. Staphopain B cleaves both CD11b and CD31 on phagocytes, which impairs phagocyte function and marks them for depletion (Smagur et al. 2009). V8 is a serine protease and, apart from cleaving complement components, is involved in cleavage of immunoglobulins (Prokesová et al. 1995). Multiple structures for most of these proteases have been described, but since they belong to different classes of proteases, there are no real structural similarities between the groups.

3.4.2 β-Hemolysin

β-hemolysin (Hlb, or also called beta toxin) is a 35-kDa protein that works as a sphingomyelinase, as it hydrolyzes a specific membrane lipid, sphingomyelin, thereby inducing cell lysis. Structurally speaking, it belongs to the DNase I folding superfamily and it was first described to lyse red blood cells and thought to be important in the acquisition of iron. It has now been recognized that β-hemolysin can also kill neutrophils, monocytes, and T cells, giving it clear immune evasive properties (Huseby et al. 2007).

3.5 The Structure–Function Relationship

Detailed insight in the above-described molecular mechanisms was largely gained through crystallographic studies of the proteins, which were feasible due to the highly stable conformation of the conserved folds. In terms of sequence similarity, pore-forming toxins are most related to each other, followed by the OB-fold/β-grasp proteins, while the triple alpha helices have the least conserved sequences within their group. The OB- and β-grasp folds are often found together, and many immune evasion molecules contain both domains, such as the SAg and SSL families. Even though these families are structurally highly similar, there are surprising differences in how they engage their binding partners. Figure 2a illustrates the generally conserved binding sites of MHC-II and TCR on the SAgs. These sites are conserved because TCRs are structurally similar and differ only in the sequence of their variable regions, and the same accounts for MHC-II. In contrast, the SSLs bind a diverse array of proteins, and consequently binding sites are found all over the protein surface (Fig. 2a). Surprisingly, members of the SSL family share similar levels of sequence homology to each other as the SAgs among themselves.

The other proteins in this group contain either an OB-domain or β-grasp domain. Interestingly, despite having completely different functions, EAP domains and CHIPS have very similar structures (Fig. 2a), likely because they both only contain the β-grasp domain. However, EAP can bind a variety of targets, due to its multi-domain structure. More surprisingly is that the SSLs and the EAP domain proteins also have somewhat similar sequences, despite their wide range in targets. The sequence of the α-helix (within the β-grasp domain) seems to be most conserved within this family of proteins (Haas et al. 2005). These residues are probably involved in maintaining the overall structure of the domain. While most of the β-grasp proteins contain five beta strands, some proteins contain only four, which may alter the domain flexibility. The biggest outlier, and the only protein that contains solely an OB-domain, is SNase. Its OB-domain likely has an altered structure to accommodate for its enzymatic activity.

For the triple alpha helices, three distinct types of folds can be distinguished (Fig. 2b). Efb-C, Ecb, and (at least domain four of) Sbi contain a continuous N-shape, whereas SCIN, SpA, and SC have a discontinuous N-shape. Although the structures are unknown, it is likely that the first two domains of Sbi have a discontinuous N-shape, due to their sequence and functional similarity to the SpA domains. The third Sbi domain is probably similar to the fourth as these are both involved in binding C3. Additionally, SC (and perhaps vWbP) has an elongated triple helix and is functionally divergent from the other two groups. Triple-helix proteins have the least conserved sequence similarity (often less than 10 % identity) of all three families; however, structural similarity is surprisingly high. The triple-helix proteins bind to a wide variety of targets, probably due to the high-sequence diversity. Only within domains that share binding partners, the sequence similarity is greater than 20 %. The amount of different targets that bind the triple alpha helix family is further increased through distinct physicochemical

properties of the proteins and individual helices themselves. Interestingly, Efb/Ecb/Sbi and SCIN all bind to complement C3 and its fragments/complexes. But, despite having highly similar structures and binding to the same protein, they function in completely different ways. For example, SCIN does not bind to native C3, in comparison with other complement inhibitors (Lambris et al. 2008). Both SCIN and Efb alter the convertase, but via completely different mechanisms.

For the pore-forming toxins, there is overall high-sequence and structural identity. The S-components all have >60 % sequence identity and so do the F-components. Between the single subunits of α-hemolysin, S-, and F-components, there is only 20–30 % sequence identity, but this is still much higher than the intragroup sequence identities of the OB-fold/β-grasp and triple-helix groups. This is not surprising since the pore-forming toxins share their general modes of action. Sequence diversity is mostly required for the S-components to bind different cell surface receptors, through which they target a variety of immune cells. In the monomers, the rim domains are most diverse as they are comprised of many flexible loops between strands. Diversity is most important here as this appears to be the region to define receptor specificity. Despite sequence variability, the α-hemolysin, S-, and F-component monomers have very high structural homology, exhibiting only subtle differences in the rim and stem domains that may account for differences in receptor selectivity and pore oligomerization/size. The PSMs are membrane disrupting toxins, but are structurally and mechanistically completely different. Their high amphipathic nature with polar residues on one side and hydrophobic on the other side results in their detergent-like properties, which is similar to human cathelicidins.

Although staphylococcal immune evasion molecules share very low sequence homology, they conform to a remarkably small number of folds. This observation underscores that during evolution, protein structure is more conserved than sequence. The structured folds clearly form ideal scaffolds for the formation of extensive variations of protein–protein interactions, highlighted by the myriad of different host target proteins and receptors. Many of these evasion proteins have most likely evolved from common ancestors and gene duplications (especially, the clusters, such as the SAgs and the SSLs). They managed to target different molecules, since the folds allow high-sequence variability without compromising stability. Related to this evolution may be the appearance of strain-specific and species-specific immune evasion molecules. We will look into this in more detail in the next chapter.

4 Genomic Location and Host Specificity

The *S. aureus* population is subdivided into lineages that evolve separately from each other. Generally, human and animal isolates belong to different lineages. However, animal lineages can also cause infections in humans (Fitzgerald 2012; McCarthy et al. 2012b). Likewise, human lineages can also be isolated from

animals, especially from animals that live in close contact with humans (McCarthy et al. 2012a; Abdelbary et al. 2014). Clearly, *S. aureus* has the ability to adapt to different host species, including cattle, small ruminants, poultry, horses, rabbits, and pigs. While the molecular mechanisms of host adaptation are largely unknown, it is likely that gene loss, allelic diversification, and the acquisition of mobile genetic elements (MGEs) are involved in this process (Guinane et al. 2011; McCarthy et al. 2012c). The *S. aureus* genome is highly variable and only 75 % of the gene content is shared between all strains (Lindsay et al. 2006b). The other 25 % comprises the accessory genome of *S. aureus* and consists of the "core variable" genome and MGEs. The core variable genome is a lineage-specific set of genes typically encoding surface proteins, secreted virulence factors, and their regulators (Lindsay and Holden 2006a; McCarthy and Lindsay 2010, 2013). MGEs are DNA segments that can move between bacteria through horizontal gene transfer, i.e., prophages, plasmids, pathogenicity islands (SaPIs), staphylococcal cassette chromosomes (SCCs), and transposons (Lindsay 2010; Malachowa and Deleo 2010). A common feature of these MGEs is that they especially encode genes involved in either immune evasion, and thus virulence, or antibiotic resistance (Lindsay and Holden 2006a; McCarthy and Lindsay 2013). The distribution of the MGEs is highly host-specific, indicating that the immune evasion genes encoded by these MGEs could be determinants of host adaptation of *S. aureus*. We set out to understand the relation between the function, host specificity, and the genomic distribution of the staphylococcal immune evasion proteins. While most animal experiments have been performed in mice, we focused on the functionality of the immune evasion proteins in natural hosts of *S. aureus* (e.g., ruminants, rabbits, pigs, and poultry) to elucidate their potential role in *S. aureus* diseases in these species. First, we discuss the immune evasion genes that are immobile and localized on the core variable genome of *S. aureus*. Next, the MGE-encoded immune evasion genes are discussed.

4.1 Core Variable Genome

4.1.1 Genomic Islands

νSaα and νSaβ are the two major genomic islands of *S. aureus* that encode immune evasion genes. They are present in virtually every *S. aureus* isolate and are part of the core variable genome, but their gene content is highly variable and prone to recombination (Everitt et al. 2014; Moon et al. 2015). νSaα and νSaβ are flanked by a broken transposase gene and a partial restriction–modification system downstream, and the GC content of these islands differs from the core genome (Baba et al. 2002, 2008). Therefore, they were presumably introduced in the *S. aureus* genome by horizontal gene transfer (Malachowa and Deleo 2010). The acquisition of νSaα and νSaβ is thought to have played a role in successful evolution of *S. aureus* as a pathogen, since they are absent in the genomes of the nonpathogenic

Staphylococcus epidermidis and other coagulase-negative staphylococci (Gill et al. 2005; Kuroda et al. 2005; Takeuchi et al. 2005; Baba et al. 2008; Holt et al. 2011). While νSaα and νSaβ are considered to have lost their mobility, it has recently been shown that νSaβ can be horizontally transferred in strains that have a resident prophage located downstream of the genomic island (Moon et al. 2015). The immune evasion proteins encoded by νSaα and νSaβ are generally not human specific (Fig. 3), a feature that is reflected by their broad distribution in both human and animal lineages.

νSaα encodes the *ssl* genes 1 through 11, which are likely a product of duplication of *ssl* genes in an ancestral *S. aureus* strain. The *ssl1, 2, 3,* and *11* genes are present in almost every *S. aureus* strain, but their coding sequence is highly diverse due to a high degree of allelic variance. The allelic variation in *ssl4–ssl10* is less but the presence of these genes is more variable among strains, due to parallel gene loss in strains of different clonal lineages (Fitzgerald et al. 2003; McCarthy and Lindsay 2013). Allelic variation in the *ssl* genes can result in functional differences of the encoded proteins, as is seen for SSL3 and SSL4. SSL3 is generally a far stronger TLR2 inhibitor than SSL4, but there is an exception in one specific strain (MRSA252) that expresses specific allelic variants of the genes. In this strain, the two proteins appear to have switched their TLR2 inhibiting potential through changes in their functional domains (Koymans et al. 2015). The host specificity of the SSLs, as far as examined, is not restricted to humans (Fig. 3). SSL3 and SSL4 target TLR2 of both humans and mice; however, they appear unable to inhibit bovine TLR2. This is probably linked to three TLR2 tyrosines in the binding interface that are conserved between human and mouse, but differ in the bovine TLR2 (Koymans et al., unpublished observations). The functionality of SSL5 has only been studied in humans and mice and activates platelets in both species (Armstrong et al. 2012; Langley and Fraser 2013). SSL7 binds IgA and complement factor C5 from several species (Langley et al. 2005; Bestebroer et al. 2010). However, up to 39 % of the common inbred mice are genetically deficient for C5 and not suitable to study the effects of SSL7 (Cinader et al. 1964; Bestebroer et al. 2010). SSL10 exclusively binds human IgG1, while its other functions, like the binding of prothrombin, have a broader species range (Patel et al. 2010; Itoh et al. 2013b).

The gene content of the νSaβ locus is also highly variable. It can include *lukED*, several serine proteases that are putatively involved in virulence, and the enterotoxin gene cluster (*egc*) (Fig. 3) (Baba et al. 2008). The *egc* contains the genes of several SAgs of which the combination of *seg, sei, selm, seln,* and *selo* seems to be most common (Jarraud et al. 2001; Grumann et al. 2014). Like the *ssl* genes, the SAg genes within the *egc* are likely to be a product of distant gene duplication (Jarraud et al. 2001; Thomas et al. 2006). Both human and animal isolates have been shown to carry the *egc* SAg genes with a frequency of 30-66 %, but their functional activity in animals is unknown (Becker et al. 2003; Holtfreter et al. 2004; Omoe et al. 2005; Smyth et al. 2005; Monecke et al. 2009). The presence of the *egc* cluster within νSaβ is lineage associated, as is the presence of *lukED* (McCarthy and Lindsay 2013; Grumann et al. 2014). LukED is the least host-specific leukocidin of *S. aureus*. In addition to humans, it has shown to be cytotoxic for bovine and

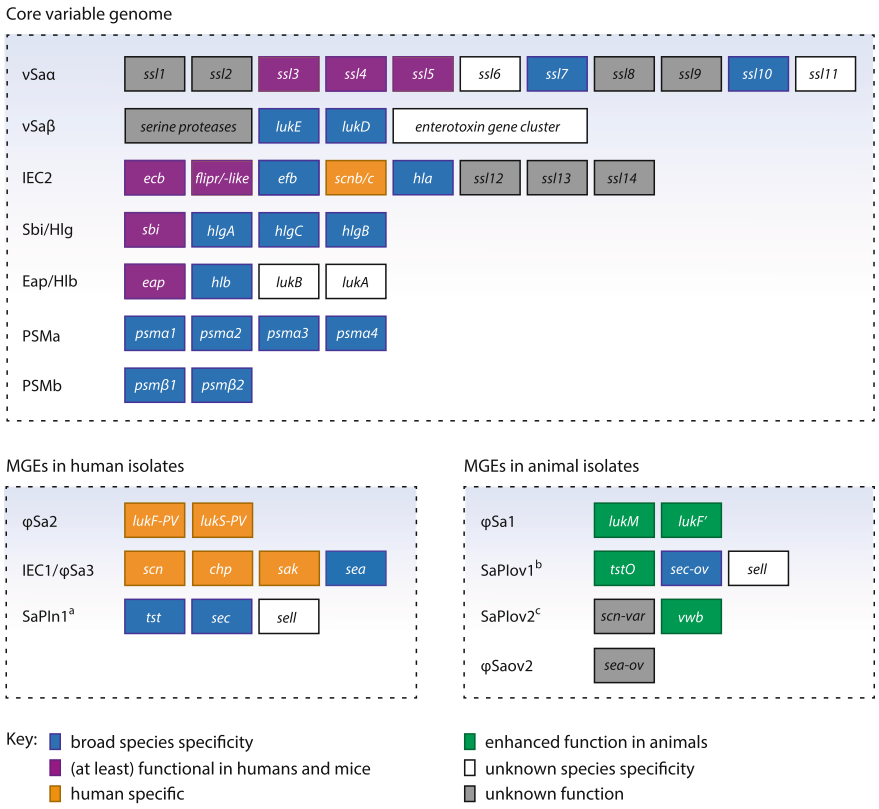


Fig. 3 Genomic distribution and species specificity of *S. aureus* immune evasion molecules. Genes are located in the core variable genome (*upper panel*), on mobile genetic elements (MGEs) specific for human isolates (*lower left panel*), or MGEs specific for animal isolates (*lower right panel*). Colors indicate the functional species specificity of the immune evasion molecules encoded by the displayed genes. Broad species specificity (*blue*) is assigned when an evasion molecule is functional in humans, rabbits, and/or mice, and in one or more large animals (e.g., pigs, sheep, goats, cattle, and horses). The specificity of evasion molecules at least functional in humans and mice (*purple*) has not been confirmed in other animals so far. [a]SaPIn1 is an example of a superantigen (SAg) encoding SaPI present in human strains, SaPIs encoding different combinations of SAgs have also been described (Novick and Subedi 2007). [b]SaPIov1 shares close homology with SaPIbov1, which encodes bovine variants of the same genes (Fitzgerald et al. 2001; Guinane et al. 2010). [c]SaPIov2 shares a considerable degree of homology with SaPIbov4, SaPIbov5, and SaPIeq1, which encode host-specific variants of the same immune evasion genes (Viana et al. 2010). Abbreviations: *chp* (chemotaxis inhibitory protein of *S. aureus*), *eap* (extracellular adherence protein), *ecb* (extracellular complement-binding protein), *efb* (extracellular fibrinogen-binding protein), *flipr* (FPR2 inhibitory protein), *flipr-like* (FPR2 inhibitory protein-like), *hla* (α-hemolysin), *hlb* (β-hemolysin), *hlg* (γ-hemolysin), *luk* (leukocidin), *psm* (phenol-soluble modulin), *sak* (staphylokinase), *sbi* (second immunoglobulin-binding protein), *scn* (staphylococcal complement inhibitor), *scnb/c* (staphylococcal complement inhibitor B/C), *scn-var* (staphylococcal complement inhibitor variant), *se* (staphylococcal enterotoxin), *sea-ov* (staphylococcal enterotoxin A-ovine), *sel* (staphylococcal enterotoxin-like), *sec-ov* (staphylococcal enterotoxin C-ovine), *ssl* (staphylococcal superantigen-like protein), *tst* (toxic shock syndrome toxin-1), *tstO* (toxic shock syndrome toxin-1-ovine), *vwb* (von Willebrand factor-binding protein)

murine neutrophils (Barrio et al. 2006; Reyes-Robles et al. 2013; Alonzo et al. 2013). LukED is the only leukocidin with a clear in vivo phenotype in mice, where survival of animals is negatively correlated with the presence of *lukED* (Reyes-Robles et al. 2013; Alonzo et al. 2013). While the sequence of LukED has been described to be highly conserved in a set of 88 isolates (McCarthy and Lindsay 2013), there are some variations to this theme. For instance, the strains Newman and V8 express a different form of LukE that has shown to have a different biological activity (Morinaga et al. 2003; Barrio et al. 2006). Also of note is the loss of function of LukE in ovine strain ED133 of the ruminant lineage CC133, which might indicate a lack of requirement of this toxin in ruminant disease pathogenesis (Guinane et al. 2010).

4.1.2 Immune Evasion Cluster 2 (IEC2)

IEC2 is a large cluster of immune evasion genes that is present in the core variable genome, while the earlier discovered immune evasion cluster 1 (IEC1) is located on a MGE and will be discussed later (van Wamel et al. 2006). Like the genomic islands, IEC2 contains mobile elements and bacteriophage remnants and is therefore suggested to be introduced by horizontal gene transfer (Jongerius et al. 2007). IEC2 encodes genes of immune evasion proteins that target a broad host range such as *efb*, *ecb*, *flipr/flipr-like,* and *hla* (Fig. 3). Ecb and Efb are functional complement inhibitors in both humans and mice (Jongerius et al. 2007; Ko et al. 2011; Jongerius et al. 2012), and Efb can also inhibit complement-mediated phagocytosis in cattle (Boerhout et al. 2015). FLIPr and FLIPr-like both efficiently inhibit phagocytosis by human neutrophils, while only FLIPr-like has similar efficiency in mice (Stemerding et al. 2013). The *hla* gene encodes for α-hemolysin which has a very broad host range including cattle, rabbits, guinea pigs, horses, and pigs (Phimister and Freer 1984; Cifrian et al. 1996; Inoshima et al. 2011; Anderson et al. 2012; Berube and Bubeck Wardenburg 2013). Of note is the extreme sensitivity of rabbits and rabbit erythrocytes to α-hemolysin (Cooper et al. 1964; Arbuthnott 1970). In several experimental rodent and rabbit models, α-hemolysin has been shown to contribute to *S. aureus* pneumonia, peritonitis, infections of the skin, cornea, endocardium, central nervous system, and the mammary gland (Berube and Bubeck Wardenburg 2013; Salgado-Pabón and Schlievert 2014). In hospitalized patients, α-hemolysin production has been associated with *S. aureus* peritonitis and ventilator-associated pneumonia (Barretti et al. 2009; Stulik et al. 2014). Together, this suggests that α-hemolysin is indeed an important virulence factor in human *S. aureus* disease.

The only human-specific immune evasion gene on IEC2 is *scn-b/c*. IEC2 contains either one of both allelic variants, *scn-b* or *c* (McCarthy and Lindsay 2013) and the activity of SCIN-B/C corresponds to that of SCIN located on immune evasion cluster 1 (IEC1) (Jongerius et al. 2007; Garcia et al. 2012b). The IEC2 genes are present in almost every *S. aureus* strain and the allelic variants of its genes are distributed in a lineage-specific manner. Of note is the occurrence of a

nonsense mutation in the *hla* gene in some strains, resulting in the expression of a non-functional protein (DeLeo et al. 2011). The *ssl12-14* genes are also located on IEC2 and thereby lie 700 kb upstream of the other *ssl* genes, but no function has been assigned to them yet.

4.1.3 Other Clustered Immune Evasion Genes

All *S. aureus* strains harbor the genes of at least three leukocidins in their core variable genome; HlgAB, HlgCB, and LukAB. The γ-hemolysin (*hlgABC*) genes are part of the *sbi/hlg* locus (Fig. 3) (Cooney et al. 1993). There is some genetic diversity among coding sequences of the *hlgABC* genes (McCarthy and Lindsay 2013), while the adjacent *sbi* genomic sequence is highly conserved throughout (both animal and human) isolates (Atkins et al. 2008). Sbi is functional in humans and rodents, but its activity in large animals (e.g., cow, sheep, horse, and goat) seems to be limited (Atkins et al. 2008; Haupt et al. 2008). The γ-hemolysins are active on neutrophils of humans, rabbits, and cattle (Spaan et al. 2015b). Murine neutrophils, however, are not susceptible, which complicates the use of mouse models to study the effects of γ-hemolysins in vivo (Spaan et al. 2014). In mice, the effects of γ-hemolysins are limited to $CCR2^+$ cells (e.g., inflammatory macrophages). While HlgAB still contributes to *S. aureus* bacteremia in this animal model, the full-blown in vivo effects of both HlgAB and HlgCB are probably severely underestimated. The genes for *lukAB* are located adjacent to the *eap/hlb* locus (Fig. 3). LukAB specifically targets the I-domain of human but not murine CD11b (DuMont et al. 2013). While there is low-to-intermediate toxicity observed in rabbits, murine neutrophils and macrophages are highly resistant to LukAB-mediated killing (Malachowa et al. 2012; DuMont et al. 2013). Surprisingly, two studies did find an in vivo effect of LukAB in a murine renal abscess (Dumont et al. 2011) and an orthopedic implant biofilm model (Scherr et al. 2015), indicating that LukAB still has some residual activity toward murine leukocytes. However, as posed earlier, mouse models do not provide a suitable context to study the complete effects of, or therapeutic approaches involving, leukocidins such as LukAB and HlgABC, because of their strongly reduced affinity for murine leukocytes. The susceptibility of large animal leukocytes to the toxic effects of LukAB, unknown as yet, will provide an answer to the question if LukAB is a human-specific toxin. The *eap* gene is very versatile and has 15 different alleles (McCarthy and Lindsay 2013). The number of EAP domains (ranging 4–6) encoded by the *eap* gene differs per allele and might have functional implications for the resulting Eap protein (Stapels et al. 2014). The host specificity of Eap has not been extensively studied, but Eap is at least also functional in mice (Cheng et al. 2009). Eap contributes to an increased bacterial load in *S. aureus* infections in mice, in synergy with its homologs EapH1 and EapH2 (Stapels et al. 2014).

The *psmα1–4* genes are located together and encode for the α-type PSMs, whereas the *psmβ1–2* genes lie on another cluster and encode for the β-type PSMs (Fig. 3). Not all *S. aureus* lineages encode the *psmβ2* gene (McCarthy and Lindsay

2013). The *hld* gene is located separately on the chromosome and encodes for δ-toxin, also a member of the PSMα family. Recently, an allelic variant of PSMα3 has been described that has differential cytolytic properties (Cheung et al. 2014a). PSMs are not species specific, which can be explained by the fact that there is no receptor involved in the cytolytic process (Wang et al. 2007; Löffler et al. 2010). Therefore, mouse models could successfully be implemented and have shown that PSMs modulate immune responses and contribute to *S. aureus* disease in vivo (Wang et al. 2007; Kretschmer et al. 2010; Nakamura et al. 2013).

The *spa* gene is an example of a core variable genome-encoded immune evasion protein that is not located in a cluster. The *spa* gene is ubiquitous in *S. aureus* and highly variable and therefore often used for strain typing. Its length can differ between isolates resulting in truncation of the protein or expression of different numbers of IgG-binding domains (Herron-Olson et al. 2007; Atkins et al. 2008; Stutz et al. 2011). SpA has immune modulating functions in multiple species and contributes to *S. aureus* abscess formation in mice (Goodyear and Silverman 2003; Atkins et al. 2008; Kim et al. 2011, 2015). Recently, it was shown that guinea pigs provide a suitable model to evaluate therapeutic approaches directed toward SpA in vivo (Kim et al. 2015). The *selx* gene encoding for the superantigen SElX is also not located in a cluster and is presumed to have been horizontally acquired by a distant progenitor of *S. aureus*. This gene has subsequently diversified among *S. aureus* lineages and has multiple allelic variants that differ in coding sequence. SElX variants from human and ruminant isolates differ in mitogenicity toward human or bovine T cells, indicating that the allelic diversification of *selx* has had functional properties for the encoded protein (Wilson et al. 2011). Other singly occurring immune evasion genes are *coa* and *vwb*. These genes encoding for, respectively, SC and vWbp are found in most *S. aureus* strains and their proteins effectively coagulate human and murine plasma (Cheng et al. 2010), but not ruminant plasma (Friedrich et al. 2006; Viana et al. 2010).

4.2 Mobile Genetic Elements

4.2.1 SaPIs

The SaPIs belong to the family of mobile phage-related pathogenicity islands that are found primarily in *S. aureus* but also in other Gram-positive bacteria, including streptococci and lactococci (Lindsay et al. 1998; Novick et al. 2010). The SaPIs are not mobile by themselves, but hijack the capsid of phages for their horizontal transfer (Novick et al. 2010; Quiles-Puchalt et al. 2014). To date, at least 16 different SaPIs have been identified in the genome of *S. aureus* (Subedi et al. 2007). Most of the SaPIs carry a combination of two or three SAg genes (Novick and Subedi 2007) (Fig. 3). However, the gene content of SaPIs is variable as a result of intensive recombination (Subedi et al. 2007). For example, the SaPIn1 in strain N315 carries the SAg-encoding genes *sell*, *sec*, and *tst*, while the SaPI3 encoded by

COL carries *seb*, *selk,* and *selq* (Baba et al. 2002; Novick and Subedi 2007; Alibayov et al. 2014). The *tst* gene is exclusively encoded by SaPIs, and carriage of specific SaPIs is therefore directly linked to the ability of a strain to cause menstrual toxic shock syndrome (Penadés and Fitzgerald 2009). As discussed, all SAgs can stimulate T cells but exhibit different preferences for TCR Vβ and MHC class II profiles (Fraser and Proft 2008). Therefore, the effect of SAgs can differ between human individuals and also between different species (Wilson et al. 2011). Mouse models have been hampered by the fact that murine MHC-II responds differently to SAgs than human MHC-II (Yeung et al. 1996). Mice are $>10^9$ times more resistant to SAgs than humans, while the sensitivity of rabbits resembles that of humans (Schlievert 2009). In rabbit models, it has been shown that the SaPI-encoded SAgs contribute to *S. aureus* toxic shock syndrome, endocarditis, sepsis, and kidney injury in vivo (Dinges et al. 2003; Buonpane et al. 2007; Salgado-Pabón et al. 2013; Spaulding et al. 2013). SAg-sensitive transgenic mice expressing HLA-DR4 can also be used to study the effects of SAgs in vivo (Xu et al. 2014, 2015). In addition, cattle are also reported to be susceptible to the effects of several SAgs (Yokomizo et al. 1995; Tollersrud et al. 2006; Wilson et al. 2011).

Genome analysis of ruminant *S. aureus* strains ED133 (Guinane et al. 2010) and RF122 (Herron-Olson et al. 2007) led to the discovery of specific SaPIs of animal isolates. These SaPIs, SaPIovine1 (SaPIov1) and SaPIbovine1, (SaPIbov1) encode host-specific variants of *tst*, *sec*, and *sell* (Fitzgerald et al. 2001) of which the SEC and TSST-1 variants have shown to vary in biological activity in comparison with the proteins encoded by human strains (Lee et al. 1992; Deringer et al. 1997) (Fig. 3). Other animal-related SaPIs (SaPIov2, SaPIbov4, SaPIbov5, and SaPIequine1 (SaPIeq1)) encode allelic variants of the *vwb* gene that differ in sequence from the chromosomally encoded gene that is present in all *S. aureus* isolates (Bjerketorp et al. 2002, 2004; Guinane et al. 2010; Viana et al. 2010). While the chromosomally encoded vWbp is mostly unable to coagulate animal blood, the SaPI-encoded vWbp variants of equine and ruminant isolates do have the ability to coagulate blood of their specific host (Fig. 3). Also, the SaPI-carried *vwp* genes are differentially regulated than the chromosomally encoded ones. Together, this indicates that acquisition of these SaPIs might be important for adaptation to a specific host (Viana et al. 2010). Finally, the ruminant, porcine, and equine SaPIs also carry homologs of three other putative virulence genes, including SCIN (Rooijakkers et al. 2005a; Viana et al. 2010; Schijffelen et al. 2010), but their function is yet to be determined.

4.2.2 Prophages

Bacteriophages can transfer their DNA from one bacterial cell to another, where it is integrated at a specific site in the chromosome. Integrated bacteriophage genomes (prophages) of *S. aureus* also encode immune evasion genes that usually have conserved coding sequences and display little allelic variance (McCarthy and Lindsay 2013). These genes can be disseminated horizontally through transduction,

as reviewed in detail by (Lindsay 2014). The leukocidins PVL and LukMF' are examples of prophage-encoded virulence factors (Zou et al. 2000; McCarthy et al. 2012c). The *pvl* gene is located on ϕSa2 and present in only 2–3 % of the *S. aureus* isolates (Kuehnert et al. 2006). However, in community-acquired (CA)-MRSA strains and strains causing necrotizing pneumonia, the prevalence of PVL is extremely high (>85 %) (Lina et al. 1999; Gillet et al. 2002; Hidron et al. 2009). Also, the successful spread of the PVL-carrying clone USA300 in the USA has increased the prevalence of *pvl* in CA-MRSA strains (Vandenesch et al. 2003; DeLeo et al. 2010). It is unclear whether carriage of the PVL encoding ϕSa2 phage is responsible for the enhanced virulence of CA-MRSA strains (DeLeo et al. 2010). In other countries, i.e., Korea and the UK, an increasing number of CA-MRSA clones are found that do not contain the PVL genes (Otter and French 2008; Lee et al. 2010). PVL is a predominantly human-specific toxin, and in line with this, the ϕSa2 phage is only found in human *S. aureus* isolates (McCarthy and Lindsay 2013) (Fig. 3). The incompatibility of murine C5aR1 with PVL has been a great setback for studying the contribution of PVL to *S. aureus* pathogenesis in in vivo models. Only rabbits are described to confer some degree of susceptibility to this toxin (Spaan et al. 2013). While an isogenetic deletion mutant of *pvl* does seem to benefit rabbit survival in a pneumonia model (Diep et al. 2010), other in vivo infection models in rabbits have shown more ambiguous results (Diep et al. 2008; Crémieux et al. 2009; Lipinska et al. 2011; Kobayashi et al. 2011). To date, the role of *pvl* in *S. aureus* pathogenesis remains elusive.

The *lukmf'* genes are located on the ϕSa1 phage, which has a different genetic makeup and distribution than the ϕSa2 phage encoding *pvl* (Zou et al. 2000; Guinane et al. 2010; Schlotter et al. 2012). The *lukmf'* genes are mainly harbored by isolates from ruminant origin and are absent in human strains (Yamada et al. 2005; Herron-Olson et al. 2007; Schlotter et al. 2012). While LukMF' has a very profound lytic effect on bovine neutrophils, human neutrophils are fully resistant to its toxicity because they lack expression of CCR1 (Vrieling et al. 2015) (Fig. 3). Apart from cattle, other ruminants such as goats, sheep, and rodents are also susceptible to LukMF'-mediated cytotoxicity (Rainard et al. 2003; Guinane et al. 2008; Fromageau et al. 2010; Simpson et al. 2013). The prevalence of *lukmf'* in bovine isolates ranges from 10 to 86 % and differs per geographic region and site of isolation (Rainard et al. 2003; Yamada et al. 2005; Monecke et al. 2007; Schlotter et al. 2012; Padmaja and Halami 2013). Carriage of *lukmf'* has been associated with mastitis in ruminants (Rainard et al. 2003; Yamada et al. 2005); however, its exact role in the pathogenesis, severity, or persistence of mastitis is yet to be determined experimentally in vivo.

The IEC1 cluster is carried on the ϕSa3 prophage and harbors a combination of the *scn*, *sak,* and *chp* genes (van Wamel et al. 2006). These genes encode immune evasion proteins that are highly human specific. SCIN, like SCIN-B/C, is unable to inhibit complement activation of mouse, rat, dog, sheep, guinea pig, goat, and cow (Rooijakkers et al. 2005a). SAK can activate plasminogen of humans, dogs, and baboons, but not of large animals (horses, pigs, cows, and sheep), rabbits, or mice (Gladysheva et al. 2003). CHIPS does not bind to neutrophils of rabbit, pig, mouse,

guinea pig, rat, and dog and the functional blockade of the C5aR1 is inefficient in these species (Postma et al. unpublished observations). In accordance with the human-specific function of the genes on IEC1, the carriage of ϕSa3 is associated with human isolates of *S. aureus* (Sung et al. 2008; Verkaik et al. 2011) (Fig. 3). An exceptional high percentage of human clinical isolates (>90 %) carry IEC1, indicating that virulence factors encoded by this cluster might be important for infection of humans (van Wamel et al. 2006). The prevalence of the IEC1 in animal isolates is generally low (Monecke et al. 2007; Kumagai et al. 2007; Cuny et al. 2015). During evolution of *S. aureus*, IEC1 is lost in strains that become associated with animals, while it remains conserved in human-associated strains (Ward et al. 2014; Viana et al. 2015). Insertion of the IEC1 cluster results in disruption of the *hlb* gene and therefore the phages encoding IEC1 are also known as β-hemolysin converting bacteriophages (van Wamel et al. 2006). There is some evidence that β-hemolysin plays a role in animal diseases like bovine mastitis (Cifrian et al. 1996; Aarestrup et al. 1999). However, the exact role of β-hemolysin in bacterial pathogenesis in animals (and humans) is still unclear. In addition to *scn*, *sak,* and *chp*, ϕSa3 can harbor several SAg genes including *sea*, *selk, selq*, and *selp* (Baba et al. 2002; van Wamel et al. 2006; McCarthy et al. 2012c). While *sea* was observed to be absent in most animal isolates (Smyth et al. 2005), ovine strain ED133 encodes a variant of *sea* (*sea-ov*, 87 % identity with *sea*) on a newly identified phage named ϕSaovine2 (ϕSaov2) (Guinane et al. 2010) (Fig. 3). Also of interest is the discovery of a novel member of the β-converting phage family (ϕAvβ), a prophage harbored by avian isolates of *S. aureus*. This prophage lacks the genes of IEC1 but encodes other virulence factors putatively involved in avian-niche adaptation (Lowder et al. 2009; Price et al. 2012).

4.3 Host Adaptation of Immune Evasion Molecules

Immune evasion genes play a major role in host adaptation. While some immune evasion clusters are on MGEs and have retained their ability to move, other clusters, such as the genomic islands and IEC2, have become immobile and are now considered to be part of the core variable genome of *S. aureus*. Allelic variation of immune evasion genes and host specificity of the encoded proteins are related to the mobility of their gene cluster. The immobile genomic clusters are present in virtually all strains, and their genes display a large degree of allelic variance between lineages (McCarthy and Lindsay 2013). The encoded immune evasion proteins generally have a broad species specificity, but allelic diversification may influence the function of these proteins in different hosts (Wilson et al. 2011). The broad species specificity of these immune evasion proteins makes it possible to study their function in common animal models, such as mice and rabbits (Jongerius et al. 2007; Wang et al. 2007; Alonzo et al. 2013).

In contrast to the gene clusters in the core variable genome, MGEs have a lineage as well as a host-restricted distribution and their genes generally show little

allelic variation (McCarthy and Lindsay 2013). Interestingly, the function of the MGE-encoded immune evasion proteins is also highly species specific. These evasion proteins typically have a high affinity for their host-specific targets (de Haas et al. 2004; Rooijakkers et al. 2005a; Spaan et al. 2013; Vrieling et al. 2015). Coevolution of MGEs with a specific host may have increased the affinity of encoded immune evasion proteins for the host's immune system, in turn coinciding with a loss of function in other species. This might be an explanation for the human-restricted functions of the IEC1-encoded proteins SCIN, CHIPS, and SAK. The lack of animal models to study these human-specific proteins has limited the understanding of their importance in the pathogenesis of *S. aureus* disease.

Future research should be directed toward explaining the molecular basis of host adaptation and the role of MGEs in this process. The identification of more MGE-encoded immune evasion molecules will provide insights into the pathogenesis in different hosts and may provide new therapeutic approaches to treat *S. aureus* disease in humans and animals.

5 Future Perspectives

It is clear that *S. aureus* has evolved multiple ways to prevent immune recognition and sabotage immune cell function, on a much larger scale than any other bacterium. Knowledge of these immune evasion strategies, their structure–function, and host relationships can aid in the development of new therapeutic strategies. A cocrystal structure can provide a highly visual and comprehensive definition of an interaction interface (Malito et al. 2015) and host specificity is important to take into account when performing in vivo studies in animals and translating these findings to other species. The immune evasion proteins are interesting for the development of novel pharmaceutical compounds in two distinct ways. Firstly, they can be used to interfere in staphylococcal virulence. Secondly, the immune evasive properties can be used in situations where there is aberrant activation of the immune system that leads to inflammatory diseases. We will review these two potential uses briefly in the next chapter.

5.1 *Therapeutic Strategies Based on Evasion Molecules for* S. aureus *Infections*

Novel therapeutic strategies to combat *S. aureus* infections are critically needed because of the rise in antibiotic-resistant strains, the limited availability of therapeutics, and the absence of a working vaccine. The limited therapeutic options are in part due to the interference of *S. aureus* in both innate and adaptive immune responses. This is because successful therapies, such as vaccination, rely on robust

immune responses to take effect (Alonzo and Torres 2014; Scully et al. 2014). Thus, the staphylococcal evasion proteins provide promising candidates for new anti-staphylococcal approaches. If neutralization of these proteins can be induced, either through vaccination, monoclonal antibodies (mAbs), or through the use of inhibitory small molecules, the immune system might be able to battle off the infection and even generate a successful immune memory.

Therapeutic strategies always have to be evaluated in vivo. One major obstacle of studying these virulence factors in vivo is that often mice are used as model organisms. Mice are highly resistant to staphylococcal infections. To illustrate, for most mouse models up to 10^8 bacteria are required to reach infection, whereas less than 100 bacteria can be enough to cause disease in a natural host. This discrepancy in doses may, in part, be explained by the activity of the immune evasion molecules that, as discussed in Chap. 4, often do not function in mice. Indeed, a lot of vaccine studies that have been tested in mice and conferred protection in these models could not be successfully translated to humans (Fowler and Proctor 2014). A way to overcome this could be to generate humanized mice that express human receptors and factors that are critical for disease. However, even here the problem remains that only the effect of an isolated protein is studied. It would be better to move toward species that are more susceptible to *S. aureus* infections and are a natural host, such as cattle, poultry, and rabbits. The principles found in studies directed toward preventing disease in these natural hosts might be translated into the human system, as long as species-specific factors are taken into account. Most of the newer vaccine designs aim at simultaneously targeting multiple virulence factors, since it was found that neutralizing one virulence factor was probably not enough given the great number of immune evasion molecules secreted by *S. aureus* (Fowler and Proctor 2014; Scully et al. 2014).

Besides vaccination, there are ongoing studies seeking to develop mAbs or small molecules targeted against host receptors or evasion proteins. Treatment with maraviroc, the CCR5 inhibitor often used in the treatment of HIV, protects T cells and myeloid cells from LukED-mediated toxicity (Alonzo et al. 2013) and treatment of bovine neutrophils with a CCR1 antagonist protects these cells from LukMF'-mediated lysis (Vrieling et al. 2015). Thus, targeting the toxin receptors might be a good therapeutic strategy. The toxins themselves can also be targeted and may make good mAb or vaccine candidates. Several studies showed that neutralizing antibodies against α-hemolysin conferred protection in mice models (Adhikari et al. 2012b; Foletti et al. 2013). These studies might be translatable to humans since antibody levels were found to be correlated with protection against *S. aureus* infections in children (Fritz et al. 2013), while low anti-α-hemolysin antibody levels were found to be associated with sepsis in hospitalized adults (Adhikari et al. 2012a). Correlates between protection and antibody levels have also been found for PVL, some superantigens, and several PSMs (Adhikari et al. 2012a).

Structural studies can be useful in understanding mechanisms of protection and allow for the bench-to-bedside transition. mAbs can be developed that target multiple *S. aureus* proteins, based on a shared structure. A single mAb has already been developed that can neutralize both α-hemolysin and four bicomponent

leukotoxins (Rouha et al. 2015). Neutralization is based on the recognition of a shared conformational epitope. These strategies are promising because they target multiple proteins at once, which might be necessary for good therapeutic efficiency because of the redundancy of the evasion proteins. Overall, therapeutic targeting of staphylococcal toxins is an interesting strategy that should be explored further.

5.2 *Therapeutic Strategies for Other Inflammatory Conditions and Cancer*

The anti-inflammatory properties of the evasion proteins can be used to combat diseases in which there is aberrant immune activation. Often, the actual evasion molecules cannot be used directly because they are too immunogenic. This creates a need for the construction of hypoimmunogenic derivatives. To attain this, (co)-crystal structures are extremely valuable as they provide us with the exact binding interface and the involved amino acid through which the construction of derivatives can be facilitated. Based on the crystallographic data, structured peptide derivatives can be designed.

Apart from being crucial in antimicrobial defense, immune receptors can be involved in the development of disease. In these situations, where the balance has tipped the wrong way, the evasion molecules provide us with highly specific strategies to inhibit these aberrant responses. For example, complement, several GPCRs, and Toll-like receptors can play central roles in human inflammatory diseases and could therefore be interesting therapeutic targets (Allegretti et al. 2005; Liu et al. 2013; Ricklin and Lambris 2013). A lot of immune evasion molecules target these systems and hence might be used in therapeutic settings and some studies have already explored this concept. Through a phage display approach, SCIN-derived peptides were identified that retained the complement inhibitory activity of SCIN (Summers et al. 2015). In a more targeted approach, a non-immunogenic mimic of the C5aR inhibitor CHIPS was produced. It was designed to include all important amino acids based on the C5aR-CHIPS cocrystal structure and the 50-residue sulfated peptide showed formation of structural elements (Bunschoten et al. 2011). Similar strategies could be used to generate more anti-inflammatory molecules. For example, SSL3-derived peptides could be able to block aberrant TLR2 activation, whereas SSL5-derived peptides could inhibit PSGL-1.

The other approach that is being explored is to use the molecules or derivatives in anticancer strategies. Some of the immune receptors are involved in the growth or metastasis of different types of cancers. Activation of FPR1 can promote brain tumor growth. Treatment of mice implanted with human, FPR1 expressing, astrocytoma cells with CHIPS reduced tumor growth and prolonged survival of the mice (Boer et al. 2013). SSL5 has also been explored for its antitumor activities. Rolling and extravasation of cancer cells, processes involved in tumor metastasis, can be

mediated through PSGL-1 and P-selectin. SSL5 showed to inhibit the P-selectin and PSGL-1 interaction on leukemic cells (Walenkamp et al. 2010). Furthermore, SSL5 can inhibit tumor cell to platelet adherence, a process described to induce tumor growth and metastasis (Walenkamp et al. 2010). Its family members SSL6 and SSL10 could be interesting because they block CD47 and CXCR4, respectively (Walenkamp et al. 2009; Fevre et al. 2014). CD47 expression is increased on various tumor cells and this protects them from phagocytosis (Willingham et al. 2012). The same study showed that blocking CD47 with a mAb in mouse models results in attenuated tumor growth, reduced metastasis, and increased survival. Similarly, blockage of CXCR4 could be beneficial since its expression has been linked to many different types of cancer and it is involved in tumor dissemination (Walenkamp et al. 2009). Altogether, immune evasion proteins might provide novel therapeutic options in the treatments of many different kinds of cancer.

6 Conclusions

For pathogenic bacteria, immune evasion is crucial, especially in the early phase of invasion, to escape the acute attack of the immune system and to create a window of opportunity in order to divide. *S. aureus* has evolved at least 35 proteins to assist in this process. These structurally highly related evasion molecules have distinct molecular targets within the host immune system. Despite the very low sequence homology, these proteins conform to a remarkably small number of folds, thus protein structure is more conserved than sequence. These structured folds form ideal scaffolds for amino acid variation to favor adjustments in protein–protein interactions. Most immune evasion molecules have evolved from common ancestors and gene duplications. The target range and specificity is explained by high-affinity protein–protein interactions that over time, resulted in the host adaptation we observe today. This is aided by the flexibility of the *S. aureus* genome and its MGEs. Phages and pathogenicity islands especially contribute to the spread of evasion molecules through different lineages of *S. aureus* and form the foundation of host adaptation. Future identification of novel MGE-encoded virulence genes and of the function of the encoded proteins contributes to our understanding of the coevolution of pathogen and host. Unraveling the pathogenesis of *S. aureus* disease in different hosts will pave the way for the development of new therapeutics in humans and animals.

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References

Aarestrup FM, Larsen HD, Eriksen NH et al (1999) Frequency of alpha- and beta-haemolysin in *Staphylococcus aureus* of bovine and human origin. A comparison between pheno- and genotype and variation in phenotypic expression. APMIS 107:425–430

Abdelbary MMH, Wittenberg A, Cuny C et al (2014) Phylogenetic analysis of *Staphylococcus aureus* CC398 reveals a sub-lineage epidemiologically associated with infections in horses. PLoS ONE 9:e88083

Adhikari RP, Ajao AO, Aman MJ et al (2012a) Lower antibody levels to *Staphylococcus aureus* exotoxins are associated with sepsis in hospitalized adults with invasive *S. aureus* infections. J Infect Dis 206:915–923

Adhikari RP, Karauzum H, Sarwar J et al (2012b) Novel structurally designed vaccine for *s. aureus* α-hemolysin: protection against bacteremia and pneumonia. PLoS One 7:e38567

Alibayov B, Zdenkova K, Sykorova H, Demnerova K (2014) Molecular analysis of *Staphylococcus aureus* pathogenicity islands (SaPI) and their superantigens combination of food samples. J Microbiol Methods 107:197–204

Allegretti M, Moriconi A, Beccari AR et al (2005) Targeting C5a: recent advances in drug discovery. Curr Med Chem 12:217–236

Alonzo F, Torres VJ (2014) The bicomponent pore-forming leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78:199–230

Alonzo F, Kozhaya L, Rawlings SA et al (2013) CCR5 is a receptor for *Staphylococcus aureus* leukotoxin ED. Nature 493:51–55

Anderson MJ, Lin Y-C, Gillman AN et al (2012) Alpha-toxin promotes *Staphylococcus aureus* mucosal biofilm formation. Front Cell Infect Microbiol 2:1–10

Arbuthnott J (1970) Staphylococcal alpha toxin. Microbial Toxins 189–236

Arcus V (2002) OB-fold domains: a snapshot of the evolution of sequence, structure and function. Curr Opin Struct Biol 12:794–801

Armstrong PCJ, Hu H, Rivera J et al (2012) Staphylococcal superantigen-like protein 5 induces thrombotic and bleeding complications in vivo: Inhibition by an anti-SSL5 antibody and the glycan Bimosiamose. J Thromb Haemost 10:2607–2609

Atkins KL, Burman JD, Chamberlain ES et al (2008) S. aureus IgG-binding proteins SpA and Sbi: Host specificity and mechanisms of immune complex formation. Mol Immunol 45:1600–1611

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

Baba T, Bae T, Schneewind O et al (2008) Genome sequence of *Staphylococcus aureus* strain newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J Bacteriol 190:300–310

Badarau A, Rouha H, Malafa S et al (2015) Structure-function analysis of heterodimer formation, oligomerization, and receptor binding of the *Staphylococcus aureus* bi-component toxin LukGH. J Biol Chem 290:142–156

Baker HM, Basu I, Chung MC et al (2007) Crystal structures of the staphylococcal toxin SSL5 in complex with sialyl Lewis X reveal a conserved binding site that shares common features with viral and bacterial sialic acid binding proteins. J Mol Biol 374:1298–1308

Bardoel BW, Vos R, Bouman T et al (2012) Evasion of toll-like receptor 2 activation by staphylococcal superantigen-like protein 3. J Mol Med 90:1109–1120

Barretti P, Montelli AC, Batalha JEN et al (2009) The role of virulence factors in the outcome of staphylococcal peritonitis in CAPD patients. BMC Infect Dis 9:212

Barrio M, Rainard P, Prévost G (2006) LukM/LukF′-PV is the most active *Staphylococcus aureus* leukotoxin on bovine neutrophils. Microbes Infect 8:2068–2074

Bassetti M, Nicco E, Mikulska M (2009) Why is community-associated MRSA spreading across the world and how will it change clinical practice? Int J Antimicrob Agents 34:S15–S19

Becker K, Friedrich AW, Lubritz G 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 41:1434–1439

Becker REN, Berube BJ, Sampedro GR et al (2014a) Tissue-specific patterning of host innate immune responses by *Staphylococcus aureus* α-toxin. J Innate Immun 6:619–631

Becker S, Frankel MB, Schneewind O, Missiakas D (2014b) Release of protein A from the cell wall of *Staphylococcus aureus*. Proc Natl Acad Sci USA 111:1574–1579

Berends ETM, Horswill AR, Haste NM et al (2010) Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. J Innate Immun 2:576–586

Berends ETM, Kuipers A, Ravesloot MM et al (2014) Bacteria under stress by complement and coagulation. FEMS Microbiol Rev 38:1146–1171

Berube BJ, Bubeck Wardenburg JB (2013) *Staphylococcus aureus* α-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166

Bestebroer J, Poppelier MJJG, Ulfman LH et al (2007) Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. Blood 109:2936–2943

Bestebroer J, Van Kessel KPM, Azouagh H et al (2009) Staphylococcal SSL5 inhibits leukocyte activation by chemokines and anaphylatoxins. Blood 113:328–337

Bestebroer J, Aerts PC, Rooijakkers SHM et al (2010) Functional basis for complement evasion by staphylococcal superantigen-like 7. Cell Microbiol 12:1506–1516

Bjerketorp J, Nilsson M, Ljungh A et al (2002) A novel von Willebrand factor binding protein expressed by *Staphylococcus aureus*. Microbiology 148:2037–2044

Bjerketorp J, Jacobsson K, Frykberg L (2004) The von Willebrand factor-binding protein (vWbp) of *Staphylococcus aureus* is a coagulase. FEMS Microbiol Lett 234:309–314

Boer JC, Domanska UM, Timmer-Bosscha H et al (2013) Inhibition of formyl peptide receptor in high-grade astrocytoma by chemotaxis inhibitory protein of *S. aureus*. Br J Cancer 108:587–596

Boerhout E, Vrieling M, Benedictus L et al (2015) Immunization routes in cattle impact the levels and neutralizing capacity of antibodies induced against *S. aureus* immune evasion proteins. Vet Res 46:115

Bunschoten A, Ippel JH, Kruijtzer JAW et al (2011) A peptide mimic of the chemotaxis inhibitory protein of *Staphylococcus aureus*: towards the development of novel anti-inflammatory compounds. Amino Acids 40:731–740

Buonpane RA, Churchill HRO, Moza B et al (2007) Neutralization of staphylococcal enterotoxin B by soluble, high-affinity receptor antagonists. Nat Med 13:725–729

Burman JD, Leung E, Atkins KL et al (2008) Interaction of human complement with Sbi, a staphylococcal immunoglobulin-binding protein: indications of a novel mechanism of complement evasion by *Staphylococcus aureus*. J Biol Chem 283:17579–17593

Burroughs M, Balaji S, Iyer LM, Aravind L (2007) Small but versatile: the extraordinary functional and structural diversity of the beta-grasp fold. Biol Direct 2:18

Cheng AG, Kim HK, Burts ML et al (2009) Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. FASEB J 23:3393–3404

Cheng AG, McAdow M, Kim HK et al (2010) Contribution of coagulases towards *Staphylococcus aureus* disease and protective immunity. PLoS Pathog 6:e1001036

Cheung GYC, Kretschmer D, Duong AC et al (2014a) Production of an attenuated phenol-soluble modulin variant unique to the MRSA clonal complex 30 increases severity of bloodstream infection. PLoS Pathog 10:e1004298

Cheung GYC, Kretschmer D, Queck SY et al (2014b) Insight into structure-function relationship in phenol-soluble modulins using an alanine screen of the phenol-soluble modulin (PSM) α3 peptide. FASEB J 28:153–161

Choi YW, Herman A, DiGiusto D et al (1990) Residues of the variable region of the T-cell-receptor beta-chain that interact with *S. aureus* toxin superantigens. Nature 346:471–473

Chung MC, Wines BD, Baker H et al (2007) The crystal structure of staphylococcal superantigen-like protein 11 in complex with sialyl Lewis X reveals the mechanism for cell binding and immune inhibition. Mol Microbiol 66:1342–1355

Cifrian E, Guidry AJ, Bramley AJ et al (1996) Effect of staphylococcal beta toxin on the cytotoxicity, proliferation and adherence of *Staphylococcus aureus* to bovine mammary epithelial cells. Vet Microbiol 48:187–198

Cinader B, Dubiski S, Wardlaw AC (1964) Distribution, inheritance, and properties of an antigen, MUB1, and its relation to hemolytic complement. J Exp Med 120:897–924

Cooney J, Kienle Z, Foster TJ, O'Toole PW (1993) The gamma-hemolysin locus of *Staphylococcus aureus* comprises three linked genes, two of which are identical to the genes for the F and S components of leukocidin. Infect Immun 61:768–771

Cooper LZ, Madoff MA, Weinstein L (1964) Hemolysis of rabbit erythrocytes by purified staphylococcal alpha-toxin. I. Kinetics of the lytic reaction. J Bacteriol 87:127–135

Crémieux A-C, Dumitrescu O, Lina G et al (2009) Panton-valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. PLoS ONE 4:e7204

Cuny C, Abdelbary M, Layer F et al (2015) Prevalence of the immune evasion gene cluster in *Staphylococcus aureus* CC398. Vet Microbiol 177:219–223

De Haas CJC, Veldkamp KE, Peschel A et al (2004) Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J Exp Med 199:687–695

De Haas CJC, Weeterings C, Vughs MM et al (2009) Staphylococcal superantigen-like 5 activates platelets and supports platelet adhesion under flow conditions, which involves glycoprotein Ibalpha and alpha IIb beta 3. J Thromb Haemost 7:1867–1874

Deis LN, Pemble CW, Qi Y et al (2014) Multiscale conformational heterogeneity in staphylococcal protein A: possible determinant of functional plasticity. Structure 22:1467–1477

Deis LN, Wu Q, Wang Y et al (2015) Suppression of conformational heterogeneity at a protein–protein interface. Proc Natl Acad Sci USA 112:9028–9033

DeLeo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet 375:1557–1568

DeLeo FR, Kennedy AD, Chen L et al (2011) Molecular differentiation of historic phage-type 80/81 and contemporary epidemic *Staphylococcus aureus*. Proc Natl Acad Sci USA 108:18091–18096

Deringer JR, Ely RJ, Monday SR et al (1997) Vbeta-dependent stimulation of bovine and human T cells by host-specific staphylococcal enterotoxins. Infect Immun 65:4048–4054

Diep BA, Palazzolo-Ballance AM, Tattevin P et al (2008) Contribution of Panton-Valentine leukocidin in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. PLoS ONE 3:e3198

Diep BA, Chan L, Tattevin P et al (2010) Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. Proc Natl Acad Sci USA 107:5587–5592

Dinges MM, Gregerson DS, Tripp TJ et al (2003) Effects of total body irradiation and cyclosporin a on the lethality of toxic shock syndrome toxin-1 in a rabbit model of toxic shock syndrome. J Infect Dis 188:1142–1145

Dumont AL, Nygaard TK, Watkins RL et al (2011) Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. Mol Microbiol 79:814–825

DuMont AL, Yoong P, Day CJ et al (2013) *Staphylococcus aureus* LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci USA 110:10794–10799

Economou A (2002) Bacterial secretome: the assembly manual and operating instructions (review). Mol Membr Biol 19:159–169

Everitt RG, Didelot X, Batty EM et al (2014) Mobile elements drive recombination hotspots in the core genome of *Staphylococcus aureus*. Nat Commun 5:3956

Falugi F, Kim HK, Missiakas DM, Schneewind O (2013) Role of protein a in the evasion of host adaptive immune responses by *Staphylococcus aureus*. mBio 4:e00575-13

Fevre C, Bestebroer J, Mebius MM et al (2014) *Staphylococcus aureus* proteins SSL6 and SElX interact with neutrophil receptors as identified using secretome phage display. Cell Microbiol 16:1646–1665

Fitzgerald JR, Monday SR, Foster TJ et al (2001) Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. J Bacteriol 183:63–70

Fitzgerald JR, Reid SD, Ruotsalainen E et al (2003) Genome diversification in *Staphylococcus aureus*: molecular evolution of a highly variable chromosomal region encoding the Staphylococcal exotoxin-like family of proteins. Infect Immun 71:2827–2838

Fitzgerald JR (2012) Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. Trends Microbiol 20:192–198

Foletti D, Strop P, Shaughnessy L et al (2013) Mechanism of action and in vivo efficacy of a human-derived antibody against *Staphylococcus aureus* α-hemolysin. J Mol Biol 425:1641–1654

Fowler VG, Proctor RA (2014) Where does a *Staphylococcus aureus* vaccine stand? Clin Microbiol Infect 20:66–75

Fraser JD, Proft T (2008) The bacterial superantigen and superantigen-like proteins. Immunol Rev 225:226–243

Friedrich R, Panizzi P, Fuentes-Prior P et al (2003) Staphylocoagulase is a prototype for the mechanism of cofactor-induced zymogen activation. Nature 425:535–539

Friedrich R, Panizzi P, Kawabata SI et al (2006) Structural basis for reduced staphylocoagulase-mediated bovine prothrombin activation. J Biol Chem 281:1188–1195

Fritz SA, Tiemann KM, Hogan PG et al (2013) A serologic correlate of protective immunity against community-onset *Staphylococcus aureus* infection. Clin Infect Dis 56:1554–1561

Fromageau A, Gilbert FB, Prévost G, Rainard P (2010) Binding of the *Staphylococcus aureus* leucotoxin LukM to its leucocyte targets. Microb Pathog 49:354–362

Galdiero S, Gouaux E (2004) High resolution crystallographic studies of alpha-hemolysinphospholipid complexes define heptamer–lipid head group interactions: Implication for understanding protein–lipid interactions, pp 1503–1511

Garcia BL, Ramyar KX, Tzekou A et al (2010) Molecular basis for complement recognition and inhibition determined by crystallographic studies of the Staphylococcal complement inhibitor (SCIN) bound to C3c and C3b. J Mol Biol 402:17–29

Garcia BL, Ramyar KX, Ricklin D et al (2012a) Advances in understanding the structure, function, and mechanism of the SCIN and Efb families of Staphylococcal immune evasion proteins. Adv Exp Med Biol 946:113–133

Garcia BL, Summers BJ, Lin Z et al (2012b) Diversity in the C3b convertase contact residues and tertiary structures of the staphylococcal complement inhibitor (SCIN) protein family. J Biol Chem 287:628–640

Garcia BL, Summers BJ, Ramyar KX et al (2013) A structurally dynamic n-terminal helix is a key functional determinant in staphylococcal complement inhibitor (SCIN) proteins. J Biol Chem 288:2870–2881

Geisbrecht BV, Hamaoka BY, Perman B et al (2005) The crystal structures of EAP domains from *Staphylococcus aureus* reveal an unexpected homology to bacterial superantigens. J Biol Chem 280:17243–17250

Gill SR, Fouts DE, Archer GL et al (2005) Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J Bacteriol 187:2426–2438

Gillet Y, Issartel B, Vanhems P, et al (2002) Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet (London, England) 359:753–759

Gladysheva IP, Turner RB, Sazonova IY et al (2003) Coevolutionary patterns in plasminogen activation. Proc Natl Acad Sci USA 100:9168–9172

Gómez MI, Lee A, Reddy B et al (2004) *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat Med 10:842–848

Gómez MI, O'Seaghdha M, Magargee M et al (2006) *Staphylococcus aureus* protein A activates TNFR1 signaling through conserved IgG binding domains. J Biol Chem 281:20190–20196

Goodyear CS, Silverman GJ (2003) Death by a B cell superantigen: In vivo VH-targeted apoptotic supraclonal B cell deletion by a staphylococcal toxin. J Exp Med 197:1125–1139

Graille M, Stura EA, Corper AL et al (2000) Crystal structure of a *Staphylococcus aureus* protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci USA 97:5399–5404

Grumann D, Nübel U, Bröker BM (2014) *Staphylococcus aureus* toxins—their functions and genetics. Infect Genet Evol 21:583–592

Guillet V, Roblin P, Werner S et al (2004) Crystal structure of leucotoxin S component: New insight into the staphylococcal β-barrel pore-forming toxins. J Biol Chem 279:41028–41037

Guinane CM, Sturdevant DE, Herron-Olson L et al (2008) Pathogenomic analysis of the common bovine *Staphylococcus aureus* clone (ET3): emergence of a virulent subtype with potential risk to public health. J Infect Dis 197:205–213. doi:10.1086/524689

Guinane CM, Ben Zakour NL, Tormo-Mas MA et al (2010) Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol Evol 2:454–466

Guinane CM, Penadés JR, Fitzgerald JR (2011) The role of horizontal gene transfer in *Staphylococcus aureus* host adaptation. Virulence 2:241–243

Haas PJ, de Haas CJC, Kleibeuker W et al (2004) N-terminal residues of the chemotaxis inhibitory protein of *Staphylococcus aureus* are essential for blocking formylated peptide receptor but not C5a receptor. J Immunol 173:5704–5711

Haas PJ, de Haas CJC, Poppelier MJJC et al (2005) The structure of the C5a receptor-blocking domain of chemotaxis inhibitory protein of *Staphylococcus aureus* is related to a group of immune evasive molecules. J Mol Biol 353:859–872

Hammel M, Nemecek D, Keightley JA et al (2007) The *Staphylococcus aureus* extracellular adherence protein (Eap) adopts an elongated but structured conformation in solution. Protein Sci 16:2605–2617

Hartleib J, Köhler N, Dickinson RB et al (2000) Protein A is the von Willebrand factor binding protein on *Staphylococcus aureus*. Blood 96:2149–2156

Haupt K, Reuter M, Van Den Elsen J et al (2008) The *Staphylococcus aureus* protein Sbi acts as a complement inhibitor and forms a tripartite complex with host complement factor H and C3b. PLoS Pathog 4:e10000250

Henson SE, Smith D, Boackle SA et al (2001) Generation of recombinant human C3dg tetramers for the analysis of CD21 binding and function. J Immunol Methods 258:97–109

Hermans SJ, Baker HM, Sequeira RP et al (2012) Structural and functional properties of staphylococcal superantigen-like protein 4. Infect Immun 80:4004–4013

Herron-Olson L, Fitzgerald JR, Musser JM, Kapur V (2007) Molecular correlates of host specialization in *Staphylococcus aureus*. PLoS ONE 2:e1120

Hidron AI, Low CE, Honig EG, Blumberg HM (2009) Emergence of community-acquired meticillin-resistant *Staphylococcus aureus* strain USA300 as a cause of necrotising community-onset pneumonia. Lancet Infect Dis 9:384–392

Holt DC, Holden MTG, Tong SYC et al (2011) A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol Evol 3:881–895

Holtfreter S, Bauer K, Thomas D 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 72:4061–4071

Hu H, Armstrong PCJ, Khalil E et al (2011) GPVI and GPIbα mediate staphylococcal superantigen-like protein 5 (SSL5) induced platelet activation and direct toward glycans as potential inhibitors. PLoS ONE 6:e19190

Huseby M, Shi K, Kent Brown C et al (2007) Structure and biological activities of beta toxin from *Staphylococcus aureus*. J Bacteriol 189:8719–8726

Hussain M, Haggar A, Peters G et al (2008) More than one tandem repeat domain of the extracellular adherence protein of *Staphylococcus aureus* is required for aggregation, adherence, and host cell invasion but not for leukocyte activation. Infect Immun 76:5615–5623

Inoshima I, Inoshima N, Wilke GA et al (2011) A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med 17:1310–1314

Inoshima N, Wang Y, Wardenburg JB (2012) Genetic requirement for ADAM10 in severe *Staphylococcus aureus* skin infection. J Invest Dermatol 132:1513–1516

Ippel JH, de Haas CJC, Bunschoten A et al (2009) Structure of the tyrosine-sulfated C5a receptor N terminus in complex with chemotaxis inhibitory protein of *Staphylococcus aureus*. J Biol Chem 284:12363–12372

Itoh S, Hamada E, Kamoshida G et al (2010a) Staphylococcal superantigen-like protein 5 inhibits matrix metalloproteinase 9 from human neutrophils. Infect Immun 78:3298–3305

Itoh S, Hamada E, Kamoshida G et al (2010b) Staphylococcal superantigen-like protein 10 (SSL10) binds to human immunoglobulin G (IgG) and inhibits complement activation via the classical pathway. Mol Immunol 47:932–938

Itoh S, Yamaoka N, Kamoshida G et al (2013a) Staphylococcal superantigen-like protein 8 (SSL8) binds to tenascin C and inhibits tenascin C-fibronectin interaction and cell motility of keratinocytes. Biochem Biophys Res Commun 433:127–132

Itoh S, Yokoyama R, Kamoshida G et al (2013b) Staphylococcal superantigen-like protein 10 (SSL10) inhibits blood coagulation by binding to prothrombin and factor Xa via their γ-carboxyglutamic acid (Gla) domain. J Biol Chem 288:21569–21580

Jarraud S, Peyrat MA, Lim A et al (2001) egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J Immunol 166:669–677

Jin T, Bokarewa M, Foster T et al (2004) *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J Immunol 172:1169–1176

Jongerius I, Köhl J, Pandey MK et al (2007) Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med 204:2461–2471

Jongerius I, Puister M, Wu J et al (2010) Staphylococcal complement inhibitor modulates phagocyte responses by dimerization of convertases. J Immunol 184:420–425

Jongerius I, von Köckritz-Blickwede M, Horsburgh MJ et al (2012) *Staphylococcus aureus* virulence is enhanced by secreted factors that block innate immune defenses. J Innate Immun 4:301–311

Jusko M, Potempa J, Kantyka T et al (2014) Staphylococcal proteases aid in evasion of the human complement system. J Innate Immun 6:31–46

Kim HK, Kim HY, Schneewind O, Missiakas D (2011) Identifying protective antigens of *Staphylococcus aureus*, a pathogen that suppresses host immune responses. FASEB J 25:3605–3612

Kim HK, Falugi F, Thomer L, Missiakas DM (2015) Protein A suppresses immune responses during *Staphylococcus aureus* bloodstream infection in guinea pigs. mBio 6:1–11

Ko YP, Liang X, Smith CW et al (2011) Binding of Efb from *Staphylococcus aureus* to fibrinogen blocks neutrophil adherence. J Biol Chem 286:9865–9874

Ko YP, Kuipers A, Freitag CM et al (2013) Phagocytosis escape by a *Staphylococcus aureus* protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog 9:1–13

Kobayashi SD, Malachowa N, Whitney AR et al (2011) Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. J Infect Dis 204:937–941

Koymans KJ, Feitsma LJ, Brondijk THC et al (2015) Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). Proc Natl Acad Sci USA 112:11018–11023

Kretschmer D, Gleske AK, Rautenberg M et al (2010) Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. Cell Host Microbe 7:463–473

Kuehnert MJ, Kruszon-Moran D, Hill HA et al (2006) Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. J Infect Dis 193:172–179

Kumagai R, Nakatani K, Ikeya N et al (2007) Quadruple or quintuple conversion of hlb, sak, sea (or sep), scn, and chp genes by bacteriophages in non-beta-hemolysin-producing bovine isolates of *Staphylococcus aureus*. Vet Microbiol 122:190–195

Kuroda M, Yamashita A, Hirakawa H et al (2005) Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc Natl Acad Sci USA 102:13272–13277

Laarman AJ, Ruyken M, Malone CL et al (2011) *Staphylococcus aureus* metalloprotease aureolysin cleaves complement C3 to mediate immune evasion. J Immunol 186:6445–6453

Laarman AJ, Mijnheer G, Mootz JM et al (2012) *Staphylococcus aureus* Staphopain A inhibits CXCR2-dependent neutrophil activation and chemotaxis. EMBO J 31:3607–3619

Lambris JD, Ricklin D, Geisbrecht BV (2008) Complement evasion by human pathogens. Nat Rev Microbiol 6:132–142

Langley R, Wines B, Willoughby N et al (2005) The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. J Immunol 174:2926–2933

Langley R, Patel D, Jackson N et al (2010) Staphylococcal superantigen super-domains in immune evasion. Crit Rev Immunol 30:149–165

Langley R, Fraser JD (2013) The staphylococcal Superantigen-like toxins. Bact Toxins: Genet Cell Biol Pract Appl 129–156

Laursen NS, Gordon N, Hermans S et al (2010) Structural basis for inhibition of complement C5 by the SSL7 protein from *Staphylococcus aureus*. Proc Natl Acad Sci USA 107:3681–3686

Le KY, Dastgheyb S, Ho TV, Otto M (2014) Molecular determinants of staphylococcal biofilm dispersal and structuring. Front Cell Infect Microbiol 4:1–7

Lee PK, Kreiswirth BN, Deringer JR et al (1992) Nucleotide sequences and biologic properties of toxic shock syndrome toxin 1 from ovine- and bovine-associated *Staphylococcus aureus*. J Infect Dis 165:1056–1063

Lee SS, Kim YJ, Chung DR et al (2010) Invasive infection caused by a community-associated methicillin-resistant *Staphylococcus aureus* strain not carrying Panton-Valentine leukocidin in South Korea. J Clin Microbiol 48:311–313

Lina G, Piémont Y, Godail-Gamot F et al (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128–1132

Lina G, Bohach GA, Nair SP et al (2004) Standard nomenclature for the superantigens expressed by Staphylococcus. J Infect Dis 189:2334–2336

Lindsay JA, Ruzin A, Ross HF et al (1998) The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol Microbiol 29:527–543

Lindsay JA, Holden MTG (2006a) Understanding the rise of the superbug: Investigation of the evolution and genomic variation of *Staphylococcus aureus*. Funct Integr Genomics 6:186–201

Lindsay JA, Moore CE, Day NP et al (2006b) Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. J Bacteriol 188:669–676

Lindsay JA (2010) Genomic variation and evolution of *Staphylococcus aureus*. Int J Med Microbiol 300:98–103

Lindsay JA (2014) *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. Int J Med Microbiol 304:103–109

Lipinska U, Hermans K, Meulemans L et al (2011) Panton-Valentine leukocidin does play a role in the early stage of *Staphylococcus aureus* skin infections: a rabbit model. PLoS ONE 6:e22864

Liu ZJ, Yang YJ, Jiang L et al (2011) Tyrosine sulfation in N-terminal domain of human C5a receptor is necessary for binding of chemotaxis inhibitory protein of *Staphylococcus aureus*. Acta Pharmacol Sin 32:1038–1044

Liu Y, Yin H, Zhao M, Lu Q (2013) TLR2 and TLR4 in autoimmune diseases: a comprehensive review. Clin Rev Allergy Immunol 1–12

Löffler B, Hussain M, Grundmeier M et al (2010) *Staphylococcus aureus* panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6:e1000715
Lowder BV, Guinane CM, Ben Zakour NL et al (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci USA 106:19545–19550
Lowy F (1998) *Staphylococcus aureus* infections. N Engl J Med 339:520–532
Malachowa N, Deleo FR (2010) Mobile genetic elements of *Staphylococcus aureus*. Cell Mol Life Sci 67:3057–3071
Malachowa N, Kobayashi SD, Braughton KR et al (2012) *Staphylococcus aureus* leukotoxin GH promotes inflammation. J Infect Dis 206:1185–1193
Malito E, Carfi A, Bottomley M (2015) Protein crystallography in vaccine research and development. Int J Mol Sci 16:13106–13140
McCarthy AJ, Lindsay JA (2010) Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173
McCarthy AJ, Lindsay JA, Loeffler A (2012a) Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. Vet Dermatol 23:267–275
McCarthy AJ, van Wamel WJB, Vandendriessche S et al (2012b) *Staphylococcus aureus* CC398 clade associated with human-to-human transmission. Appl Environ Microbiol 78:8845–8848
McCarthy AJ, Witney AA, Lindsay JA (2012c) *Staphylococcus aureus* temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. Front Cell Infect Microbiol 2:6
McCarthy AJ, Lindsay JA (2013) *Staphylococcus aureus* innate immune evasion is lineage-specific: a bioinfomatics study. Infect Genet Evol 19:7–14
Monecke S, Kuhnert P, Hotzel H et al (2007) Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. Vet Microbiol 125:128–140
Monecke S, Luedicke C, Slickers P, Ehricht R (2009) Molecular epidemiology of *Staphylococcus aureus* in asymptomatic carriers. Eur J Clin Microbiol Infect Dis 28:1159–1165
Moon BY, Park JY, Hwang SY et al (2015) Phage-mediated horizontal transfer of a *Staphylococcus aureus* virulence-associated genomic island. Sci Rep 5:9784
Morinaga N, Kaihou Y, Noda M (2003) Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. Microbiol Immunol 47:81–90
Murzin AG (1993) OB(oligonucleotide/oligosaccharide binding)-fold: common structural and functional solution for non-homologous sequences. EMBO J 12:861–867
Nakamura Y, Oscherwitz J, Cease KB et al (2013) Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. Nature 503:397–401
Nguyen T, Ghebrehiwet B, Ellinor IB (2000) *Staphylococcus aureus* Protein A recognizes platelet gC1qR/ p33: a novel mechanism for staphylococcal interactions with platelets. Infect Immun 68:2061–2068
Novick RP, Subedi A (2007) The SaPIs: mobile pathogenicity islands of Staphylococcus. Chem Immunol Allergy 93:42–57
Novick RP, Christie GE, Penadés JR (2010) The phage-related chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol 8:541–551
Nygaard TK, Pallister KB, DuMont AL et al (2012) Alpha-toxin induces programmed cell death of human T cells, B cells, and monocytes during USA300 infection. PLoS ONE 7:e36532
Okumura CYM, Nizet V (2014) Subterfuge and sabotage: evasion of host innate defenses by invasive gram-positive bacterial pathogens. Annu Rev Microbiol 68:439–458
Olson R, Nariya H, Yokota K et al (1999) Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel. Nat Struct Biol 6:134–140

Omoe K, Hu DL, Takahashi-Omoe H et al (2005) Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. FEMS Microbiol Lett 246:191–198

Ono HK, Sato'o Y, Narita K et al (2015) Identification and characterization of a novel staphylococcal emetic toxin. Appl Environ Microbiol 81:7034–7040

Otter JA, French GL (2008) The emergence of community-associated methicillin-resistant *Staphylococcus aureus* at a London teaching hospital, 2000–2006. Clin Microbiol Infect 14:670–676

Padmaja RJ, Halami PM (2013) Molecular characterization and toxicity confirmation of LukM/F'-PV producing *Staphylococcus aureus* isolated from bovine mastitis samples in Mysore, India. Indian J Microbiol 53:276–282

Palma M, Shannon O, Quezada HC et al (2001) Extracellular fibrinogen-binding protein, Efb, from *Staphylococcus aureus* blocks platelet aggregation due to its binding to the alpha-chain. J Biol Chem 276:31691–31697

Panizzi P, Friedrich R, Fuentes-Prior P et al (2006) Fibrinogen substrate recognition by staphylocoagulase·(Pro)thrombin complexes. J Biol Chem 281:1179–1187

Parry MA, Fernandez-Catalan C, Bergner A et al (1998) The ternary microplasmin-staphylokinase-microplasmin complex is a proteinase-cofactor-substrate complex in action. Nat Struct Biol 5:917–923

Patel D, Wines BD, Langley RJ, Fraser JD (2010) Specificity of staphylococcal superantigen-like protein 10 toward the human IgG1 Fc domain. J Immunol 184:6283–6292

Pédelacq JD, Maveyraud L, Prévost G et al (1999) The structure of a *Staphylococcus aureus* leucocidin component (LukF-PV) reveals the fold of the water-soluble species of a family of transmembrane pore-forming toxins. Structure 7:277–287

Penadés JR, Fitzgerald JR (2009) Toxins encoded by mobile genetic elements. Current research and future trends, Microbial toxins, pp 1–13

Peton V, Le Loir Y (2014) *Staphylococcus aureus* in veterinary medicine. Infect Genet Evol 21:602–615

Phimister GM, Freer JH (1984) Binding of 125I-alpha toxin of *Staphylococcus aureus* to erythrocytes. J Med Microbiol 18:197–204

Postma B, Poppelier MJ, van Galen JC et al (2004) Chemotaxis inhibitory protein of *Staphylococcus aureus* binds specifically to the C5a and formylated peptide receptor. J Immunol 172:6994–7001

Postma B, Kleibeuker W, Poppelier MJJG et al (2005) Residues 10-18 within the C5a receptor N terminus compose a binding domain for chemotaxis inhibitory protein of *Staphylococcus aureus*. J Biol Chem 280:2020–2027

Powers ME, Kim HK, Wang Y, Wardenburg JB (2012) ADAM10 mediates vascular injury induced by *Staphylococcus aureus* α-hemolysin. J Infect Dis 206:352–356

Prat C, Bestebroer J, de Haas CJC et al (2006) A new staphylococcal anti-inflammatory protein that antagonizes the formyl peptide receptor-like 1. J Immunol 177:8017–8026

Prat C, Haas PJ, Bestebroer J et al (2009) A homolog of formyl peptide receptor-like 1 (FPRL1) inhibitor from *Staphylococcus aureus* (FPRL1 inhibitory protein) that inhibits FPRL1 and FPR. J Immunol 183:6569–6578

Price LB, Stegger M, Hasman H, et al (2012) Adaptation and emergence of *Staphylococcus aureus* CC39: Host adaptation and emergence of methicillin resistance in livestock. mBio 3:e00305-11

Prokesová L, Potuzníkovà B, Potempa J et al (1995) Cleavage of human immunoglobulins by proteinase from *Staphylococcus aureus*. Adv Exp Med Biol 371A:613–616

Quiles-Puchalt N, Carpena N, Alonso JC et al (2014) Staphylococcal pathogenicity island DNA packaging system involving cos-site packaging and phage-encoded HNH endonucleases. Proc Natl Acad Sci USA 111:6016–6021

Rabijns A, De Bondt H, De Ranter C (1997) Three-dimensional structure of staphylokinase, a plasminogen activator with therapeutic potential. Nat Struct Biol 4:357–360

Rahimpour R, Mitchell G, Khandaker MH et al (1999) Bacterial superantigens induce down-modulation of CC chemokine responsiveness in human monocytes via an alternative chemokine ligand-independent mechanism. J Immunol 162:2299–2307

Rainard P, Corrales JC, Barrio MB et al (2003) Leucotoxic activities of *Staphylococcus aureus* strains isolated from cows, ewes, and goats with mastitis: importance of LukM/LukF'-PV leukotoxin. Clin Diagn Lab Immunol 10:272–277

Ramsland PA, Willoughby N, Trist HM et al (2007) Structural basis for evasion of IgA immunity by *Staphylococcus aureus* revealed in the complex of SSL7 with Fc of human IgA1. Proc Natl Acad Sci USA 104:15051–15056

Reyes-Robles T, Alonzo F, Kozhaya L et al (2013) *Staphylococcus aureus* Leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to Kill leukocytes and promote infection. Cell Host Microbe 14:453–459

Ricklin D, Ricklin-Lichtsteiner SK, Markiewski MM et al (2008) Cutting edge: members of the *Staphylococcus aureus* extracellular fibrinogen-binding protein family inhibit the interaction of C3d with complement receptor 2. J Immunol 181:7463–7467

Ricklin D, Tzekou A, Garcia BL et al (2009) A molecular insight into complement evasion by the staphylococcal complement inhibitor protein family. J Immunol 183:2565–2574

Ricklin D, Lambris JD (2013) Complement in immune and inflammatory disorders: pathophysiological mechanisms. J Immunol 190:3831–3838

Rödström KEJ, Elbing K, Lindkvist-Petersson K (2014) Structure of the superantigen staphylococcal enterotoxin B in complex with TCR and peptide-MHC demonstrates absence of TCR-peptide contacts. J Immunol 193:1998–2004

Rooijakkers SHM, Ruyken M, Roos A et al (2005a) Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol 6:920–927

Rooijakkers SHM, van Wamel WJB, Ruyken M et al (2005b) Anti-opsonic properties of staphylokinase. Microbes Infect 7:476–484

Rooijakkers SHM, Milder FJ, Bardoel BW et al (2007) Staphylococcal complement inhibitor: structure and active sites. J Immunol 179:2989–2998

Rooijakkers SHM, Wu J, Ruyken M et al (2009) Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. Nat Immunol 10:721–727

Rouha H, Badarau A, Visram ZC et al (2015) Five birds, one stone: neutralization of α-hemolysin and 4 bi-component leukocidins of *Staphylococcus aureus* with a single human monoclonal antibody. MAbs 7:243–254

Salgado-Pabón W, Breshears L, Spaulding AR, et al (2013) Superantigens are critical for *Staphylococcus aureus* infective endocarditis, sepsis, and acute kidney injury. mBio 4: e00494-13

Salgado-Pabón W, Schlievert PM (2014) Models matter: the search for an effective *Staphylococcus aureus* vaccine. Nat Rev Microbiol 12:585–591

Schad EM, Zaitseva I, Zaitsev VN et al (1995) Crystal structure of the superantigen staphylococcal enterotoxin type A. EMBO J 14:3292–3301

Scherr TD, Hanke ML, Huang O et al (2015) *Staphylococcus aureus* biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. mBio 6:e01021-15

Schijffelen MJ, Boel CHE, van Strijp JAG, Fluit AC (2010) Whole genome analysis of a livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 isolate from a case of human endocarditis. BMC Genom 11:376

Schlievert PM (2009) Cytolysins, superantigens, and pneumonia due to community-associated methicillin-resistant *Staphylococcus aureus*. J Infect Dis 200:676–678

Schlotter K, Ehricht R, Hotzel H 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 43:42

Schneewind O, Model P, Fischetti VA (1992) Sorting of Protein-A to the staphylococcal cell-wall. Cell 70:267–281

Schreiner J, Kretschmer D, Klenk J et al (2013) *Staphylococcus aureus* phenol-soluble modulin peptides modulate dendritic cell functions and increase in vitro priming of regulatory T cells. J Immunol 190:3417–3426

Scully IL, Liberator PA, Jansen KU, Anderson AS (2014) Covering all the bases: preclinical development of an effective *Staphylococcus aureus* vaccine. Front Immunol 5:1–7

Sieprawska-lupa M, Mydel P, Wójcik K et al (2004) Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. Antimicrob Agents Chemother 48:4673–4679

Simpson VR, Davison NJ, Kearns AM et al (2013) Association of a lukM-positive clone of *Staphylococcus aureus* with fatal exudative dermatitis in red squirrels (Sciurus vulgaris). Vet Microbiol 162:987–991

Smagur J, Guzik K, Bzowska M et al (2009) Staphylococcal cysteine protease staphopain B (SspB) induces rapid engulfment of human neutrophils and monocytes by macrophages. Biol Chem 390:361–371

Smith EJ, Visai L, Kerrigan SW et al (2011) The Sbi protein is a multifunctional immune evasion factor of *Staphylococcus aureus*. Infect Immun 79:3801–3809

Smyth DS, Hartigan PJ, Meaney WJ et al (2005) Superantigen genes encoded by the egc cluster and SaPlbov are predominant among *Staphylococcus aureus* isolates from cows, goats, sheep, rabbits and poultry. J Med Microbiol 54:401–411

Song L, Hobaugh MR, Shustak C et al (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866

Spaan AN, Henry T, Van Rooijen WJM et al (2013) The staphylococcal toxin panton-valentine leukocidin targets human C5a receptors. Cell Host Microbe 13:584–594

Spaan AN, Vrieling M, Wallet P et al (2014) The staphylococcal toxins γ-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. Nat Commun 5:5438

Spaan AN, Reyes-Robles T, Badiou C et al (2015a) *Staphylococcus aureus* targets the duffy antigen receptor for chemokines (DARC) to lyse erythrocytes. Cell Host Microbe 18:363–370

Spaan AN, Schiepers A, de Haas CJC et al (2015b) Differential interaction of the staphylococcal toxins Panton-Valentine leukocidin and γ-hemolysin CB with human C5a receptors. J Immunol 195:1034–1043

Spaulding AR, Salgado-Pabón W, Kohler PL et al (2013) Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 26:422–447

Stapels DAC, Ramyar KX, Bischoff M et al (2014) *Staphylococcus aureus* secretes a unique class of neutrophil serine protease inhibitors that promotes bacterial infection. Proc Natl Acad Sci USA 111:13187–13192

Stapels DAC, Kuipers A, von Köckritz-Blickwede M et al (2015) *Staphylococcus aureus* protects its immune-evasion proteins against degradation by neutrophil serine proteases. Cell Microbiol

Stemerding AM, Köhl J, Pandey MK et al (2013) *Staphylococcus aureus* formyl peptide receptor-like 1 inhibitor (FLIPr) and its homologue FLIPr-like are potent FcγR antagonists that inhibit IgG-mediated effector functions. J Immunol 191:353–362

Stulik L, Malafa S, Hudcova J et al (2014) α-hemolysin activity of methicillin-susceptible *Staphylococcus aureus* predicts ventilator-associated pneumonia. Am J Respir Crit Care Med 190:1139–1148

Stutz K, Stephan R, Tasara T (2011) SpA, ClfA, and FnbA genetic variations lead to Staphaurex test-negative phenotypes in bovine mastitis *Staphylococcus aureus* isolates. J Clin Microbiol 49:638–646

Subedi A, Ubeda C, Adhikari RP et al (2007) Sequence analysis reveals genetic exchanges and intraspecific spread of SaPl2, pathogenicity island involved in menstrual toxic shock. Microbiology 153:3235–3245

Summers BJ, Garcia BL, Woehl JL et al (2015) Identification of peptidic inhibitors of the alternative complement pathway based on *Staphylococcus aureus* SCIN proteins. Mol Immunol 67:193–205

Sundberg EJ, Deng L, Mariuzza RA (2007) TCR recognition of peptide/MHC class II complexes and superantigens. Semin Immunol 19:262–271

Sung JML, Lloyd DH, Lindsay JA (2008) *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154:1949–1959

Surewaard BGJ, Nijland R, Spaan AN et al (2012) Inactivation of staphylococcal phenol soluble modulins by serum lipoprotein particles. PLoS Pathog 8:e1002606

Surewaard BGJ, De Haas CJC, Vervoort F et al (2013) Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. Cell Microbiol 15:1427–1437

Takeuchi F, Watanabe S, Baba T et al (2005) Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. J Bacteriol 187:7292–7308

Tanaka Y, Hirano N, Kaneko J et al (2011) 2-Methyl-2,4-pentanediol induces spontaneous assembly of staphylococcal α-hemolysin into heptameric pore structure. Protein Sci 20:448–456

Thammavongsa V, Missiakas D, Schneewind O (2013) *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death. Science (80-) 342:863–866

Thomas DY, Jarraud S, Lemercier B et al (2006) Staphylococcal enterotoxin-like toxins U2 and V, two new staphylococcal superantigens arising from recombination within the enterotoxin gene cluster. Infect Immun 74:4724–4734

Thomer L, Schneewind O, Missiakas D (2013) Multiple ligands of von willebrand factor-binding protein (vWbp) promote *Staphylococcus aureus* clot formation in human plasma. J Biol Chem 288:28283–28292

Thurlow LR, Hanke ML, Fritz T et al (2011) *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol 186:6585–6596

Tollersrud T, Kampen AH, Kenny K (2006) *Staphylococcus aureus* enterotoxin D is secreted in milk and stimulates specific antibody responses in cows in the course of experimental intramammary infection. Infect Immun 74:3507–3512

Tuchscherr L, Heitmann V, Hussain M et al (2010) *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. J Infect Dis 202:1031–1040

van Wamel WJB, Rooijakkers SHM, Ruyken M et al (2006) The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188:1310–1315

Vandenesch F, Naimi T, Enright MC et al (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984

Verkaik NJ, Benard M, Boelens HA et al (2011) Immune evasion cluster-positive bacteriophages are highly prevalent among human *Staphylococcus aureus* strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect 17:343–348

Viana D, Blanco J, Tormo-Más MÁ et al (2010) Adaptation of *Staphylococcus aureus* to ruminant and equine hosts involves SaPI-carried variants of von Willebrand factor-binding protein. Mol Microbiol 77:1583–1594

Viana D, Comos M, McAdam PR et al (2015) A single natural nucleotide mutation alters bacterial pathogen host tropism. Nat Genet 47:361–366

Vrieling M, Koymans KJ, Heesterbeek DAC et al (2015) Bovine *Staphylococcus aureus* secretes the leukocidin LukMF' to kill migrating neutrophils through CCR1. mBio 6:e00335

Walenkamp AME, Boer IGJ, Bestebroer J et al (2009) Staphylococcal superantigen-like 10 inhibits CXCL12-induced human tumor cell migration. Neoplasia 11:333–344

Walenkamp AME, Bestebroer J, Boer IGJ et al (2010) Staphylococcal SSL5 binding to human leukemia cells inhibits cell adhesion to endothelial cells and platelets. Cell Oncol 32:1–10

Wang R, Braughton KR, Kretschmer D et al (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 13:1510–1514

Ward MJ, Gibbons CL, McAdam PR et al (2014) Time-scaled evolutionary analysis of the transmission and antibiotic resistance dynamics of *Staphylococcus aureus* clonal complex 398. Appl Environ Microbiol 80:7275–7282

Watson E, Matousek WM, Irimies EL, Alexandrescu AT (2007) Partially folded states of staphylococcal nuclease highlight the conserved structural hierarchy of OB-fold proteins. Biochemistry 46:9484–9494

Wilke G , Bubeck Wardenburg J (2010) Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci USA 107:13473–13478

Williams RJ, Ward JM, Henderson B et al (2000) Identification of a novel gene cluster encoding staphylococcal exotoxin-like proteins : characterization of the prototypic gene and its protein product, SET1. Infect Immun 68:4407–4415

Willingham SB, Volkmer JP, Gentles AJ et al (2012) The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci USA 109:6662–6667

Wilson GJ, Seo KS, Cartwright RA et al (2011) A novel core genome-encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia. PLoS Pathog 7:e1002271

Woehl JL, Stapels DAC, Garcia BL et al (2014) The extracellular adherence protein from *Staphylococcus aureus* inhibits the classical and lectin pathways of complement by blocking formation of the C3 proconvertase. J Immunol 193:6161–6171

Xu SX, Gilmore KJ, Szabo PA et al (2014) Superantigens subvert the neutrophil response to promote abscess formation and enhance *Staphylococcus aureus* survival in vivo. Infect Immun 82:3588–3598

Xu SX, Kasper KJ, Zeppa JJ, McCormick JK (2015) Superantigens modulate bacterial density during *Staphylococcus aureus* nasal colonization. Toxins (Basel) 7:1821–1836

Yamada T, Tochimaru N, Nakasuji S et al (2005) Leukotoxin family genes in *Staphylococcus aureus* isolated from domestic animals and prevalence of lukM-lukF-PV genes by bacteriophages in bovine isolates. Vet Microbiol 110:97–103

Yamashita K, Kawai Y, Tanaka Y et al (2011) Crystal structure of the octameric pore of staphylococcal γ-hemolysin reveals the β-barrel pore formation mechanism by two components. Proc Natl Acad Sci USA 108:17314–17319

Yamashita D, Sugawara T, Takeshita M et al (2014) Molecular basis of transmembrane beta-barrel formation of staphylococcal pore-forming toxins. Nat Commun 5:4897

Yeung RS, Penninger JM, Kündig T et al (1996) Human CD4 and human major histocompatibility complex class II (DQ6) transgenic mice: supersensitivity to superantigen-induced septic shock. Eur J Immunol 26:1074–1082

Yokomizo Y, Mori Y, Shimoji Y et al (1995) Proliferative response and cytokine production of bovine peripheral blood mononuclear cells induced by the superantigens staphylococcal enterotoxins and toxic shock syndrome toxin-1. J Vet Med Sci 57:299–305

Yokoyama R, Itoh S, Kamoshida G et al (2012) Staphylococcal superantigen-like protein 3 binds to the toll-like receptor 2 extracellular domain and inhibits cytokine production induced by *Staphylococcus aureus*, cell wall component, or lipopeptides in murine macrophages. Infect Immun 80:2816–2825

Zhang L, Jacobson K, Vasi J et al (1995) A second IgG-binding protein in *Staphylococcus aureus*. Microbiology 408:985–991

Zhang L, Jacobsson K, Ström K et al (1999) *Staphylococcus aureus* expresses a cell surface protein that binds both IgG and beta2-glycoprotein I. Microbiology 145:177–183

Zou D, Kaneko J, Narita S, Kamio Y (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 64:2631–2643

Vaccines for *Staphylococcus aureus* and Target Populations

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Abstract *Staphylococcus aureus* is a leading pathogen in surgical site, intensive care unit, and skin infections, as well as healthcare-associated pneumonias. These infections are associated with an enormous burden of morbidity, mortality, and increase of hospital length of stay and patient cost. *S. aureus* is impressively fast in acquiring antibiotic resistance, and multidrug-resistant strains are a serious threat to human health. Due to resistance or insufficient effectiveness, antibiotics and bundle measures leave a tremendous unmet medical need worldwide. There are no licensed vaccines on the market despite the significant efforts done by public and private initiatives. Indeed, vaccines tested in clinical trials in the last two decades have failed to show efficacy. However, they targeted single antigens and contained no adjuvants and efficacy trials were performed in severely ill subjects. Herein, we provide a comprehensive evaluation of potential target populations for efficacy trials taking into account key factors such as population size, incidence of *S. aureus* infection, disease outcome, primary endpoints, as well as practical advantages and disadvantages. We describe the whole-blood assay as a potential surrogate of protection, and we show the link between phase III clinical trial data of failed vaccines with their preclinical observations. Finally, we give our perspective on how new vaccine formulations and clinical development approaches may lead to successful *S. aureus* vaccines.

Abbreviations

BSI	Bloodstream infection
CA	Community acquired
CAI	Community acquired infection
CA-SSTI	Community acquired skin and soft tissue infection
ClfA	Clumping factor A
CP	Capsular polysaccharide
Csa1A	Conserved staphylococcal antigen 1A

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CT	Cardiothoracic
EPA	Enterotoxin protein A
ESRD	End-stage renal disease
EsxAB	Ess extracellular A and B
FhuD2	Ferric hydroxamate uptake D2
HA	Hospital acquired
HAI	Hospital acquired infection
HCA	Healthcare associated
Hla	Alpha hemolysin
HlgAB/ HlgCB	Gamma hemolysin AB/CB
LukED	Leukocidins E and D
ICU	Intensive care unit
IsdB	Iron-surface determinant B
MntABC	ATP-binding cassette (ABC)
MntC	Manganese transport protein C
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NEAT	NEAr iron transporter
NS	Neurosurgical
OP	Orthopaedic
OPA	Opsonophagocytic Assay
PMN	Polymorphonuclear cells
PVL	Panton–Valentine leukocidin
SA-SSTI	*Staphylococcus aureus* skin and soft tissue infection
SpA	Staphylococcal protein A
SSI	Surgical site infection
SSTI	Skin and soft tissue infection
Th1	T helper cell 1
Th17	T helper cell 17
TLR7	Toll-like receptor 7
TT	Tetanus toxoid
WBA	Whole-blood assay

Contents

1 Introduction

Staphylococcus aureus is associated with hospital-acquired and community-acquired infections (HAI and CAI, respectively). It is a leading nosocomial pathogen in both developed and developing countries. Surgical site infections are among the most common *S. aureus* HAI and include orthopaedic patients (e.g., knee and hip replacements), cardiothoracic surgery (e.g., coronary artery bypass graft and cardiac valve), neurosurgery (e.g., instrumented lumbar fusion and laminectomy), and plastic surgery (e.g., breast and facial surgery) (Anderson et al. 2010; Noskin et al. 2007). A study conducted in 1265 intensive care units (ICUs) from 75 countries and collecting data from 14,414 patients showed that *S. aureus* was associated with the greatest proportion of infections (20% of the infections) (Vincent et al. 2009). The most common infectious disease in ICUs is pneumonia for which this pathogen is also the leading cause (Magill et al. 2014). *S. aureus* can colonize and form biofilms on indwelling catheters. This is a major issue especially for end-stage renal disease patients undergoing hemodialysis.

Among CAI, *S. aureus* is the main pathogen of skin and soft tissue infections (SSTIs) (Lorette et al. 2009; Sader et al. 2010; Moran et al. 2006; Zhao et al. 2012). The incidence of community-acquired SSTI (CA-SSTI) is generally greater in crowded communities, in the elderly, and when the members of the community are subject to skin injuries. Indeed, CA-SSTI is particularly high in infantry trainees, athletes, and nursing homes residents (David and Daum 2010; Ellis et al. 2014; Morrison-Rodriguez et al. 2010; Tong et al. 2015).

Several vaccine candidates have been tested in clinical trials in recent years (see Table 1), but none of them succeeded in showing efficacy against the infection in phase III trials (Bagnoli et al. 2012; Daum and Spellberg 2012). However, vaccines tested so far in efficacy trials, which targeted single antigens and contained no adjuvants, were likely insufficient to cope with the complexity of this pathogen. Indeed, its pathogenicity is due to several virulence factors, each playing different roles in the disease progression (Bagnoli et al. 2012). Based on this observation, we, as well as other researchers, focused research on vaccine combinations. Two different protein combinations were shown to elicit superior protection to IsdB in different

Table 1 *S. aureus* antigens tested in clinical trials

Antigen	Vaccine	Sponsor	Clinical phase
Toxins			
LukS-PV	*S. aureus* Toxoids	USUHS[a]	I
α-hemolysin	4C-Staph, GSK2392, *S. aureus* Toxoids	USUHS, Novartis, GSK	I
Enterotoxin B	STEBvax	NIAID[b]	I
Adhesion factors			
ClfA	SA3Ag, SA4Ag, GSK2392	Pfizer, GSK	I, II, IIb
Als3	NDV-3	NovaDigm	I
Nutrient-scavenging factors			
IsdB	V710	Merck	I, II, III
MntC	SA4Ag	Pfizer	I, II, IIb
FhuD2	4C-Staph	Novartis	I
Capsular polysaccharides			
CP5 and CP8	SA3Ag, SA4Ag, GSK2392, StaphVAX	Pfizer, GSK, NABI	I, II, III
Antigens with other/unknown function			
EsxA and EsxB	4C-Staph	Novartis	I
Csa1A	4C-Staph	Novartis	I

LukS-PV Panton–Valentine leukocidin component *S*; *ClfA* clumping factor A; *Als3* agglutinin-like sequence protein 3 (from Candida albicans); *IsdB* iron-regulated surface determinant protein B; *MntC* manganese transport protein C; *FhuD2* ferric hydroxamate uptake D2; *CP* capsular polysaccharide; *Esx* ess extracellular; *CSA* conserved staphylococcal antigen
[a]Uniformed Services University of the Health Sciences
[b]National Institute of Allergy and Infectious Diseases

animal models (Bagnoli et al. 2015; Stranger-Jones et al. 2006). IsdB is a surface antigen recently tested in a phase III trial, which failed to reach the primary endpoints of efficacy. While the combinations conferred highly significant protective immunity, IsdB was found not to be significantly protective in most of the experiments. Based on the preclinical observations mentioned above, the outcome in humans is not completely surprising. More recently, an important role of cell-mediated immunity against this pathogen has also been demonstrated. In particular, lack of Th17 responses (e.g., Job's syndrome, HIV, as well as subjects with auto-antibodies against IL-17) has been associated with higher risk of *S. aureus* infections (Pozzi et al. 2015; Miller and Cho 2011). The protective role of IL-17 has also been confirmed in animal models (Cho et al. 2010). Furthermore, in 2015, an innovative adjuvant able to stimulate the toll-like receptor 7 (Alum-TLR7) was shown to induce a Th1/Th17 response and significantly increase protection of a tetravalent antigen combination as compared to Alum formulation (Bagnoli et al. 2015).

All these observations suggest that vaccination continues to represent one of the most promising solutions to this deadly pathogen. Indeed, a new phase IIb trial with

a vaccine combination is currently ongoing (https://clinicaltrials.gov/ct2/show/NCT02388165?term=pfizer+staphylococcus+vaccine&rank=1), and research on new vaccine candidates has been recently published (Bagnoli et al. 2015; Aman and Adhikari 2014; Torre et al. 2015; Narita et al. 2015; van den Berg et al. 2015; Kim et al. 2010).

The aim of this manuscript is to provide an updated perspective on the rationale behind the need of a vaccine against *S. aureus* and its current medical need, provide an extensive evaluation of target populations suitable for efficacy trials, link phase III data to preclinical data, and review recent research and development efforts.

2 Rationale Behind the Need of a *S. Aureus* Vaccine

A vaccine against *S. aureus* is urgently needed because this pathogen causes a large array of diseases worldwide and no sufficiently efficacious medical treatments available. *S. aureus* is the leading cause of SSI and ICU infections, HCA pneumonia, CA-SSTI, and mortality worldwide (Table 2). Patients often get infected with the strain that they carry on their skin. This suggested that decolonization of the patients could prevent invasive infections. Bundle measures including decolonization procedures to prevent postoperative infections have been implemented in several hospitals. These measures have provided modest yet still significant reductions in infection (Schweizer et al. 2015). The effort necessary for decolonizing the patients can be quite substantial. Indeed, decolonization studies that demonstrated efficacy required that the elective surgery patients were screened for carriage status and strain type, as to determine the appropriate antibiotic prophylaxis. Carriers were then treated with mupirocin for nasal decolonization and asked to bath with chlorhexidine for five days before the operation. Patients carrying methicillin-resistant strains (MRSA) received vancomycin and cefazolin or cefuroxime for perioperative prophylaxis, while carriers of methicillin-susceptible strains received cefazolin or cefuroxime (Schweizer et al. 2015).

Similar attempts have been performed to reduce or prevent CA-SSTI. In this case, no consistent reduction of the infection has been observed despite the numerous attempts and substantial investments on this approach (Creech et al. 2015; Miller et al. 2015). Indeed, although a trend towards reduction of postoperative infections has been observed, that is not true for CA-SSTI (Suaya et al. 2014).

Therefore, bundle measures are not likely to decrease CA-SSTI, which in turn could continue to function as an incubator for epidemics and evolution of antibiotic resistance. This has already happened with two major outbreaks (for which we have documented information) being attributed to CAIs. The first one was the epidemic in Australia, Great Britain, Canada, and the USA caused by the penicillin-resistant *S. aureus* clone known as phage-type 80/81 which lasted for ten years from 1953 to 1963 and then declined for unknown reasons (DeLeo et al. 2011). Another more recent outbreak started in the USA around the year 2000 and was associated with the MRSA strain USA300 (Chambers and Deleo 2009). Interestingly, both the

Table 2 Proportional contribution and mortality of CA and HA *S. aureus* infections

Region	Percentage of *S. aureus* infection				*S. aureus*-associated mortality
	CA-SSTI	SSI	HCA pneumonia	ICU infections	
Europe	>50 (Lorette et al. 2009; Sader et al. 2010)	21.6 (Anonymous 2013)	14.5–31 (Dandagi 2010; Chastre and Fagon 2002; Koulenti et al. 2009; Jones 2010)	>19 Vincent et al. (2009)	5400 extra deaths attributable to MRSA BSI per year (ECDC/EMEA 2009)
North America	53–84 (Moran et al. 2006)	15.5–30 (Magill et al. 2014; Hidron et al. 2008)	16–36.3 (Magill et al. 2014; Jones 2010; Hidron et al. 2008; Kollef et al. 2005)	26.9 Vincent et al. (2009)	11,000 deaths per year with invasive MRSA (USA) (CDC 2013)
Asia	32.7 (Zhao et al. 2012)	NA	$\geq$15 (Chi et al. 2012; Chung et al. 2011), 16 (Nickerson et al. 2009) 20.3 (Diekema et al. 2001)	16.1 Vincent et al. (2009)	Mortality rate associated with BSI: 15.3% (Turkey) (Yilmaz et al. 2016); 16.4% (Japan) (Nagao et al. 2010); 44% (Thailand) (Nickerson et al. 2009)
Africa	NA	>20 (Ojulong et al. 2009; Mawalla et al. 2011; Anguzu and Olila 2007)	20.3 (Diekema et al. 2001	29.6 Vincent et al. (2009)	Mortality rate associated with BSI: 8.8% (South Africa) (Naidoo et al. 2013)
Latin America	NA	NA	20.1 (Jones 2010)	19.2 Vincent et al. (2009)	Mortality rate associated with BSI: 35.1% (Brazil) (Guilarde et al. 2006); 4–13% (Argentina) (Paganini et al. 2010)
Australia/New Zealand	NA	>50% (Chen et al. 2008; Harrington et al. 2004; Spelman et al. 2000)	20.3 (Diekema et al. 2001)	27.5 Vincent et al. (2009)	Mortality rate to BSI: 11.2% (Australia) (Turnidge et al. 2007); 18.9% (New Zealand) (Hill et al. 2001)

epidemics started as CAI and then entered the hospital setting. USA300 is now the predominant strain in the USA for CAI and is expected to replace typical HA strains such as USA100 in the hospitals (D'Agata et al. 2009).

Given the extraordinary ability of *S. aureus* in developing antibiotic resistance, bundle measures could pose an additional pressure towards its increase. Indeed,

isolates resistant to penicillin and methicillin, for example, were observed approximately one year after the commercial introduction of the two antibiotics in 1944 and 1960, respectively (Chambers and Deleo 2009). Resistant strains to vancomycin are emerging fortunately more slowly, but strains resistant to new-generation antibiotics (e.g., daptomycin and linezolid) have also already been observed. In addition, antibiotic therapy is not always effective even against susceptible strains. This may depend on many factors including the site of the infection and the presence of bacterial biofilms.

Once proven efficacious against *S. aureus* infections in humans, vaccines may be able to prevent a further increase of antibiotic resistance, provide herd immunity and decrease likelihood of outbreaks, as well as synergize with all existing medical treatments. These reasons clearly advocate for the need of a vaccine against *S. aureus.* However, *S. aureus* is a complex pathogen and a vaccine against it is difficult to develop given also the lack of a known correlate of protection. Furthermore, the cost-effectiveness of such a vaccine is another important parameter to evaluate for supporting its rationale. Vaccine impact should be evaluated considering diverse variables, such as reduction of infection-associated morbidity and mortality, length of hospital stay, and hospital expenses. In principle, prophylactic vaccination has the great advantage of being able to block initial infection phases, establishment of biofilm and other chronic infectious forms as well as prevent sequelae decreasing the risk of permanent impairments. All these aspects may significantly reduce the length of hospital stay and healthcare costs. Computer simulation modelling predicted a favourable cost-effectiveness of a hypothetical *S. aureus* vaccine across a wide range of MRSA prevalence, vaccine efficacy (10–90%), and vaccine cost (50–1000$) for orthopaedic surgery patients (Lee et al. 2010). Similar estimates were also done for hemodialysis patients (Song et al. 2012).

3 Target Populations Suitable for *S. Aureus* Vaccine Efficacy Trials

Ideally, the target population selected for efficacy trials should be the one that will facilitate successful completion of clinical trials as well as have a significant medical need and will be cost-effective for vaccination strategies. With these concepts in mind, we selected target populations that may fulfil these requirements and we highlighted the up- and downsides (Table 3).

3.1 End-Stage Renal Disease (ESRD) Patients

One of the first target groups coming to mind is the end-stage renal disease (ESRD) patients receiving hemodialysis. These patients are at increased risk of *S. aureus*

Table 3 Target population selected for efficacy trials

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
Hemodialysis patients	There are more than 870,000 ESRD sufferers living in the USA	4%	In the USA, between 1/3 and 1/2 of *S. aureus* bacteremias in hemodialysis patients are due to MRSA	Mainly BSI. Mortality up to 20%	Average age during dialysis >60 years	Population at a high risk of *S. aureus* infection	Secondary to frequent dialysis, there is reduced half-life of serum Ig.	Reduction of *S. aureus* BSI	Chan et al. (2012)
					Patients are to be considered immunocompromised during hemodialysis	Efficacy could be shown in relatively short period and with small number of patients	Fast antibody decline rate following a *S. aureus* glycoconjugated vaccine has been observed		http://www.usrds.org/2015/view/v2_01.aspx
						Patients are well monitored (3 times/week in dialysis centre)	Impaired neutrophil function		Fattom et al. (2015)
						and epidemiology established			Fattom et al. (2004)
									https://pharm.ucsf.edu/kidney/need/statistics
									http://www.niddk.nih.gov/health-information/health-statistics/Pages/kidney-disease-statistics-united-states.aspx

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
ICU patients	More than 5 million patients are admitted annually to ICUs in the USA	11%	*S. aureus* is the most common pathogen (20%)	The ICU mortality rate of infected patients was more than twice that of non-infected patients	Average age >60 years	High infection rate	Extremely ill patients	Reduction of all-case mortality	(Vincent et al. 2009)
				Respiratory infections are the most prevalent	Extremely ill patients and may undergo multiple complex interventions at the same time	High prevalence of *S. aureus*	Short time for inducing protective immunity	Reduction of pneumonia	http://www.sccm.org/Communications/Pages/CriticalCareStats.aspx
				Sepsis is the leading cause of death in non-cardiac ICUs, with mortality rates that reach 60%		High unmet medical need		Reduction of *S. aureus* BSI	http://healthpolicy.ucsf.edu/content/icu-outcomes Mullins et al. (2013)

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
Neurosurgery	>280,000	0.6–1.8%	11–39% considering all surgical site infections (most important pathogen)	BSI and SSI with greater incidence of the latter (deep infections more frequent than superficial)	Average age at surgery >50 years	This target group includes a substantial proportion of relatively young and otherwise healthy subjects	Size of the population relatively small	Reduction of *S. aureus* SSI and BSI	Noskin et al. (2007)
	operations per year in USA	(including BSI and SSI)		Mortality rate: 5.7%			Wide range of incidence has been observed (infection rates vary greatly according to the type of initial surgical intervention) complicating clinical trial design		Anderson et al. (2010)
							Population size relatively small		Hidron et al. (2008)
									Magill et al. (2014)
									Chahoud et al. (2014)
									Mullins et al. (2013)
									Magill et al. (2014)
									Chen et al. (2008)
									Harrington et al. (2004)
									Spelman et al. (2000)

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
Orthopaedic	>2,400,000	0.4–1.8%	11–39% considering all surgical site infections (most important pathogen)	BSI and SSI with greater incidence of the latter	Average age at surgery >50 years	Large target group with number of operations steadily increasing	Wide range of incidence has been observed (infection rates vary greatly according to the type of initial surgical intervention) complicating clinical trial design	Reduction of *S. aureus* SSI and BSI	Noskin et al. (2007)
	operations per year in USA	(including BSI and SSI)		Mortality rate 2.5%		Infection rate is increasing due to greater number of implants used			Anderson et al. (2010)
									Del Pozo and Patel (2009)
Cardiothoracic	>2,200,000	0.8–1%	11–39% considering all surgical site infections (most important pathogen)	BSI and SSI with greater incidence of the former. High proportion of BSI is associated with high mortality rate (mortality rate 9.5%)	Average age at surgery > 60 years	Large target group and number of procedures increasing	Implementation of less invasive procedures reduces infection rate and hospitalization time	Reduction of *S. aureus* BSI and SSI	Noskin et al. (2007)
	operations per year in USA	(including BSI and SSI)							Anderson et al. (2010)
									Segers et al. (2006)
									Iribarne et al. (2011)

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
Patients with recurrent SSTI	14 million outpatient and emergency department visits annually in the USA for primary or recurrent SSTI	9–70% of recurrences after a primary SSTI	Virtually 100% (in recurrent patients after a primary infection with *S. aureus*)	Mainly uncomplicated SSTI such as abscess and cellulitis	Children and adults	By enrolling patients with a primary SSTI or with ongoing recurrent SSTI, the incidence in this target group can be as high as 70%	High variability in incidence reported between different hospitals	Reduction of incidence of *S. aureus* SSTI Reduction of superficial skin infections	Hersh et al. (2008)
						Rising hospitalization and recurrence rates have been observed	This target group is usually not associated with severe infections, and medical need is relatively lower than other targets		Knox et al. (2016), Fritz et al. (2013), Fritz et al. (2012), Miller et al. (2015)
						Increased disease severity observed recently in the UK. More severe and recurrent staphylococcal skin diseases have limited treatment options			Shallcross et al. (2015)

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
Atopic dermatitis (AD) patients	AD affects 15 to 20% of children and 1 to 3% of adults worldwide (over 17 million in USA)	>90% of patients with AD are colonized with *S. aureus* in the lesional skin	*S. aureus* is the infectious agent most often involved in AD	Infection can worsen AD symptoms	Mainly infants and children	High attack rate	Altered tegument (filaggrin) and T cell response in atopic dermatitis may affect vaccine efficacy against skin infections		Nutten (2015)
				Superficial skin infections, i.e., impetigo (very common and requiring repeated antibiotic courses)		Patients are under constant observation		Reduction of AD symptoms	Hanifin et al. (2007)
				Increased risk of invasive disease in AD patients (i.e., bacteremia, osteomyelitis, and endocarditis)		Improvements of AD symptoms following vaccination can be easily tracked			Rudikoff and Lebwohl (1998)
						AD patients respond well to common recommended vaccines and with no adverse events, indicating that potential underlying immune defects of these patients			Benenson et al. (2005)

(continued)

Table 3 (continued)

Target Population	Target population size	Infection rate	*S. aureus* relative abundance	Disease outcome	Patients' characteristics	Advantages	Disadvantages	Trial primary endpoint	References
						does not alter responses to vaccination			
						Eradication of *S. aureus* is difficult in AD patients using available therapies			Watson and Kapur (2011)
									(Goss and Beninger 2005)
									Marin et al. (2005)
									Kienast et al. (2007)
Military recruits	US Army active-duty force 490,000 in 2015	3.5-4% in US Army infantry	85% of SSTI in the US Army infantry were due to *S. aureus*	Most common manifestations are cellulitis and abscesses	Average age 20 years	Easy access to vaccinees (all infantry trainees). Constant monitoring of the vaccinees.	This target group is usually not associated with severe infections, and medical need is relatively lower than other targets	Reduction of *S. aureus* SSTI	Frydenberg et al. (2005)
						High disease incidence. Healthy and young subjects			Morrison-Rodriguez et al. (2010)

infections due to violation of the protective skin barrier for vascular access several times a week for a long time. The type of vascular access may predispose the patient towards bacteraemia, with higher rates observed in patients with venous catheters in situ than in patients with arteriovenous fistulas (Crowley et al. 2012). Among 293,000 patients receiving chronic hemodialysis in the USA, *S. aureus* bacteraemia was observed in 4.0 per 100 outpatient-years (Chan et al. 2012). Of these, infections between one-third and one-half are due to MRSA strains. Complications include endocarditis, osteomyelitis, discitis, and soft tissue abscesses, and the case fatality rate is as high as 20% (Fitzgerald et al. 2011).

However, ESRD patients respond poorly to vaccination (Shinefield et al. 2002; Fattom et al. 2015). Indeed, hemodialysis has a debilitating impact on the performance of their PMNs and complement, both essential components of the opsonophagocytosis process. Furthermore, it also reduces half-life of serum Ig with the consequent fast decline of antibody titres following vaccination.

3.2 Intensive Care Unit (ICU) Patients

ICU admissions have almost doubled in recent years with more than 5 million patients admitted annually to ICUs in the USA (Carr 2009). Infections in ICUs are associated with considerable morbidity, mortality, and costs worldwide (Vincent et al. 2009). 65% of healthcare-associated infections were reported to occur in the critical care setting by the centres for disease control and prevention in the period 2009–2010 (Sievert et al. 2013). An extended prevalence of infection in intensive care (EPIC II) study conducted in 1,265 participating ICUs from 75 countries with 14,414 patients gave a global picture of the epidemiology in ICUs (Vincent et al. 2009). Seven geographical regions were included in the study: North America, Central and South America, Western Europe, Eastern Europe, Asia, Oceania, and Africa. Central and South America had the highest infection rate (60%), and Africa had the lowest (46%). Pneumonia was the most common disease, accounting for 64% of infections, followed by the abdomen (20%), the bloodstream (15%), and the renal tract/genitourinary system (14%). Forty seven per cent of the positive isolates were gram-positive with the most common organism being *S. aureus* (20%).

However, ICU patients are severely ill and they often need complex medical interventions, which may hinder the success of a trial in this target group. In addition, in several cases, time available to vaccinate patients and mount a protective response could be too short (Table 3).

3.3 Surgery Patients

S. aureus is a major cause of infection in surgical patients and those hospitalized for planned surgery may have time to develop effective immune response upon

vaccination before surgery. Among the surgery patients most frequently infected by *S. aureus*, the largest group is the orthopaedic (OP), followed by cardiothoracic (CT) and neurosurgical (NS) [see Table 3 and reference Noskin et al. (2007)]. Given the increasing size of the elderly population worldwide, this number is expected to further increase, especially the ones associated with orthopaedic surgery for hip and knee replacement. Consequently, the number of infections in this target group is also expected to increase. In the recent literature, *S. aureus* infection incidence goes from 0.4 to 1.8% for OP, 0.8–1% for CT, and 0.6–1.8% for NS (Anderson et al. 2010; Noskin et al. 2007). Infections in OP and NS patients mainly occur due to infection of the surgical wound (surgical site infection (SSI)). SSI is the third most frequent healthcare-associated events. These infections can be superficial (involving only skin or subcutaneous tissue of the incision), can be deep-seated (involving fascia and/or muscular layers), or can reach the organ space (any other anatomic site that was opened or manipulated during surgery). Among the pathogens that can cause SSI, *S. aureus* accounts for the greater number. Furthermore, although to a lesser extent, *S. aureus* causes also bloodstream infections (BSI) in OP and NS. On the other hand, CT patients have BSI as major infection outcome. Indeed, this latter group has the highest mortality rate (9.5%), followed by NS (5.7%) and OP (2.5%) (Noskin et al. 2007).

The high medical need makes these three groups certainly important targets for clinical trials. However, they present different characteristics that one should consider for selecting the target. CT patients have the highest mortality and they are also a large group. However, the average age of these patients is higher than the other two groups, and the infection outcome is usually more severe putting the vaccine trial in a complicated scenario. Furthermore, infection incidence in this group is declining because of the implementation of less invasive procedure, which reduces the infection rate and hospitalization time (Iribarne et al. 2011). On the other hand, concerning OP patients, the number of hip and knee replacements is increasing in parallel with population age (Del Pozo and Patel 2009). NS patients have a high infection and mortality rate, but they are by far the smaller group (Table 3).

3.4 Community-Acquired Skin and Soft Tissue Infections (CA-SSTIs) Patients

S. aureus is the most common pathogen associated with CA-SSTI worldwide (Moet et al. 2007). The prevalence of CA-MRSA is higher in the USA when compared to Europe, but the prevalence in the latter is rising (Ibler and Kromann 2014). *S. aureus* SSTI incidence doubled from 57 in 2001 to 117 in 2009 (per 100,000 persons) and hospitalizations due to these infections increased by 123% representing a significant share of *S. aureus* hospitalizations (51%) in the USA. Total annual cost of staphylococcal SSTI hospitalizations has been reported to be $4.5

billion, while $14.5 billion was the cost due to all staphylococcal infections (Suaya et al. 2014). CA-SSTIs have a vast and diverse presentation going from superficial infections such as impetigo, ecthyma, and erysipelas to deeper infections such as abscess (carbuncles and furuncles) and cellulitis. The majority of CA-SSTIs are cellulitis and abscess and almost half of these infections are caused by MRSA in the USA (Ray et al. 2013). Skin infections with MRSA can be complicated by systemic infections including pneumonia, necrotizing fasciitis, and myositis (Ibler and Kromann 2014).

Importantly, there are individuals at higher risk of infection than the general population and present themselves as potential target populations for clinical trials: patients with recurrent SSTI, atopic dermatitis patients, and military recruits. Recurrent staphylococcal SSTIs are common and problematic, with rates ranging between 9 and 70% in the USA (Creech et al. 2015; Miller et al. 2015; Fridkin et al. 2005; Miller et al. 2007; Fritz et al. 2012; Montgomery et al. 2015). As many as 70% of patients with CA-MRSA SSTI experience recurrent SSTI over 1 year even after successful initial treatment (Kaplan et al. 2014; Duong et al. 2010; Chen et al. 2009; Williams et al. 2011). In addition, recurrent infections are usually more common after infection by MSRA than with MSSA (Chen et al. 2009; Sutter et al. 2011). Therefore, although recurrent SSTIs are not usually associated with severe disease outcomes, they represent a significant medical issue deserving attention, and in view of clinical trials, they have the notable advantages of the high infection incidence and the possibility to enrol the subjects when they present to the hospital with a primary SSTI. In addition, recurrence seems not to be due to underlying health defects of the subjects, but rather to their colonization status and exposure to environmental sources of the bacterium (Montgomery et al. 2015).

Staphylococcal infection of atopic dermatitis lesions occurs in around 90% of the patients, and this leads to a worsening of the skin syndrome and delayed healing (Bieber 2008). The prevalence of eczema has substantially increased in industrialized countries during the past three decades with 15–30% of children and 2–10% of adults being affected (Bieber 2008). Therefore, a vaccine able to reduce the staphylococcal burden on the eczematous lesions would be highly beneficial to these patients. It would be obviously easy to recruit and follow such patients during clinical trials, and the high prevalence of staphylococcal infection would facilitate the studies. On the other hand, these patients could have underlying immunological and skin defects that might hinder the success of the trials (Bieber 2008). However, there are evidences indicating that they respond normally to marketed vaccines, such as the one against varicella (Marin et al. 2005; Goss and Beninger 2005; Kienast et al. 2007; Frydenberg et al. 2005).

Military recruits and in particular US army infantry have an unusually high incidence (4%) of staphylococcal SSTI (Ellis et al. 2014; Morrison-Rodriguez et al. 2010). This is likely due to the relatively crowded living conditions of the trainees and to the frequent skin abrasions and traumas that they experience during training. Given that these subjects are generally healthy and that they can be constantly monitored by the military medical personnel, they may represent a good target for vaccine trials.

4 Preclinical Research on Antigens Selected for Clinical Development

A number of vaccine candidates have been tested in phase I clinical trials through active immunization. The antigens tested so far are mainly bacterial surface-associated and secreted proteins or capsular polysaccharides (CPs). In Table 1, we summarize the antigens tested so far in clinical trials. Of these vaccine candidates, only V710 (containing IsdB) and StaphVax (containing CP type 5 and CP type 8) have been tested up to phase III trials. Recently, Pfizer announced plans to advance to a phase IIb trial its 4-component vaccine candidate SA4ag (containing CP5, CP8, ClfA, and MntC). Here, we briefly review the essential preclinical data on the antigens tested so far, or currently being tested, in phase II and III trials in the attempt to link preclinical observations with human efficacy data. In addition, we discuss antigens recently proposed as additional vaccine candidates, but that are still in preclinical phase or in early clinical development.

4.1 Antigens that Reached Phase III Trials

The iron-regulated surface determinant (Isd) proteins compose an iron-scavenging system that is capable of stealing the iron-containing heme from host haemoglobin and transporting it to the cytoplasm (Mazmanian et al. 2003). IsdB acts as the first player in a cascade of Isd proteins of which all harbour NEAr iron transporter (NEAT) domains that directly bind the heme from host haemoglobin (Gaudin et al. 2011). IsdB was shown to be the main haemoglobin receptor for the bacterium and inactivation of the IsdB gene impaired the ability of the bacterium to use haemoglobin as iron source and mutants lacking *isdB* were less virulent than the wild type in the mouse abscess model (Torres et al. 2006). However, immunization of mice with IsdB was shown to confer inconsistent protection from infection, and it was shown to be significantly inferior to antigen combinations (Bagnoli et al. 2015; Stranger-Jones et al. 2006). On the other hand, immunization of rhesus macaques elicited high antibody titres against the protein (Stranger-Jones et al. 2006; Kuklin et al. 2006). These preclinical results suggest that although highly immunogenic, IsdB is not sufficient to confer protective immunity.

Capsular polysaccharides constitute a way of immune evasion for *S. aureus* (Nanra et al. 2013). CP5 and CP8 serotype strains are the most common within clinically relevant strains and therefore have been of interest for vaccine research (Fattom et al. 1993). Either CP5 or CP8 is expressed by most strains, but importantly not by USA300, the most common community-associated MRSA strain in the USA (Boyle-Vavra et al. 2015). The capsular polysaccharides are thought to prevent effective phagocytosis by interfering with antibody and complement C3b deposited on the bacterial surface (O'Riordan and Lee 2004; Thakker et al. 1998). Nevertheless, they themselves constitute a target for antibodies, and antibodies

against CP5 and CP8 have been shown to enhance opsonophagocytotic killing of the bacteria (Nanra et al. 2013; Fattom et al. 1993). In mice, active immunization with *Pseudomonas aeruginosa* exotoxin A (EPA)-conjugated CP5 and passive immunization with CP5 antibodies were shown to confer protection against *S. aureus* challenge (Fattom et al. 1996). However, *S. aureus* strains lacking capsule expression show only a modest reduction in virulence in animal models of abscess formation, arthritis, wound infection, and bacteraemia (O'Riordan and Lee 2004; Cheng et al. 2009). In addition, the level of protective immunity elicited by CP8 antibodies in animal models of infection appears inconsistent in the literature (Fattom et al. 2015; Cook et al. 2009). Although a comparison of the efficacy of the CP antigens with antigen combinations has never been published, given the lack of capsular expression in USA300 and the inconsistent observations in animal models, it is plausible to assume that higher protection could be raised by combining the CPs with other vaccine targets.

4.2 Antigens Currently Being Tested in Phase II Trials

The candidate vaccine being developed at Pfizer includes CP5, CP8, ClfA, and MntC (Anderson et al. 2012; Dayan et al. 2016). We have already described the capsular antigens above. However, in the case of Pfizer vaccine, the polysaccharides are conjugated to the carrier protein CRM, instead of EPA as in the case of StaphVax. Clumping factor A (ClfA) is a surface-exposed adherence protein that binds human fibrinogen and fibrin causing clumping of blood platelets and bacteria (McDevitt et al. 1994), and it is also capable of inhibiting complement-mediated phagocytosis (Hair et al. 2010). ClfA has been shown to be important in the early stages of infection (Hair et al. 2010), sepsis (McAdow et al. 2011), arthritis (Josefsson et al. 2001), skin infection (Kwiecinski et al. 2014), and endocarditis (Moreillon et al. 1995; Que et al. 2001) in small animal models. Both passive and active immunization studies with ClfA have shown protectivity against staphylococcal infection in mice (Arrecubieta et al. 2008; Hall et al. 2003; Tuchscherr et al. 2008). The majority of humans have anti-ClfA antibodies from previous exposure to *S. aureus*, but generally the antibodies do not functionally block the binding of bacteria to fibrinogen. On the other hand, vaccination of mice and humans with combinations containing ClfA, however, was able to elicit antibodies that were able to block the bacterium from binding to fibrinogen (Hawkins et al. 2012).

Manganese transport protein C (MntC) functions together with MntABC, an ATP-binding cassette (ABC) transporter complex important for manganese transport across the bacterial cell membrane. MntC has been shown to be highly conserved across different *S. aureus* strains, and it is also relatively conserved across other staphylococcal species (Anderson et al. 2012). In mouse bacteremia model, MntC was expressed early during infection, in many cases detectable 1 h after

infection. Immunization of mice with MntC indicated protection against both *S. aureus* and *S. epidermidis* in an acute bacteremia model, and immunization-derived monoclonal antibodies were shown to cause a reduction in bacterial load in infant rats and to be able to elicit a neutrophil respiratory burst. MntC has also been shown to have a role in protecting the bacterium from oxidative bursts, one of the means neutrophils use for bacterial killing (Handke et al. 2013). MntC provides an access for the bacterium to manganese, the only known cofactor for superoxide dismutases that neutralize neutrophil superoxide radicals. Antibodies against MntC may thus have a dual function: opsonizing the bacteria and sensitizing them to neutrophil killing. This antigen has been included in the four-valent vaccine being developed by Pfizer which is now in phase IIb trial (https://clinicaltrials.gov/ct2/show/NCT02388165?term=pfizer+staphylococcus+vaccine&rank=1).

4.3 Antigen Combinations that Reached Phase I Trials

There are a number of antigens that were tested in phase I trials, and their full review is beyond the scope of the present manuscript (see Table 1). Herein, we will focus on combo vaccines. The GSK candidate that reached phase I includes three out of four of the Pfizer antigens: CP5, CP8, and ClfA (Anderson et al. 2012; Levy et al. 2015). The differences are in that GSK has additionally included a detoxified α-hemolysin (HlaH35R), whereas Pfizer has included MntC. Furthermore, polysaccharides developed by GSK are conjugated to the carrier protein TT, instead of CRM as in the case of Pfizer.

α-Hemolysin (Hla), also known as α-toxin, is one of the major virulence factors of *S. aureus.* The toxin is secreted as a water-soluble monomer and can bind to erythrocytes, platelets, monocytes, lymphocytes, and endothelial as well as epithelial cells (Bhakdi and Tranum-Jensen 1991; Berube and Bubeck 2013). Monomers assemble into a membrane-perforating homoheptamer upon binding to its eukaryotic proteinaceous cellular receptor, ADAM10 (Bhakdi and Tranum-Jensen 1991; Inoshima et al. 2011; Song et al. 1996; Kawate and Gouaux 2003). Hla appears to play a prominent role in causing pneumonia and skin lesions in animal models of *S. aureus* infection (Berube and Bubeck 2013). The antigen used in clinical trials was detoxified by an amino acid substitution at position 35 resulting in a mutated protein unable to form pores and lyse host cells and to cause cell junction dissolution (Wilke and Bubeck 2010).

Novartis Vaccines (now part of GSK) has been developing a considerably different quadrivalent vaccine (4C-Staph), in which only one of the antigens, α-hemolysin, is common to the GSK combination tested in phase I. The combination included two surface (FhuD2 and Csa1A) and three secreted factors (HlaH35L and a fusion of EsxA and EsxB).

FhuD2 (ferric hydroxamate uptake D2) is a ferric hydroxamate-binding lipoprotein involved in iron uptake and in early stages of invasive *S. aureus* infection (Mishra et al. 2012; Mariotti et al. 2013; Sebulsky and Heinrichs 2001).

Its role in virulence appears to be particularly associated with abscess formation as shown by vaccination studies and through the use of FhuD2 knockout mutants in mice (Bagnoli et al. 2015; Mishra et al. 2012). Csa1A (conserved staphylococcal antigen 1A) is a putative lipoprotein highly conserved across different *S. aureus* isolates and belongs to a family of proteins encoded in at least four distinct loci sharing from 54 to 91% sequence identity (Schluepen et al. 2013). Regarding the three secreted virulence factors included in 4C-Staph, Hla was already described above, while EsxA (ess extracellular A) and EsxB (ess extracellular B) are two factors secreted through the ESAT-6 secretion system (ESS) of *S. aureus*. They are associated with abscess formation and may facilitate persistence and spread of the pathogen in the infected host (Burts et al. 2005; Korea et al. 2014). To be exploited as vaccine components, EsxA and EsxB were fused together, creating a recombinant 24-kDa EsxAB chimera, which was stable and well expressed in *Escherichia coli* unlike the individual proteins. That is why the Novartis vaccine candidate was named 4C-Staph (four-component *S. aureus* vaccine), although it contains five antigens.

This composition was shown to be highly protective in different animal models, its efficacy to be significantly augmented by a novel TLR7-dependent adjuvant and to be clearly superior to IsdB (Bagnoli et al. 2015; Korea et al. 2014).

Another combination that has been brought to phase I by Uniformed Services University of the Health Sciences in collaboration with Nabi Biopharmaceutical contained Hla and LukS-PV, a component of Panton–Valentine leukocidin (PVL) (https://clinicaltrials.gov/ct2/show/NCT01011335?term=nabi+staphylococcus&rank=1). Hla has been described above, while LukS-PV is one of the two components that together with LukF-PV form a pore-forming octameric toxin. The two components are secreted by the bacterium separately as monomers. A molecule of LukS-PV binds to its receptor on the target host cell, and this event triggers the binding of four LukF-PV molecules to an equivalent number of LukS-PV leading to the formation of the octameric complex. The toxin targets polymorphonuclear phagocytes (PMN) and monocytes (DuMont and Torres 2014). Additional information about leukocidins as vaccine targets is included in the next paragraph.

It is encouraging for vaccine development that such varied compositions show promising results in preclinical experiments. Furthermore, it seems likely now that a combination of several antigens is required for an effective vaccine and it is the path most companies have decided to follow.

4.4 Recently Proposed Antigens that Are Still in Preclinical Phase

Since *S. aureus* encodes a number of virulence factors and other surface-exposed or secreted proteins, there are likely other, currently less explored proteins that could

be promising vaccine antigens. These include the staphylococcal protein A (SpA) and the bicomponent leukocidins. SpA is a logical target as it is capable of binding to IgG Fc region possibly making any valuable antibodies against the vaccine antigens incapable of inducing opsonophagocytosis. SpA also functions as a B cell superantigen and through B cell clonal expansion and apoptosis can impair humoral responses (Silverman and Goodyear 2006). Vaccination with a functionally impaired SpA mutant (SpA_{KKAA}) was indeed shown to increase antibodies against other *S. aureus* antigens upon experimental infection of mice and guinea pigs (Bagnoli et al. 2015; Kim et al. 2010, 2015). Thus, a vaccine that includes SpA should work by inducing antibodies able to neutralize SpA activity and virtually increasing the efficacy of antibodies against any other bacterial antigens. Furthermore, inhibition of B cell deletion activity is expected to unleash the response of the host towards the pathogen (Fig. 1).

Bicomponent leukocidins are potent secreted toxins that can effectively kill various types of leucocytes as well as erythrocytes (Alonzo and Torres 2014). They are reviewed in detail in this volume. The pathogenic role of bicomponent leukocidins in mice has been demonstrated for LukED (Alonzo et al. 2012) and LukAB

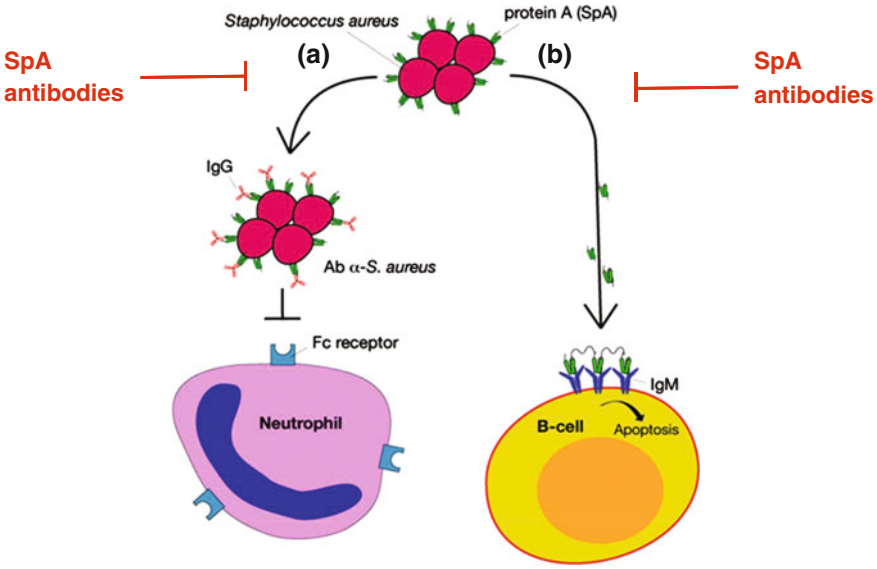

Fig. 1 Blocking the activity of staphylococcal protein A (SpA) through vaccination. SpA plays a dual role in suppressing immune responses to *S. aureus* infection: **a** prevents phagocytosis by binding to the Ig Fcγ domain and impeding its interaction with Fc receptors on phagocytes; **b** inhibits humoral response against staphylococcal antigens by cross-linking B cell receptors and inducing apoptosis of affected cells. Functional SpA antibodies are expected to block both of these activities allowing any existing staphylococcal antibodies to bind properly to the bacterium and potentially induce phagocytosis as well as to unleash B cell response against virtually any immunogenic staphylococcal antigen

(Dumont et al. 2011). An attenuated LukS-PV antigen was shown to protect mice from intraperitoneal infection and was able to induce cross-neutralizing antibodies against LukED, HlgAB, and HlgCB (Karauzum et al. 2013; Adhikari et al. 2015). Leukocidins have partly overlapping targets, and most of them can form non-canonical pairs increasing the number of possible permutations to 13 different toxins. Thus, in order to completely block their function, it would be instrumental to elicit antibodies that can neutralize all of them in order to fully understand their relevance for pathogenesis and consequently for vaccination.

We summarized the main features of the antigens described above and others in various stages of development in Table 1.

5 Clinical Data on Vaccine Candidates that Reached Phase III

Phase I–III data on IsdB (V710) and the CP5/CP8-based vaccine (StaphVax) have been published (Shinefield et al. 2002; Fattom et al. 2015; Fowler et al. 2013; Harro et al. 2010, 2012; Moustafa et al. 2012; Fattom et al. 2004). These data are certainly among the most important available in the literature for understanding human response to *S. aureus* vaccines and deserve attention.

5.1 Summary of Phase I–III Trial Data on V710

V710 was immunogenic within 14 days after a single dose of either an adjuvanted or non-adjuvanted formulation in healthy volunteers (Harro et al. 2010, 2012; Moustafa et al. 2012). Antibody response to V710 was similar in younger and older participants. Elevated antibody responses persisted for at least one year after vaccination in most patients. The vaccine was shown to be safe in Phase I and II trials (Harro et al. 2010; Harro et al. 2012; Moustafa et al. 2012).

Phase I testing led to the selection of a 60-μg dose of lyophilized, non-adjuvanted antigen for efficacy evaluation. A pivotal phase 2b/3 study was initiated to evaluate the efficacy and safety of preoperative vaccination with V710 in patients undergoing cardiothoracic surgery. The primary efficacy objectives of the study were to demonstrate a reduction in the proportion of patients with postoperative *S. aureus* bacteremia and/or *S. aureus* deep sternal wound infections through 90 postoperative days by at least 20% relative to a placebo group. Secondary efficacy objectives included demonstrating a reduction in the proportion of patients who developed any invasive or surgical site infection with *S. aureus* through 90 postoperative days. Patients were vaccinated 14–60 days prior to surgery and monitored for safety and *S. aureus* infections for 90 days postsurgery.

During an interim analysis, a Data Monitoring Committee (DMC) recommended to terminate the trial due to a low probability of achieving vaccine efficacy as well as safety concerns. Indeed, the incidence of multiple organ failure and mortality in patients with staphylococcal infections was found to be significantly higher among V710 vaccine than placebo recipients during the entire study. Despite the fact the vaccine failed to reach its primary efficacy endpoints, it elicited robust antibody responses and reduced the number of infections at the saphenous vein donor site.

There could be multiple reasons behind the failure of V710 vaccine (Bagnoli et al. 2012; McNeely et al. 2014). First of all, it is unlikely that an approach targeting a single antigen can cope with the complex pathogenic armamentarium of *S. aureus*. Indeed, as described above, IsdB was not consistently protective in animal models. Then, the surgery complexity, the old age (65 years on average), and the poor health status of the patients enrolled in the trial may have also contributed to this failure. Possible explanations for the apparent increased mortality in the vaccine recipients who got infected with *S. aureus* come from a retrospective analysis of the trial, which had the aim to explore possible associations between immune markers at baseline and death after postoperative *S. aureus* infection in V710 recipients (McNeely et al. 2014). The following ten cytokines were analysed: interferon-γ, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17a, and TNF-α. Undetectable IL-2 levels before and after vaccination and a low IL-17a level at hospital admission showed a statically significant association with postoperative mortality in V710 recipients after subsequent *S. aureus* infection. On the other hand, this association was not found with placebo recipients. This observation could, at least partially, explain not only the increased mortality observed with the vaccine, but also the lack of efficacy. Importantly, in line with this observation, we have observed that a protein vaccine formulated with a new TLR7-dependent adjuvant is able to significantly increase protective efficacy, the level of IL-2, and skew the response towards Th1/Th17 in mice (Bagnoli et al. 2015).

5.2 Summary of Phase I–III Trial Data on StaphVax

StaphVax was shown to be highly immunogenic in healthy subjects, and following a single dose of a non-adjuvanted formulation, containing 25 μg of each CP5 and CP8 conjugates per dose, vaccination elicited a 10–20-fold increase in antibody levels. Antibody concentration peaked within 10–14 days after immunization, and a second injection of the vaccine 6 weeks later did not further increase antibody titres. Furthermore, a long-term immune response with a slow decline over time was observed in healthy subjects. On the other hand, when ESRD patients (the population used in phase III trials conducted with StaphVax) were immunized with the same vaccine lot, IgG levels were 56% of that achieved in healthy volunteers (Fattom et al. 2004). Moreover, a faster decline in antibody titres was observed in ESRD patients compared to healthy subjects receiving the same vaccine six months after vaccination. Higher doses of the vaccine increased antibody levels in ESRD

patients; however, they remained generally lower than in healthy volunteers and they declined more rapidly.

The first efficacy trial included 1804 adult patients randomly assigned to receive a single intramuscular injection of StaphVAX (containing 100 μg of each CP5 and CP8 without adjuvant) or saline (Shinefield et al. 2002). The primary endpoint of the trial was a significant reduction in *S. aureus* bacteraemia for 1 year. Although the study did not reach the primary endpoint because at one year reduction of bacteraemia as compared to the placebo group was only 26% and was not statistically significant, a post hoc analysis evaluating the performance of the vaccine through various earlier time points demonstrated that the vaccine did statistically reduce *S. aureus* bacteraemia by 64% through a 32-week follow-up and by 57% through a 40-week follow-up. Interestingly, efficacy appeared to decrease when antibody titres fell below approximately 80 μg/ml. These data suggested that with a booster dose efficacy of the vaccine could have been prolonged.

Indeed, a second efficacy trial was performed in which two vaccine injections were administered (Fattom et al. 2015). The trial included 3359 ESRD patients randomized (1:1) to receive vaccine or placebo at weeks 0 and 35. The primary efficacy study endpoint was the incidence of *S. aureus* bacteraemia during the 3–35-week period following the first vaccine injection. Surprisingly, no significant difference in the incidence of *S. aureus* bacteraemia was observed between vaccine and placebo groups between weeks 3 and 35 or at later time points with either one or two vaccine injections. Lack of efficacy was apparently not paralleled by a poor immune response. Comparison of the individual sera from patients enrolled in the previous study (1356) with those from patients enrolled in the second phase III trial (1371) showed no significant difference in terms of antibodies when measured by enzyme-linked immunosorbent assay (ELISA) and opsonophagocytic killing assay (OPK). However, affinity of antibodies to CP8 but not CP5 was lower in the second study compared to study 1356. In addition, anti-CP8 antibodies from study 1371 were found to act suboptimally in protecting mice from a *S. aureus* lethal challenge as compared to antibodies derived from patients in study 1356. That did not happen with CP5 antibodies. These data suggest that suboptimal vaccine quality (manufacturing) of lots used in the second phase III trial elicited antibodies with lower quality than the vaccine used in the previous 1356 study, at least for CP8.

Given the multifactorial nature of the pathogenesis of staphylococcal infections, a successful staphylococcal vaccine will likely target different virulence factors and not only one virulence factor (the polysaccharide capsule) as done in these trials. This is also suggested by data in animal models as previously discussed.

Another possible contributing factor to the failure of the vaccine is the immunological impairment associated with ESRD patients and hemodialysis as we have previously described.

One critical observation in both the phase III trials performed with StaphVax was that the vaccine was safe both in healthy subjects and in ESRD patients. Importantly, no increase of mortality has been observed after *S. aureus* infection in these trials (Shinefield et al. 2002; Fattom et al. 2015).

6 Lack of Established Correlates of Protection

Lack of known correlates of protection against *S. aureus* in humans is delaying development of efficacious vaccines and hindering interpretation of both preclinical and clinical data. *S. aureus* infection in both humans and animal models seems not to generate protective immunity against subsequent infections. Due to its ability of inhibiting opsonophagocytosis and killing B cells, SpA might represent one of the major reasons behind lack of protective immunity following infection. However, humans do normally have antibodies recognizing several antigens of the pathogen (Dryla et al. 2005; Verkaik et al. 2009; Clarke et al. 2006). We believe that lack of a clear association between humoral response and protection against *S. aureus* in humans is primarily due to B cell clonal deletion and anergy processes. These are promoted by converging factors that lead to B cell activation in concomitance with lack of T cell help due to superantigens and cytotoxins produced by the pathogen during infection (Pozzi et al. 2015). Given these phenomena, development of humoral response against most *S. aureus* antigens may be inhibited by SpA activity or characterized by B cells that do develop, but produce low-affinity antibodies due to anergic processes. This complicates interpretation of serological studies performed with the aim of identifying a correlate of protection and may prevent induction of protective immunity following *S. aureus* infection.

The other big issue that hinders the understanding of protective mechanisms against *S. aureus* is the lack of predictive functional assay. Gold standard opsonophagocytosis assays (OPA) used so far have failed to predict efficacy against *S. aureus* in clinical trials. Functional assays alternative to OPA such as whole-blood assay (WBA) (Lancefield 1957) are available and, although scarcely adopted so far by investigators, may provide important information on protective mechanisms and may be developed as a surrogate assay of protection. WBA displays many advantages over OPA (Fig. 2): no addition of exogenous and heterologous sources of constituents, and maintenance of the complex native environment such as cytokine signalling pathways, cellular types, and soluble factors (immunoglobulin and complement). Indeed, blood contains all the immunological factors (e.g., antibodies, cytokines, neutrophils, monocytes, complement, and T cells) likely necessary to contain the infection. This is particularly relevant for correlating the assay with protection against the pathogen, and such an assay could be used throughout the preclinical and clinical development. However, the assay requires fresh blood, and this might hinder its feasibility in hospitals not equipped with a proper laboratory and skilled personnel.

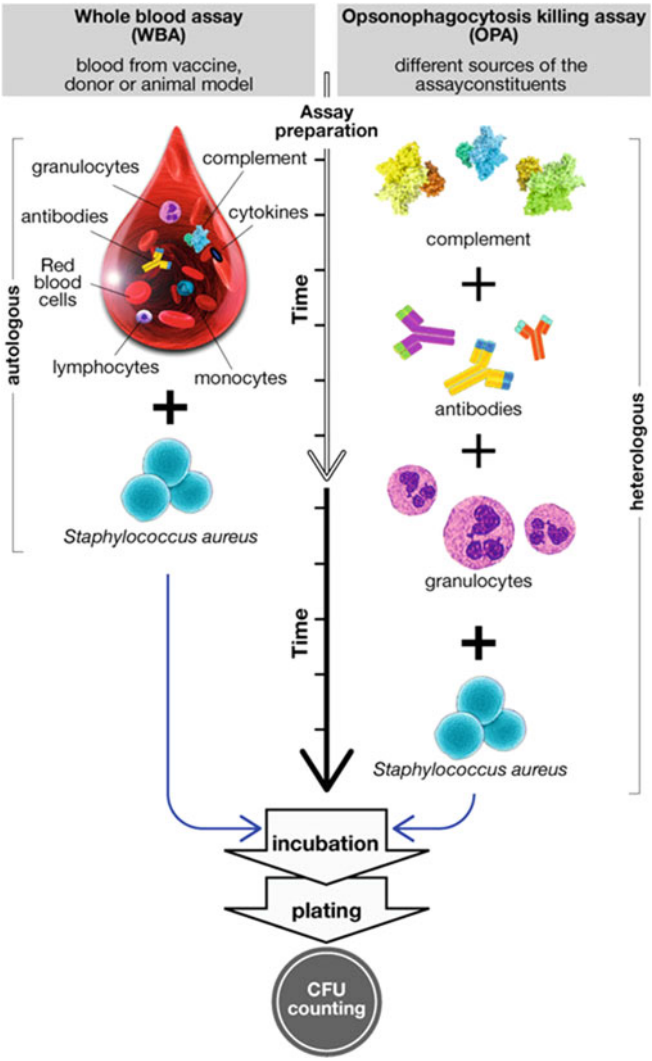

Fig. 2 Comparison of opsonophagocytic assay (OPA) and whole-blood assay (WBA) to measure the functional activity of vaccine-specific antibodies. On the left, in the WBA, all the immune components required for phagocytosis are present in blood and no exogenous constituents are necessary. On the contrary, the OPA assay, on the right panel, requires isolation of components from different sources (often from different animal species) such as complement (e.g., human, baby rabbit, rabbit, mouse, or goat), sera or purified antibodies, and granulocytes (human HL-60 or PMNs). A bacterial suspension of *S. aureus* at a concentration of 10^4–10^6 CFU/mL is added to the blood or in the OPA mixture and incubated for a time period of 60–120 min. Finally, bacteria are plated and CFU counted

7 Discussion

After the failure of two different prophylactic vaccines in phase III trials, the scientific community started to wonder whether a vaccine against *S. aureus* was really feasible. Others begun to believe that even if feasible, it was not worth pursuing costly research and development of such a complex vaccine.

Instead, our answer is yes. Yes, we still believe that vaccination represents a promising approach to meet the tremendous medical need associated with *S. aureus* infections. Safety concerns observed with V710 vaccine appear to be related uniquely to that particular vaccine and study. Indeed, no increase of disease severity or mortality was observed with two independent efficacy trials performed with the capsular-based vaccine StaphVax. This is key information for vaccine development and suggests that the increase of mortality observed with IsdB is not a common phenomenon triggered by any surface antigen. Post hoc analysis of the V710 efficacy trial suggested that deaths were significantly associated with low IL-2 response before and after vaccination. This is another invaluable observation obtained with the clinical trials. We could assume that the addition of proper antigens and adjuvants in the vaccine formulation could prevent safety issues to happen again by shifting the response towards Th1 response and increasing IL-2 levels. Interestingly, a formulation able to significantly increase IL-2 levels in mice has been recently described (Bagnoli et al. 2015). From the preclinical perspective, several publications indicate that protective efficacy of vaccines combining together important virulence factors is significantly greater that that achieved with single antigens against *S. aureus* infection in animal models (Bagnoli et al. 2015; Stranger-Jones et al. 2006). Furthermore, new promising vaccine candidates such as SpA and leukocidins and novel adjuvants able to stimulate cell-mediated immunity and increase vaccine efficacy in animal models have been identified (Bagnoli et al. 2015; Kim et al. 2010; Karauzum et al. 2013; Adhikari et al. 2015; Monaci et al. 2015).

Looking forward to clinical development of new vaccine candidates, critical aspects to consider are the selection of target populations for efficacy trials as well as endpoints and biomarkers to be assessed throughout clinical development. In the present manuscript, we have comprehensively analysed potential target populations for efficacy trials. The different target populations present both positive and negative attributes. For instance, ESRD patients while notable because of the high infection rate, their unmet medical need, and the ease of enrolment have several immune deficiencies that might hinder the success of vaccine trials as could have happened with the StaphVax trials. There are also other populations overlooked so far by vaccine developers, such as patients affected by CA-SSTI, because they are generally considered to have a lower medical need as compared to other populations who have more severe disease outcomes. However, it is emerging that CA-SSTIs do represent a significant burden to the healthcare system and that certain populations can have a very high infection rate (Creech et al. 2015; Ray et al. 2013a, b). Of note, some of the populations highly affected by CA-SSTI, such as infantry recruits, are completely healthy and young subjects.

Patients affected by SSI represent another important population. There are three major target groups in this category: neurosurgery and cardiothoracic and orthopaedic surgery patients. They are all associated with severe disease outcomes; however, they do have differences in terms of infection rate, population size, and health status, as well as average age of the patients. Selection of the target population will have to take into account and balance all these aspects.

Another fundamental criterion for the selection of the target population for efficacy trials is the estimated cost-effectiveness of the vaccine in that target group. Cost-effectiveness of a hypothetical *S. aureus* vaccine has been modelled for different target populations (Lee et al. 2010; Song et al. 2012). These predictions suggest that such a vaccine would be cost-effective for different target populations in a wide range of vaccine efficacy and price. However, a balance between the feasibility of showing vaccine efficacy in a given target population and its cost-effectiveness should be made and the possibility to perform a first efficacy trial in an "easier" population and then extend the study to a more "difficult" one could also be considered. This is particularly true for *S. aureus* for which no established correlates of protection are known. Indeed, without correlates of protection, it is impossible to predict vaccine efficacy in humans on the basis of preclinical data or immunogenicity studies in humans. Therefore, it is very important that research efforts focus on the identification and development of assays that can be used in the clinical setting in parallel with efficacy trials to establish surrogates of protection that will largely facilitate next steps of clinical development and licensing of the vaccines. One such potential assay that can recapitulate the full flavour of the vaccine immune response, and has the great advantage of being independent from heterologous constituents, is based on the use of whole blood from vaccinees. Indeed, blood, and not serum or isolated cells, contains all the immunological elements elicited by vaccination which are needed for inducing protection.

Recently bundle measures that included screening, decolonization, and targeted prophylaxis of patients prior to surgery were shown to slightly, but significantly, reduce *S. aureus* postoperative infection rate (Schweizer et al. 2015). These are great and promising interventions. On the other hand, it is likely that an efficacious vaccine would reduce the cost associated with infection control interventions and could prevent the further increase of antibiotic resistance, as observed for other vaccines in the market (Mishra et al. 2012).

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and Rino Rappuoli are employees of GSK Vaccines. Fabio Bagnoli owns patents on *S. aureus* vaccine candidates. Fabio Bagnoli and Rino Rappuoli own GSK stocks. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Authorship

Clarissa Pozzi, Reuben Olaniyi, Lassi Liljeroos, Ilaria Galgani, Rino Rappuoli and Fabio Bagnoli were involved in the conception and design of the manuscript. All authors were involved in writing the manuscript or revising it critically for important intellectual content. All authors approved the manuscript before it was submitted.

References

Adhikari RP, Kort T, Shulenin S, Kanipakala T, Ganjbaksh N, Roghmann MC, Holtsberg FW, Aman MJ (2015) Antibodies to *S. aureus* LukS-PV attenuated subunit vaccine neutralize a broad spectrum of canonical and non-canonical bicomponent leukotoxin pairs. PLoS ONE 10: e0137874

Alonzo F 3rd, Torres VJ (2014) The bicomponent pore-forming leucocidins of *Staphylococcus aureus*. Microbiol Mol Biol Rev 78:199–230

Alonzo F 3rd, Benson MA, Chen J, Novick RP, Shopsin B, Torres VJ (2012) *Staphylococcus aureus* leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. Mol Microbiol 83:423–435

Aman MJ, Adhikari RP (2014) Staphylococcal bicomponent pore-forming toxins: targets for prophylaxis and immunotherapy. Toxins (Basel) 6:950–972

Anderson DJ, Arduino JM, Reed SD, Sexton DJ, Kaye KS, Grussemeyer CA, Peter SA, Hardy C, Choi YI, Friedman JY, Fowler VG Jr (2010) Variation in the type and frequency of postoperative invasive *Staphylococcus aureus* infections according to type of surgical procedure. Infect Control Hosp Epidemiol 31:701–709

Anderson AS, Scully IL, Timofeyeva Y, Murphy E, McNeil LK, Mininni T, Nunez L, Carriere M, Singer C, Dilts DA, Jansen KU (2012) *Staphylococcus aureus* manganese transport protein C is a highly conserved cell surface protein that elicits protective immunity against *S. aureus* and *Staphylococcus epidermidis*. J Infect Dis 205:1688–1696

Anguzu JR, Olila D (2007) Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr Health Sci 7:148–154

Anonymous (2013) European Centre for Disease Prevention and Control. Surveillance of surgical site infections in Europe 2010–2011. ECDC, Stockholm

Anonymous. European Centre for Disease Prevention and Control/European Medicines Agency (ECDC/EMEA) (2009) Joint technical reportn the bacterial challenge: time to react. ECDC/EMEA, Stockholm. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

Arrecubieta C, Matsunaga I, Asai T, Naka Y, Deng MC, Lowy FD (2008) Vaccination with clumping factor A and fibronectin binding protein A to prevent *Staphylococcus aureus* infection of an aortic patch in mice. J Infect Dis 198:571–575

Bagnoli F, Bertholet S, Grandi G (2012) Inferring reasons for the failure of *Staphylococcus aureus* vaccines in clinical trials. Front Cell Infect Microbiol 2:16

Bagnoli F, Fontana MR, Soldaini E, Mishra RP, Fiaschi L, Cartocci E, Nardi-Dei V, Ruggiero P, Nosari S, De Falco MG, Lofano G, Marchi S, Galletti B, Mariotti P, Bacconi M, Torre A, Maccari S, Scarselli M, Rinaudo CD, Inoshima N, Savino S, Mori E, Rossi-Paccani S, Baudner B, Pallaoro M, Swennen E, Petracca R, Brettoni C, Liberatori S, Norais N, Monaci E, Bubeck Wardenburg J, Schneewind O, O'Hagan DT, Valiante NM, Bensi G, Bertholet S,

De Gregorio E, Rappuoli R, Grandi G (2015) Vaccine composition formulated with a novel TLR7-dependent adjuvant induces high and broad protection against *Staphylococcus aureus*. Proc Natl Acad Sci USA 112:3680–3685

Berube BJ, Bubeck Wardenburg J (2013) *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166

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

Bieber T (2008) Atopic dermatitis. N Engl J Med 358:1483–1494

Boyle-Vavra S, Li X, Alam MT, Read TD, Sieth J, Cywes-Bentley C, Dobbins G, David MZ, Kumar N, Eells SJ, Miller LG, Boxrud DJ, Chambers HF, Lynfield R, Lee JC, Daum RS (2015) USA300 and USA500 clonal lineages of *Staphylococcus aureus* do not produce a capsular polysaccharide due to conserved mutations in the cap5 locus. mBio 6

Burts ML, Williams WA, DeBord K, Missiakas DM (2005) EsxA and EsxB are secreted by an ESAT-6-like system that is required for the pathogenesis of *Staphylococcus aureus* infections. Proc Natl Acad Sci USA 102:1169–1174

Carr DD (2009) Building collaborative partnerships in critical care: the RN case manager/social work dyad in critical care. Prof Case Manag 14:121–132; quiz 133–124

CDC (2013) Antibiotic resistance threats in the United States

Chambers HF, Deleo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7:629–641

Chan KE, Warren HS, Thadhani RI, Steele DJ, Hymes JL, Maddux FW, Hakim RM (2012) Prevalence and outcomes of antimicrobial treatment for *Staphylococcus aureus* bacteremia in outpatients with ESRD. J Am Soc Nephrol 23:1551–1559

Chastre J, Fagon J-Y (2002) Ventilator-associated pneumonia. Am J Respir Crit Care Med 165:867–903

Chen Y, Almeida AA, Mitnovetski S, Goldstein J, Lowe C, Smith JA (2008) Managing deep sternal wound infections with vacuum-assisted closure. ANZ J Surg 78:333–336

Chen AE, Cantey JB, Carroll KC, Ross T, Speser S, Siberry GK (2009) Discordance between *Staphylococcus aureus* nasal colonization and skin infections in children. Pediatr Infect Dis J 28:244–246

Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM (2009) Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. FASEB J 23:3393–3404

Chi SY, Kim TO, Park CW, Yu JY, Lee B, Lee HS, Kim YI, Lim SC, Kwon YS (2012) Bacterial pathogens of ventilator associated pneumonia in a tertiary referral hospital. Tuberc Respir Dis 73:32–37

Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, Magorien JE, Blauvelt A, Kolls JK, Cheung AL, Cheng G, Modlin RL, Miller LS (2010) IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. J Clin Invest 120:1762–1773

Chung DR, Song J-H, Kim SH, Thamlikitkul V, Huang S-G, Wang H, So TM-k, Yasin RMD, Hsueh P-R, Carlos CC, Hsu LY, Buntaran L, Lalitha MK, Kim MJ, Choi JY, Kim SI, Ko KS, Kang C-I, Peck KR (2011) High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med 184:1409–1417

Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, Stapleton MR, Acevedo J, Read RC, Day NP, Peacock SJ, Mond JJ, Kokai-Kun JF, Foster SJ (2006) Identification of in vivo-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. J Infect Dis 193:1098–1108

Cook J, Hepler R, Pancari G, Kuklin N, Fan H, Wang XM, Cope L, Tan C, Joyce J, Onishi J, Montgomery D, Anderson A, McNeely T (2009) *Staphylococcus aureus* capsule type 8 antibodies provide inconsistent efficacy in murine models of staphylococcal infection. Hum Vaccin 5:254–263

Creech CB, Al-Zubeidi DN, Fritz SA (2015) Prevention of recurrent staphylococcal skin infections. Infect Dis Clin North Am 29:429–464

Crowley L, Wilson J, Guy R, Pitcher D, Fluck R (2012) Chapter 12 Epidemiology of *Staphylococcus aureus* bacteraemia amongst patients receiving dialysis for established renal failure in England in 2009 to 2011: a joint report from the Health Protection Agency and the UK Renal Registry. Nephron Clin Pract 120(Suppl 1):c233–c245

D'Agata EM, Webb GF, Horn MA, Moellering RC Jr, Ruan S (2009) Modeling the invasion of community-acquired methicillin-resistant *Staphylococcus aureus* into hospitals. Clin Infect Dis 48:274–284

Dandagi GL (2010) Nosocomial pneumonia in critically ill patients. Lung India 27:149–153 (Official Organ of Indian Chest Society)

Daum RS, Spellberg B (2012) Progress toward a *Staphylococcus aureus* vaccine. Clin Infect Dis 54:560–567

David MZ, Daum RS (2010) Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23:616–687

Dayan GH, Mohamed N, Scully IL, Cooper D, Begier E, Eiden J, Jansen KU, Gurtman A, Anderson AS (2016) *Staphylococcus aureus*: the current state of disease, pathophysiology and strategies for prevention. Expert Rev Vaccines. doi:10.1080/14760584.2016.1179583:1-20

Del Pozo JL, Patel R (2009) Clinical practice. Infection associated with prosthetic joints. N Engl J Med 361:787–794

DeLeo FR, Kennedy AD, Chen L, Bubeck Wardenburg J, Kobayashi SD, Mathema B, Braughton KR, Whitney AR, Villaruz AE, Martens CA, Porcella SF, McGavin MJ, Otto M, Musser JM, Kreiswirth BN (2011) Molecular differentiation of historic phage-type 80/81 and contemporary epidemic *Staphylococcus aureus*. Proc Natl Acad Sci USA 108:18091–18096

Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M, Group SP (2001) Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis 32(Suppl 2):S114–S132

Dryla A, Prustomersky S, Gelbmann D, Hanner M, Bettinger E, Kocsis B, Kustos T, Henics T, Meinke A, Nagy E (2005) Comparison of antibody repertoires against *Staphylococcus aureus* in healthy individuals and in acutely infected patients. Clin Diagn Lab Immunol 12:387–398

DuMont AL, Torres VJ (2014) Cell targeting by the *Staphylococcus aureus* pore-forming toxins: it's not just about lipids. Trends Microbiol 22:21–27

Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, Shopsin B, Unutmaz D, Voyich JM, Torres VJ (2011) Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. Mol Microbiol 79:814–825

Duong M, Markwell S, Peter J, Barenkamp S (2010) Randomized, controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. Ann Emerg Med 55:401–407

Ellis MW, Schlett CD, Millar EV, Wilkins KJ, Crawford KB, Morrison-Rodriguez SM, Pacha LA, Gorwitz RJ, Lanier JB, Tribble DR (2014) Hygiene strategies to prevent methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections: a cluster-randomized controlled trial among high-risk military trainees. Clin Infect Dis 58:1540–1548

Fattom A, Schneerson R, Watson DC, Karakawa WW, Fitzgerald D, Pastan I, Li X, Shiloach J, Bryla DA, Robbins JB (1993) Laboratory and clinical evaluation of conjugate vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides bound to *Pseudomonas aeruginosa* recombinant exoprotein A. Infect Immun 61:1023–1032

Fattom AI, Sarwar J, Ortiz A, Naso R (1996) A *Staphylococcus aureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. Infect Immun 64:1659–1665

Fattom AI, Horwith G, Fuller S, Propst M, Naso R (2004) Development of StaphVAX, a polysaccharide conjugate vaccine against *S. aureus* infection: from the lab bench to phase III clinical trials. Vaccine 22:880–887

Fattom A, Matalon A, Buerkert J, Taylor K, Damaso S, Boutriau D (2015) Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: Phase III randomized study. Hum Vaccin Immunother 11:632–641

Fitzgerald SF, O'Gorman J, Morris-Downes MM, Crowley RK, Donlon S, Bajwa R, Smyth EG, Fitzpatrick F, Conlon PJ, Humphreys H (2011) A 12-year review of *Staphylococcus aureus* bloodstream infections in haemodialysis patients: more work to be done. J Hosp Infect 79:218–221

Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, Corey GR, Carmeli Y, Betts R, Hartzel JS, Chan IS, McNeely TB, Kartsonis NA, Guris D, Onorato MT, Smugar SS, DiNubile MJ, Sobanjo-ter Meulen A (2013) Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. JAMA 309:1368–1378

Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM (2005) Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med 352:1436–1444

Fritz SA, Hogan PG, Hayek G, Eisenstein KA, Rodriguez M, Epplin EK, Garbutt J, Fraser VJ (2012) Household versus individual approaches to eradication of community-associated *Staphylococcus aureus* in children: a randomized trial. Clin Infect Dis 54:743–751

Frydenberg AR, Buttery JP, Royle J (2005) Determining the rate of varicella vaccine rash in children with moderate-severe eczema. J Paediatr Child Health 41:561–563

Gaudin CF, Grigg JC, Arrieta AL, Murphy ME (2011) Unique heme-iron coordination by the hemoglobin receptor IsdB of *Staphylococcus aureus*. Biochemistry 50:5443–5452

Goss MA, Beninger PR (2005) Eczema and postvaccination varicella breakthrough. Pediatrics 116:1613

Guilarde AO, Turchi MD, Martelli CM, Primo MG (2006) *Staphylococcus aureus* bacteraemia: incidence, risk factors and predictors for death in a Brazilian teaching hospital. J Hosp Infect 63:330–336

Hair PS, Echague CG, Sholl AM, Watkins JA, Geoghegan JA, Foster TJ, Cunnion KM (2010) Clumping factor A interaction with complement factor I increases C3b cleavage on the bacterial surface of *Staphylococcus aureus* and decreases complement-mediated phagocytosis. Infect Immun 78:1717–1727

Hall AE, Domanski PJ, Patel PR, Vernachio JH, Syribeys PJ, Gorovits EL, Johnson MA, Ross JM, Hutchins JT, Patti JM (2003) Characterization of a protective monoclonal antibody recognizing *Staphylococcus aureus* MSCRAMM protein clumping factor A. Infect Immun 71:6864–6870

Handke LD, Hawkins JC, Miller AA, Jansen KU, Anderson AS (2013) Regulation of *Staphylococcus aureus* MntC expression and its role in response to oxidative stress. PLoS ONE 8:e77874

Harrington G, Russo P, Spelman D, Borrell S, Watson K, Barr W, Martin R, Edmonds D, Cocks J, Greenbough J, Lowe J, Randle L, Castell J, Browne E, Bellis K, Aberline M (2004) Surgical-site infection rates and risk factor analysis in coronary artery bypass graft surgery. Infect Control Hosp Epidemiol 25:472–476

Harro C, Betts R, Orenstein W, Kwak EJ, Greenberg HE, Onorato MT, Hartzel J, Lipka J, DiNubile MJ, Kartsonis N (2010) Safety and immunogenicity of a novel *Staphylococcus aureus* vaccine: results from the first study of the vaccine dose range in humans. Clin Vaccine Immunol 17:1868–1874

Harro CD, Betts RF, Hartzel JS, Onorato MT, Lipka J, Smugar SS, Kartsonis NA (2012) The immunogenicity and safety of different formulations of a novel *Staphylococcus aureus* vaccine (V710): results of two Phase I studies. Vaccine 30:1729–1736

Hawkins J, Kodali S, Matsuka YV, McNeil LK, Mininni T, Scully IL, Vernachio JH, Severina E, Girgenti D, Jansen KU, Anderson AS, Donald RG (2012) A recombinant clumping factor A-containing vaccine induces functional antibodies to *Staphylococcus aureus* that are not observed after natural exposure. Clin Vaccine Immunol 19:1641–1650

Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK (2008) NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the

Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 29:996–1011

Hill PC, Birch M, Chambers S, Drinkovic D, Ellis-Pegler RB, Everts R, Murdoch D, Pottumarthy S, Roberts SA, Swager C, Taylor SL, Thomas MG, Wong CG, Morris AJ (2001) Prospective study of 424 cases of *Staphylococcus aureus* bacteraemia: determination of factors affecting incidence and mortality. Intern Med J 31:97–103

Ibler KS, Kromann CB (2014) Recurrent furunculosis–challenges and management: a review. Clin Cosmet Investig Dermatol 7:59–64

Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, Bubeck Wardenburg J (2011) A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med 17:1310–1314

Iribarne A, Easterwood R, Russo MJ, Wang YC, Yang J, Hong KN, Smith CR, Argenziano M (2011) A minimally invasive approach is more cost-effective than a traditional sternotomy approach for mitral valve surgery. J Thorac Cardiovasc Surg 142:1507–1514

Jones RN (2010) Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis 51:S81–S87

Josefsson E, Hartford O, O'Brien L, Patti JM, Foster T (2001) Protection against experimental *Staphylococcus aureus* arthritis by vaccination with clumping factor A, a novel virulence determinant. J Infect Dis 184:1572–1580

Kaplan SL, Forbes A, Hammerman WA, Lamberth L, Hulten KG, Minard CG, Mason EO (2014) Randomized trial of "Bleach Baths" plus routine hygienic measures vs routine hygienic measures alone for prevention of recurrent infections. Clin Infect Dis 58:679–682

Karauzum H, Adhikari RP, Sarwar J, Devi VS, Abaandou L, Haudenschild C, Mahmoudieh M, Boroun AR, Vu H, Nguyen T, Warfield KL, Shulenin S, Aman MJ (2013) Structurally designed attenuated subunit vaccines for S. aureus LukS-PV and LukF-PV confer protection in a mouse bacteremia model. PLoS ONE 8:e65384

Kawate T, Gouaux E (2003) Arresting and releasing Staphylococcal alpha-hemolysin at intermediate stages of pore formation by engineered disulfide bonds. Protein Sci 12:997–1006

Kienast AK, Kreth HW, Hoger PH (2007) Varicella vaccination in children with atopic eczema. J Dtsch Dermatol Ges 5:875–880

Kim HK, Cheng AG, Kim HY, Missiakas DM, Schneewind O (2010) Nontoxigenic protein A vaccine for methicillin-resistant *Staphylococcus aureus* infections in mice. J Exp Med 207:1863–1870

Kim HK, Falugi F, Thomer L, Missiakas DM, Schneewind O (2015) Protein A suppresses immune responses during Staphylococcus aureus bloodstream infection in guinea pigs. mBio 6

Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS (2005) Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest 128:3854–3862

Korea CG, Balsamo G, Pezzicoli A, Merakou C, Tavarini S, Bagnoli F, Serruto D, Unnikrishnan M (2014) Staphylococcal Esx proteins modulate apoptosis and release of intracellular *Staphylococcus aureus* during infection in epithelial cells. Infect Immun 82:4144–4153

Koulenti D, Lisboa T, Brun-Buisson C, Krueger W, Macor A, Sole-Violan J, Diaz E, Topeli A, DeWaele J, Carneiro A, Martin-Loeches I, Armaganidis A, Rello J, Group E-VCS (2009) Spectrum of practice in the diagnosis of nosocomial pneumonia in patients requiring mechanical ventilation in European intensive care units. Crit Care Med 37:2360–2368

Kuklin NA, Clark DJ, Secore S, Cook J, Cope LD, McNeely T, Noble L, Brown MJ, Zorman JK, Wang XM, Pancari G, Fan H, Isett K, Burgess B, Bryan J, Brownlow M, George H, Meinz M, Liddell ME, Kelly R, Schultz L, Montgomery D, Onishi J, Losada M, Martin M, Ebert T, Tan CY, Schofield TL, Nagy E, Meineke A, Joyce JG, Kurtz MB, Caulfield MJ, Jansen KU, McClements W, Anderson AS (2006) A novel *Staphylococcus aureus* vaccine: iron surface determinant B induces rapid antibody responses in rhesus macaques and specific increased survival in a murine *S. aureus* sepsis model. Infect Immun 74:2215–2223

Kwiecinski J, Jin T, Josefsson E (2014) Surface proteins of *Staphylococcus aureus* play an important role in experimental skin infection. APMIS 122:1240–1250

Lancefield RC (1957) Differentiation of group A streptococci with a common R antigen into three serological types, with special reference to the bactericidal test. J Exp Med 106:525–544

Lee BY, Wiringa AE, Bailey RR, Lewis GJ, Feura J, Muder RR (2010) *Staphylococcus aureus* vaccine for orthopedic patients: an economic model and analysis. Vaccine 28:2465–2471

Levy J, Licini L, Haelterman E, Moris P, Lestrate P, Damaso S, Van Belle P, Boutriau D (2015) Safety and immunogenicity of an investigational 4-component *Staphylococcus aureus* vaccine with or without AS03B adjuvant: Results of a randomized phase I trial. Hum Vaccin Immunother 11:620–631

Lorette G, Beaulieu P, Allaert FA, Mahmoudi A, Jarlier V (2009) Superficial community-acquired skin infections: prevalence of bacteria and antibiotic susceptibility in France. J Eur Acad Dermatol Venereol 23:1423–1426

Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK, Emerging Infections Program Healthcare-Associated I, Antimicrobial Use Prevalence Survey T (2014) Multistate point-prevalence survey of health care-associated infections. N Engl J Med 370:1198–1208

Marin M, Nguyen HQ, Keen J, Jumaan AO, Mellen PM, Hayes EB, Gensheimer KF, Gunderman-King J, Seward JF (2005) Importance of catch-up vaccination: experience from a varicella outbreak, Maine, 2002–2003. Pediatrics 115:900–905

Mariotti P, Malito E, Biancucci M, Lo Surdo P, Mishra RP, Nardi-Dei V, Savino S, Nissum M, Spraggon G, Grandi G, Bagnoli F, Bottomley MJ (2013) Structural and functional characterization of the *Staphylococcus aureus* virulence factor and vaccine candidate FhuD2. Biochem J 449:683–693

Mawalla B, Mshana SE, Chalya PL, Imirzalioglu C, Mahalu W (2011) Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. BMC Surg 11:21

Mazmanian SK, Skaar EP, Gaspar AH, Humayun M, Gornicki P, Jelenska J, Joachmiak A, Missiakas DM, Schneewind O (2003) Passage of heme-iron across the envelope of *Staphylococcus aureus*. Science 299:906–909

McAdow M, Kim HK, Dedent AC, Hendrickx AP, Schneewind O, Missiakas DM (2011) Preventing *Staphylococcus aureus* sepsis through the inhibition of its agglutination in blood. PLoS Pathog 7:e1002307

McDevitt D, Francois P, Vaudaux P, Foster TJ (1994) Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. Mol Microbiol 11:237–248

McNeely TB, Shah NA, Fridman A, Joshi A, Hartzel JS, Keshari RS, Lupu F, DiNubile MJ (2014) Mortality among recipients of the Merck V710 *Staphylococcus aureus* vaccine after postoperative *S. aureus* infections: an analysis of possible contributing host factors. Hum Vaccin Immunother 10:3513–3516

Miller LS, Cho JS (2011) Immunity against *Staphylococcus aureus* cutaneous infections. Nat Rev Immunol 11:505–518

Miller LG, Quan C, Shay A, Mostafaie K, Bharadwa K, Tan N, Matayoshi K, Cronin J, Tan J, Tagudar G, Bayer AS (2007) A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. Clin Infect Dis 44:483–492

Miller LG, Eells SJ, David MZ, Ortiz N, Taylor AR, Kumar N, Cruz D, Boyle-Vavra S, Daum RS (2015) *Staphylococcus aureus* skin infection recurrences among household members: an examination of host, behavioral, and pathogen-level predictors. Clin Infect Dis 60:753–763

Mishra RP, Mariotti P, Fiaschi L, Nosari S, Maccari S, Liberatori S, Fontana MR, Pezzicoli A, De Falco MG, Falugi F, Altindis E, Serruto D, Grandi G, Bagnoli F (2012a) *Staphylococcus aureus* FhuD2 is involved in the early phase of staphylococcal dissemination and generates protective immunity in mice. J Infect Dis 206:1041–1049

Mishra RP, Oviedo-Orta E, Prachi P, Rappuoli R, Bagnoli F (2012b) Vaccines and antibiotic resistance. Curr Opin Microbiol 15:596–602

Moet GJ, Jones RN, Biedenbach DJ, Stilwell MG, Fritsche TR (2007) Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998–2004). Diagn Microbiol Infect Dis 57:7–13

Monaci E, Mancini F, Lofano G, Bacconi M, Tavarini S, Sammicheli C, Arcidiacono L, Giraldi M, Galletti B, Rossi Paccani S, Torre A, Fontana MR, Grandi G, de Gregorio E, Bensi G, Chiarot E, Nuti S, Bagnoli F, Soldaini E, Bertholet S (2015) MF59- and Al(OH) 3-adjuvanted *Staphylococcus aureus* (4C-Staph) vaccines induce sustained protective humoral and cellular immune responses, with a critical role for effector CD4 T cells at low antibody titers. Front Immunol 6:439

Montgomery CP, David MZ, Daum RS (2015) Host factors that contribute to recurrent staphylococcal skin infection. Curr Opin Infect Dis 28:253–258

Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA, Group EMINS (2006) Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 355:666–674

Moreillon P, Entenza JM, Francioli P, McDevitt D, Foster TJ, Francois P, Vaudaux P (1995) Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect Immun 63:4738–4743

Morrison-Rodriguez SM, Pacha LA, Patrick JE, Jordan NN (2010) Community-associated methicillin-resistant *Staphylococcus aureus* infections at an Army training installation. Epidemiol Infect 138:721–729

Moustafa M, Aronoff GR, Chandran C, Hartzel JS, Smugar SS, Galphin CM, Mailloux LU, Brown E, Dinubile MJ, Kartsonis NA, Guris D (2012) Phase IIa study of the immunogenicity and safety of the novel *Staphylococcus aureus* vaccine V710 in adults with end-stage renal disease receiving hemodialysis. Clin Vaccine Immunol 19:1509–1516

Mullins PM, Goyal M, Pines JM (2013) Academic emergency medicine 20(5). Version of record online, 14 MAY 2013

Nagao M, Iinuma Y, Saito T, Matsumura Y, Shirano M, Matsushima A, Takakura S, Ito Y, Ichiyama S (2010) Close cooperation between infectious disease physicians and attending physicians can result in better management and outcome for patients with *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 16:1783–1788

Naidoo R, Nuttall J, Whitelaw A, Eley B (2013) Epidemiology of *Staphylococcus aureus* bacteraemia at a tertiary children's hospital in Cape Town, South Africa. PLoS ONE 8:e78396

Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, Scully IL, McNeil LK, Aste-Amezaga JM, Cooper D, Jansen KU, Anderson AS (2013) Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Hum Vaccin Immunother 9:480–487

Narita K, Hu DL, Asano K, Nakane A (2015) Vaccination with non-toxic mutant toxic shock syndrome toxin-1 induces IL-17-dependent protection against *Staphylococcus aureus* infection. Pathog Dis 73

Nickerson EK, Hongsuwan M, Limmathurotsakul D, Wuthiekanun V, Shah KR, Srisomang P, Mahavanakul W, Wacharaprechasgul T, Fowler VG, West TE, Teerawatanasuk N, Becher H, White NJ, Chierakul W, Day NP, Peacock SJ (2009) *Staphylococcus aureus* bacteraemia in a tropical setting: patient outcome and impact of antibiotic resistance. PLoS ONE 4:e4308

Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, Smulders M, Gemmen E, Bharmal M (2007) National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6-year period (1998–2003). Clin Infect Dis 45:1132–1140

Ojulong J, Mwambu TP, Joloba M, Bwanga F, Kaddu-Mulindwa DH (2009) Relative prevalence of methicilline resistant *Staphylococcus aureus* and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. Tanzan J Health Res 11:149–153

O'Riordan K, Lee JC (2004) *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev 17:218–234

Paganini HR, Della Latta P, Soto A, Casimir L, Monaco A, Verdaguer V, Berberian G, Rosanova MT, Gonzalez F, Sarkis C (2010) Community-acquired *Staphylococcus aureus* bacteremia: 17 years of experience in Argentine children. Arch Argent Pediatr 108:311–317

Pozzi C, Lofano G, Mancini F, Soldaini E, Speziale P, De Gregorio E, Rappuoli R, Bertholet S, Grandi G, Bagnoli F (2015) Phagocyte subsets and lymphocyte clonal deletion behind ineffective immune response to *Staphylococcus aureus*. FEMS Microbiol Rev 39:750–763

Que YA, Francois P, Haefliger JA, Entenza JM, Vaudaux P, Moreillon P (2001) Reassessing the role of *Staphylococcus aureus* clumping factor and fibronectin-binding protein by expression in *Lactococcus lactis*. Infect Immun 69:6296–6302

Ray GT, Suaya JA, Baxter R (2013a) Incidence, microbiology, and patient characteristics of skin and soft-tissue infections in a U.S. population: a retrospective population-based study. BMC Infect Dis 13:252

Ray GT, Suaya JA, Baxter R (2013b) Microbiology of skin and soft tissue infections in the age of community-acquired methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 76:24–30

Sader HS, Farrell DJ, Jones RN (2010) Antimicrobial susceptibility of Gram-positive cocci isolated from skin and skin-structure infections in European medical centres. Int J Antimicrob Agents 36:28–32

Schluepen C, Malito E, Marongiu A, Schirle M, McWhinnie E, Lo Surdo P, Biancucci M, Falugi F, Nardi-Dei V, Marchi S, Fontana MR, Lombardi B, De Falco MG, Rinaudo CD, Spraggon G, Nissum M, Bagnoli F, Grandi G, Bottomley MJ, Liberatori S (2013) Mining the bacterial unknown proteome: identification and characterization of a novel family of highly conserved protective antigens in *Staphylococcus aureus*. Biochem J 455:273–284

Schweizer ML, Chiang HY, Septimus E, Moody J, Braun B, Hafner J, Ward MA, Hickok J, Perencevich EN, Diekema DJ, Richards CL, Cavanaugh JE, Perlin JB, Herwaldt LA (2015) Association of a bundled intervention with surgical site infections among patients undergoing cardiac, hip, or knee surgery. JAMA 313:2162–2171

Sebulsky MT, Heinrichs DE (2001) Identification and characterization of fhuD1 and fhuD2, two genes involved in iron-hydroxamate uptake in *Staphylococcus aureus*. J Bacteriol 183:4994–5000

Shinefield H, Black S, Fattom A, Horwith G, Rasgon S, Ordonez J, Yeoh H, Law D, Robbins JB, Schneerson R, Muenz L, Fuller S, Johnson J, Fireman B, Alcorn H, Naso R (2002) Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. N Engl J Med 346:491–496

Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B, Fridkin S, National Healthcare Safety Network T, Participating NF (2013) Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol 34:1–14

Silverman GJ, Goodyear CS (2006) Confounding B-cell defences: lessons from a staphylococcal superantigen. Nat Rev Immunol 6:465–475

Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866

Song Y, Tai JH, Bartsch SM, Zimmerman RK, Muder RR, Lee BY (2012) The potential economic value of a *Staphylococcus aureus* vaccine among hemodialysis patients. Vaccine 30:3675–3682

Spelman DW, Russo P, Harrington G, Davis BB, Rabinov M, Smith JA, Spicer WJ, Esmore D (2000) Risk factors for surgical wound infection and bacteraemia following coronary artery bypass surgery. Aust N Z J Surg 70:47–51

Stranger-Jones YK, Bae T, Schneewind O (2006) Vaccine assembly from surface proteins of *Staphylococcus aureus*. Proc Natl Acad Sci USA 103:16942–16947

Suaya JA, Mera RM, Cassidy A, O'Hara P, Amrine-Madsen H, Burstin S, Miller LG (2014) Incidence and cost of hospitalizations associated with *Staphylococcus aureus* skin and soft tissue infections in the United States from 2001 through 2009. BMC Infect Dis 14:296

Sutter DE, Summers AM, Keys CE, Taylor KL, Frasch CE, Braun LE, Fattom AI, Bash MC (2011) Capsular serotype of *Staphylococcus aureus* in the era of community-acquired MRSA. FEMS Immunol Med Microbiol 63:16–24

Thakker M, Park JS, Carey V, Lee JC (1998) *Staphylococcus aureus* serotype 5 capsular polysaccharide is antiphagocytic and enhances bacterial virulence in a murine bacteremia model. Infect Immun 66:5183–5189

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661

Torre A, Bacconi M, Sammicheli C, Galletti B, Laera D, Fontana MR, Grandi G, De Gregorio E, Bagnoli F, Nuti S, Bertholet S, Bensi G (2015) Four-component *Staphylococcus aureus* vaccine 4C-staph enhances Fcgamma receptor expression in neutrophils and monocytes and mitigates *S. aureus* infection in neutropenic mice. Infect Immun 83:3157–3163

Torres VJ, Pishchany G, Humayun M, Schneewind O, Skaar EP (2006) *Staphylococcus aureus* IsdB is a hemoglobin receptor required for heme iron utilization. J Bacteriol 188:8421–8429

Tuchscherr LP, Buzzola FR, Alvarez LP, Lee JC, Sordelli DO (2008) Antibodies to capsular polysaccharide and clumping factor A prevent mastitis and the emergence of unencapsulated and small-colony variants of *Staphylococcus aureus* in mice. Infect Immun 76:5738–5744

Turnidge JD, Nimmo GR, Pearson J, Gottlieb T, Collignon PJ, Australian Group on Antimicrobial R (2007) Epidemiology and outcomes for Staphylococcus aureus bacteraemia in Australian hospitals, 2005–06: report from the Australian Group on Antimicrobial Resistance. Commun Dis Intell Q Rep 31:398–403

van den Berg S, Koedijk DG, Back JW, Neef J, Dreisbach A, van Dijl JM, Bakker-Woudenberg IA, Buist G (2015) Active immunization with an octa-valent *Staphylococcus aureus* antigen mixture in models of *S. aureus* bacteremia and skin infection in mice. PLoS One 10:e0116847

Verkaik NJ, de Vogel CP, Boelens HA, Grumann D, Hoogenboezem T, Vink C, Hooijkaas H, Foster TJ, Verbrugh HA, van Belkum A, van Wamel WJ (2009) Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of *Staphylococcus aureus*. J Infect Dis 199:625–632

Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K, Investigators EIGo (2009) International study of the prevalence and outcomes of infection in intensive care units. JAMA 302:2323–2329

Vincent J-L, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K, Investigators EIGo (2009) International study of the prevalence and outcomes of infection in intensive care units. JAMA 302:2323–2329

Wilke GA, Bubeck Wardenburg J (2010) Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci USA 107:13473–13478

Williams DJ, Cooper WO, Kaltenbach LA, Dudley JA, Kirschke DL, Jones TF, Arbogast PG, Griffin MR, Creech CB (2011) Comparative effectiveness of antibiotic treatment strategies for pediatric skin and soft-tissue infections. Pediatrics 128:e479–e487

Yilmaz M, Elaldi N, Balkan II, Arslan F, Batirel AA, Bakici MZ, Gozel MG, Alkan S, Celik AD, Yetkin MA, Bodur H, Sinirtas M, Akalin H, Altay FA, Sencan I, Azak E, Gundes S, Ceylan B, Ozturk R, Leblebicioglu H, Vahaboglu H, Mert A (2016) Mortality predictors of *Staphylococcus aureus* bacteremia: a prospective multicenter study. Ann Clin Microbiol Antimicrob 15:7

Zhao C, Liu Y, Zhao M, Liu Y, Yu Y, Chen H, Sun Q, Chen H, Jiang W, Liu Y, Han S, Xu Y, Chen M, Cao B, Wang H (2012) Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL + ST398. PLoS ONE 7:e38577

Lysin Therapy for *Staphylococcus aureus* and Other Bacterial Pathogens

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Abstract Lysins are a new and novel class of anti-infectives derived from bacteriophage (or phage). They represent highly evolved enzymes produced to cleave essential bonds in the bacterial cell wall peptidoglycan for phage progeny release. Small quantities of purified recombinant lysin added externally to gram-positive bacteria results in immediate lysis causing log-fold death of the target bacterium. Lysins can eliminate bacteria both systemically and topically, from mucosal surfaces and biofilms, as evidenced by experimental models of sepsis, pneumonia, meningitis, endocarditis, and mucosal decolonization. Furthermore, lysins can act synergistically with antibiotics by resensitizing bacteria to non-susceptible antibiotics. The advantages over antibiotics are their specificity for the pathogen without disturbing the normal flora, the low chance of bacterial resistance, and their ability to kill colonizing pathogens on mucosal surfaces, a capacity previously unavailable. Lysins, therefore, may be a much-needed anti-infective in an age of mounting antibiotic resistance.

Contents

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1 Introduction

Staphylococcus aureus are responsible for severe secondary infections in immunocompromised individuals, as well as disease in otherwise healthy individuals; it is the most common cause of human bacterial infections worldwide (Lowy 1998). Besides skin and soft tissue infections (SSTIs), *S. aureus* can cause sepsis, toxic shock syndrome, pneumonia, necrotizing fasciitis, pyomyositis, endocarditis, and impetigo (White and Smith 1963; Wertheim et al. 2005). Unfortunately, many *S. aureus* strains, such as MRSA and vancomycin-resistant *S. aureus* (VRSA), have evolved resistance to one or more antibiotics used as standard therapy. MRSA account for more than 50 % of hospital isolates causing pneumonia and septicemia (Klein et al. 2007) particularly in intensive care units resulting in 30–40 % mortality (Tiemersma et al. 2004; Laupland et al. 2008). While health-care-associated MRSA infect susceptible patients, community-associated MRSA (CA-MRSA) infect healthy individuals (Herold et al. 1998; Centers for Disease Control and Prevention 1999). CA-MRSA strains are more virulent and contagious and are capable of causing more severe diseases (Miller et al. 2005; Li et al. 2009).

The long-term and large-scale use of antibiotics in human and veterinary medicine provides a powerful selective pressure for antibiotic resistance to arise and eventually dominate populations of human pathogenic microorganisms (Andersson and Hughes 2010). Spontaneous resistance to most antibiotics appears with frequencies ranging from $\leq 10^{-8}$–10^{-11} and, through a series of successive mutations, ultimately generates clinically significant resistance which can then be mobilized in an intra- and interspecies manner by genetic elements such as transposons, plasmids, integrons, and genomic islands (Woodford and Ellington 2007). The first reported human case of MRSA was in Boston in 1968 (Barrett et al. 1968) which increased over the subsequent decades. The evolution of multidrug resistance and the international dissemination of epidemic clones compound the problem, highlighting the need for new antimicrobial development strategies.

Interest in phages and phage products as antimicrobials has been recently renewed in order to address the problem of evolving resistance to antibiotics (O'Flaherty et al. 2009). The use of lysins, or bacteriophage-encoded cell wall hydrolases, has received particular attention because of a potent and often species-specific bacteriolytic activity and a notable lack of bacterial resistance to lysin activity (Fenton et al. 2010; Fischetti et al. 2006). In the context of a phage lifecycle inside a bacterial host, lysins are expressed during viral replication and are ultimately used to cleave the peptidoglycan, lyse the bacterium, and release progeny virions. Purified recombinant lysins, on the other hand, can also be potent lytic agents outside the viral context, driving "lysis from without" of target bacteria both in vitro and in experimentally infected animals (Fenton et al. 2010; Schuch et al. 2002a; Cheng et al. 2005; Ahmed et al. 2011). Potentially therapeutic lysins generally have modular structures defined by well-conserved N-terminal peptidoglycan-cleaving domains and more divergent C-terminal cell wall-binding domains (CBD) that can recognize species-specific cell wall glycopolymers.

The largely universal nature of lysin-sensitive cleavage sites in the peptidoglycan, combined with an increasing understanding of roles for cell wall glycopolymers in maintaining cell wall integrity, is cited to explain the absence of resistance to certain lysins (Fischetti et al. 2006; Fischetti 2010).

Phage lysins have been refined by phage over millions of years to lyse bacteria. They target the integrity of the cell wall and are designed to attack one of the four major bonds in the peptidoglycan. With few exceptions (Loessner et al. 1997), lysins do not have signal sequences, so they are not translocated through the cytoplasmic membrane to attack their substrate in the peptidoglycan; this movement is controlled by a second phage gene product in the lytic system, the holin (Wang et al. 2000). During phage development in the infected bacterium, lysin accumulates in the cytoplasm in anticipation of phage maturation. At a genetically specified time, holin molecules are inserted in the cytoplasmic membrane forming patches, ultimately resulting in generalized membrane disruption (Wang et al. 2003), allowing the cytoplasmic lysin to access the peptidoglycan, thereby causing cell lysis and the release of progeny phage (Wang et al. 2000). Scientists have been aware of the lytic activity of phage for nearly a century, and while whole phages have been used to control infection (Matsuzaki et al. 2005), not until recently have lytic enzymes been exploited for bacterial control in vivo (Nelson et al. 2001; Schuch et al. 2002a; Loeffler et al. 2003). Previous data indicate that lysins work only with gram-positive bacteria, since they are able to make direct contact with the cell wall carbohydrates and peptidoglycan when added externally, whereas the outer membrane of gram-negative bacteria prevents this interaction. However, recently lysins have been identified that are active against certain gram-negative pathogens (Lai et al. 2011; Lood et al. 2015).

2 Mechanism of Action

By thin-section electron microscopy of lsin-treated bacteria, it appears that lysins exert their lethal effects by forming holes in the cell wall through peptidoglycan digestion. The high internal pressure of bacterial cells (roughly 10–15 atmospheres for gram positives) is controlled by the highly cross-linked cell wall peptidoglycan (Fig. 1). Any disruption in the wall's integrity will result in the extrusion of the cytoplasmic membrane and ultimate hypotonic lysis (Fig. 2). Catalytically, a single enzyme molecule should be sufficient to cleave an adequate number of bonds to kill an organism; however, it is uncertain at this time whether this theoretical limit is possible. The reason comes from the work of Loessner (Loessner et al. 2002), showing that a listeria phage enzyme had a binding affinity approaching that of an IgG molecule for its substrate, suggesting that phage enzymes, like cellulases (Jervis et al. 1997) are one-use enzymes, likely requiring several molecules attacking a local region to sufficiently weaken the cell wall.

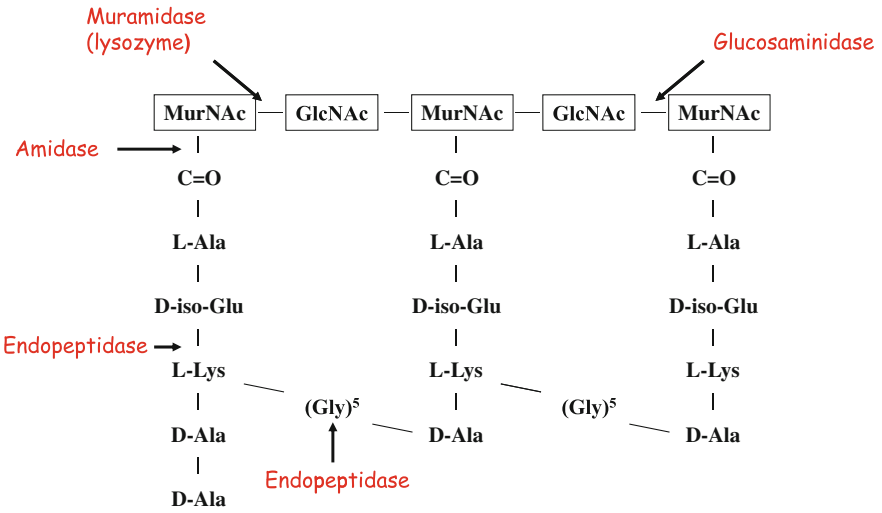

Fig. 1 Diagram of the staphylococcal cell wall peptidoglycan and bonds cleaved by phage lysins. Repeating units of MurNAc (N-acetylmuramic acid) and GlcNAc (N-acetylglucosamine) compose the glycan strands that are linked to a stem peptide through an amide bond to the MurNAc. L-Ala (L-alanine), D-iso-Glu (D-iso-glutamic acid), L-Lys (L-lysine), D-Ala (D-alanine). Stem peptides are then cross-linked through a pentaglycine (in the case of *S. aureus*) to adjacent stem peptides forming a tight stable net around the bacterium. Lysins cleave the major bonds: amidase (N-acetylmuramoyl L-alanine amidase), muramidase (N-acetylmuramidase), glucosamidase (N-acetylglucosaminidase), and endopeptidase

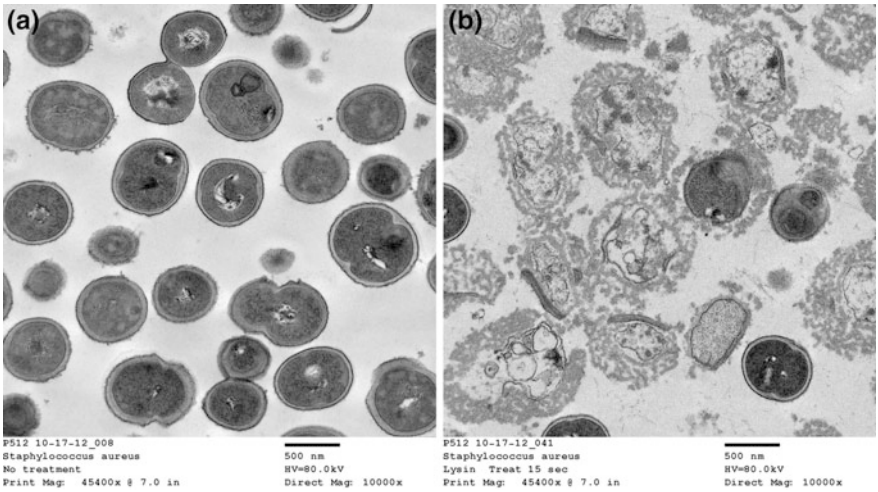

Fig. 2 Thin-section electron micrographs of *S. aureus* before (**a**) and after (**b**) treatment with phage lysin PlySs2 for 15 s

3 Lysin Efficacy

In general, lysins only kill the species (or subspecies) of bacteria from which they were produced. For instance, enzymes produced from streptococcal phage kill certain streptococci, and enzymes produced by pneumococcal phage kill pneumococci (Loeffler et al. 2001; Nelson et al. 2001). Specifically, a lysin from a group C streptococcal phage (PlyC) will kill group C streptococci, as well as groups A and E streptococci (human pathogens), the bovine pathogen *Streptococcus uberis,* the horse pathogen, *Streptococcus equi*, and the bovine pathogen *S. uberis,* but essentially no effect on streptococci normally found in the oral cavity of humans and other gram-positive bacteria. Similar results are seen with a pneumococcal-specific lysin (Cpl-1) (Loeffler et al. 2001). The most specific lysin reported to date is PlyG directed to *Bacillus anthracis* (Schuch et al. 2002a). This enzyme binds to a neutral polysaccharide found only in the cell wall of *B. anthracis* and rare "transition strains" of *B. anthracis*/*Bacillus cereus* (Schuch et al. 2013). Thus, unlike antibiotics, which are usually broad spectrum and kill many different bacteria found in the human body, some of which are beneficial, lysins may be identified which kill only the disease organism with little to no effect on the normal human bacterial flora. In some cases, however, phage enzymes may be identified with broad lytic activity. One such lysin from an enterococcal phage has recently been reported to not only kill enterococci but a number of other gram-positive pathogens such as *Streptococcus pyogen*es, group B streptococci, and *S. aureus* (Yoong et al. 2004). However, the broadest lysin identified is from a bacteriophage that infects *Streptococcus suis* (a pig pathogen). This enzyme (PlySs2) not only kills *S. suis* but other gram-positive pathogens (MRSA, vancomycin-intermediate *S. aureus* (VISA), Listeria, *Staphylococcus simulans, Staphylococcus epidermidis, S. equi, Streptococcus agalactiae*, *S. pyogenes, Streptococcus sanguinis, group G streptococci*, group E streptococci, and *Streptococcus pneumoniae*) (Gilmer et al. 2013).

4 *Staphylococcus aureus*-Specific Lysins

An important lysin with respect to infection control would be lysins directed to *S. aureus* (Sonstein et al. 1971; Clyne et al. 1992; O'Flaherty et al. 2005; Rashel et al. 2007; Manoharadas et al. 2009; Gilmer et al. 2013). In an earlier publication (Rashel et al. 2007), a staphylococcal enzyme was described that could be easily produced recombinantly and had a significant lethal effect on methicillin-resistant *S. aureus* (MRSA) both in vitro and in a mouse model. In the animal experiments, the authors show that the enzymes may be used to decolonize staphylococci from the nose of the mice as well as protect the animals from an intraperitoneal challenge with MRSA. However, in the latter experiments, the best protection was observed if the lysin was added up to 30 min after the MRSA. To help overcome insolubility

problems inherent in many staphylococcal-specific lysins, a chimeric enzyme was produced from two different *S. aureus*-specific lysins; however, though soluble, the activity did not show log-fold drop in viability or efficacy in animal models (Manoharadas et al. 2009). Subsequently, a chimeric lysin (ClyS) was produced from two staphylococcal-specific lysins (PhiNM3 and Twort). Purified ClyS efficiently lysed MRSA, VISA, and methicillin sensitive (MSSA) strains of *S. aureus* by >2-logs in vitro. In a mouse nasal decolonization model, a 2-log reduction in the viability of MRSA cells was seen 1 h following a single treatment with ClyS and one intraperitoneal dose of ClyS also protected against death by MRSA in a mouse bacteremia model. More recently, PlySs2 (described above as one of the broadest acting lysins) (Gilmer et al. 2013) was used to control MRSA. PlySs2 at 128 μg/ml in vitro reduced MRSA growth by 5-logs within 1 h and exhibited an MIC of 16 μg/ml for MRSA. A single, 2-mg dose of PlySs2 protected 92 % of the mice in a bacteremia model of mixed MRSA and *S. pyogenes* infection.

5 Synergy

Because of their action on the cell wall, when lysins are used in combination with antibiotics, they tend to work synergistically (Loeffler and Fischetti 2003; Djurkovic et al. 2005; Rashel et al. 2007; Daniel et al. 2010; Schuch et al. 2014; Schmelcher et al. 2015) (Fig. 3). For example, when the pneumococcal lysin Cpl-1 was used in combination with gentamicin, increased synergistic killing of

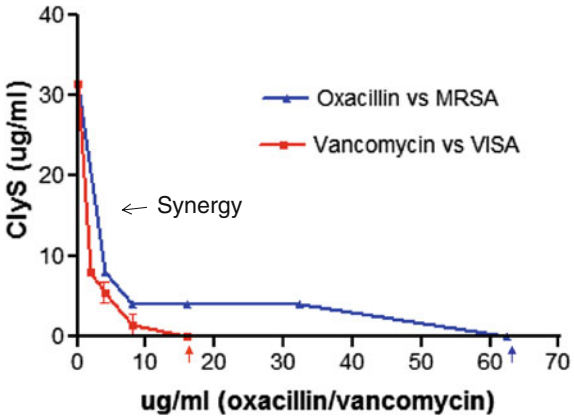

Fig. 3 Results of an isobologram with the staphylococcal-specific lysin (ClyS) and an oxacillin-resistant MRSA strain and a vancomycin-resistant VISA strain of staphylococci. The results show a typical synergistic curve for both organisms, showing that small (non-lytic) amounts of the ClyS lysin will increase the sensitivity of the resistant staphylococci to the respective antibiotics

pneumococci was observed with decreasing penicillin MIC (Djurkovic et al. 2005). Synergy was also observed with a staphylococcal-specific enzyme and glycopeptide antibiotics (Rashel et al. 2007).

In a recent report of a lysin being developed as a therapeutic by Contrafect Corporation (Schuch et al. 2014) (Contrafect.com), a lysin (CF-301) was used in combination with vancomycin or daptomycin to significantly increase the survival of mice with staphylococcal-induced bacteremia. The lysin at sublytic doses apparently weakens the bonds in the peptidoglycan allowing more efficient uptake of the antibiotics. Thus, the right combination of lysin and antibiotic could help in the control of antibiotic-resistant bacteria as well as reinstate the use of certain antibiotics for which resistance has been established.

6 Biofilms

Most bacteria can form biofilms, which are composed of bacterial cells adherent to a substrate and to each other. These adherent cells are usually embedded within a matrix of extracellular slimy material composed of DNA, protein, and polysaccharides secreted and released by the cells. Biofilms are found on living or non-living surfaces and are prevalent wherever bacteria are found. By its very nature, bacteria imbedded within biofilms are more difficult to kill with conventional antibiotics. Because bacteria are part of the biofilm matrix, the lytic action of lysins on the bacterial component of the matrix has a destabilizing effect on these structures. CF-301, the staphylococcal-specific lysin, being developed by Contrafect, was shown to be quite effective against staphylococcal biofilms produced for 24 h on polystyrene dishes and subsequently treated with CF-301. Following treatments for 2 or 4 h, residual biofilms were completely removed by 2 h, whereas standard of care antibiotics at 1000-fold higher concentration failed to remove the biomass even after 4 h of treatment (Schuch et al. 2014). These results could explain earlier studies where a pneumococcal lysin was used to successfully remove pneumococci from the heart valves in a rat endocarditis model (Entenza et al. 2005). By definition, endocarditis is a biofilm of organisms on heart valves, which is difficult to treat and has a high fatality rate, particularly in the case of MRSA endocarditis (Huang et al. 2008). In some cases, surgical intervention is required to remove the biofilm. Lysin treatment, in combination with standard of care antibiotics, could prove more effective than antibiotics alone.

7 Effects of Antibodies

The pharmacokinetics of lysins like other foreign proteins delivered systemically to animals is about 20 min (Loeffler and Fischetti 2003). Thus, if lysins are to be used systemically, they may need to be modified to extend their half-life, or they may

need to be delivered frequently or by IV infusion. However, because lysins work rapidly, more rapidly than antibiotics, perhaps one or two doses may be sufficient. Since lysins are proteins, a concern regarding their use is the development of neutralizing antibodies, which could reduce the in vivo levels and activity of the enzyme during treatment. When rabbit hyperimmune serum raised against the pneumococcal-specific enzyme Cpl-1 was assayed for its effect on lytic activity, it did not block the activity of Cpl-1 (Loeffler et al. 2003). When similar in vitro experiments were performed with antibodies directed to a *B. anthracis*- and an *S. pyogenes*-specific enzyme, similar results were obtained (Fischetti, unpublished data). These results were also verified with a staphylococcal-specific lysin (Rashel et al. 2007).

To test the relevance of this result in vivo, mice that received three intravenous doses of the Cpl-1 enzyme tested positive for IgG against Cpl-1 in 5 of 6 cases with low but measurable titers of about 1:10. Vaccinated and naïve control mice were then challenged intravenously with pneumococci and then treated by the same route with 200 μg Cpl-1 after 10 h. Within a minute, the treatment reduced the pneumococcal titer in the blood of Cpl-1-immunized mice to the same degree as the naive mice, supporting the in vitro data that antibody to lysins has little to no neutralizing effect. A similar experiment by Rashel with a staphylococcal enzyme (Rashel et al. 2007) showed the same result and that animals injected with lysin multiple times exhibited no adverse events.

This unexpected effect may be partially explained if the binding affinity of the enzyme for its substrate in the bacterial cell wall is higher than the antibody's affinity for the enzyme. This is supported by the results of Loessner (Loessner et al. 2002), showing that the cell wall-binding domain of a listeria-specific phage enzyme binds to its wall substrate at nanomolar affinities. However, while this may explain the inability of the antibody to neutralize the binding domain, it does not explain why antibodies to the catalytic domain do not neutralize. Nevertheless, these results are encouraging since it suggests that such enzymes may be used repeatedly in certain situations to control infecting or colonizing disease bacteria.

8 Bacterial Resistance to Lysins

Exposure of bacteria grown on agar plates to low concentrations of lysin did not lead to the recovery of resistant strains even after over 40 cycles. Organisms in colonies isolated at the periphery of a clear lytic zone created by a 10 μl drop of dilute lysin always resulted in enzyme-sensitive bacteria. Enzyme-resistant bacteria could also not be identified after >10 cycles of bacterial exposure to low concentrations of lysin (from 5–20 units) in liquid culture (Loeffler et al. 2001). These results may be explained by the fact that the cell wall receptor for the pneumococcal lysin is choline (Garcia et al. 1983), a molecule that is essential for pneumococcal viability, and the receptor for PlyG, the lysin against *B. anthracis,* is directed against a neutral wall polysaccharide that is essential for *B. anthracis* survival

Table 1 List of pathogens to which phage lysins have been developed

Organisms for which lysins have been developed:	
S. pyogenes	*S. uberis*
S. pneumoniae	*S. equi*
B anthracis	*B. cereus*
Gr. B streptococcus	*B. thuringiensis*
E faecalis/E faecium	*B. megaterium*
S. aureus	Gr. G streptococci
C. perfringens	*A. baumannii*
C. difficile	*S. suis*
P. acnes	

(Schuch et al. 2013). While not yet proven, it is possible that during a phage's association with bacteria over the millennia, to avoid becoming trapped inside the host, the binding domain of their lytic enzymes evolved to target unique and essential molecules in the cell wall, making resistance to these enzymes a rare event.

9 Conclusion

Lysins are a new therapeutic to control a number of bacterial pathogens (Table 1). For the first time, we may be able to specifically kill pathogens on mucous membranes without affecting the surrounding normal flora, thus reducing a significant pathogen reservoir in the population. Since this capability has not been previously available, its acceptance may not be immediate. Nevertheless, such as vaccines, we should be striving to develop methods to prevent rather than treat infection. Whenever there is a need to kill bacteria, and contact can be made with the organism, lysins may be freely utilized. Such enzymes will be of direct benefit in environments where antibiotic-resistant gram-positive pathogens are a serious problem, such as hospitals, day care centers, and nursing homes. The lysins isolated thus far are remarkably heat stable (up to 60 °C) and are relatively easy to produce in a purified state and in large quantities, making them amenable to these applications. The challenge for the future is to use this basic strategy and improve upon it, as was the case for second- and third-generation antibiotics. Protein engineering, domain swapping, and gene shuffling all could lead to better lytic enzymes to control bacterial pathogens in a variety of environments. Since it is estimated that there are 10^{31} phage on earth, the potential to identify new lytic enzymes is enormous. Perhaps, someday phage lytic enzymes will be an essential component in our armamentarium against pathogenic bacteria.

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Literature Cited

Ahmed KB, Warner SL, Chen A, Gourley ES, Liu X, Vankayalapati H, Nussenzveig R, Prchal JT, Bearss DJ, Parker CJ (2011) In vitro and in vivo characterization of SGI-1252, a small molecule inhibitor of JAK2. Exp Hematol 39(1):14–25

Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8(4):260–271

Barrett FF, McGehee RF Jr, Finland M (1968) Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital. Bacteriologic and epidemiologic observations. N Engl J Med 279 (9):441–448

Centers for Disease Control and Prevention (1999) Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*–Minnesota and North Dakota, 1997–1999. JAMA 282(12):1123–1125

Cheng Q, Nelson D, Zhu S, Fischetti V (2005) Removal of group B streptococci colonizing the vagina and oropharynx of mice with a bacteriophage lytic enzyme. Antimicrob Agents Chemother 49(1):111–117

Clyne M, Birkbeck TH, Arbuthnott JP (1992) Characterization of staphylococcal Y-lysin. J Gen Microbiol 138:923–930

Daniel A, Euler C, Collin M, Chahales P, Gorelick KJ, Fischetti VA (2010) Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 54(4):1603–1612

Djurkovic S, Loeffler JM, Fischetti VA (2005) Synergistic killing of *Streptococcus pneumoniae* with the bacteriophage lytic enzyme Cpl-1 and penicillin or gentamicin depends on the level of penicillin resistance. Antimicrob Agents Chemother 49:1225–1228

Entenza JM, Loeffler JM, Grandgirard D, Fischetti VA, Moreillon P (2005) Therapeutic effects of bacteriophage Cpl-1 lysin against *Streptococcus pneumoniae* endocarditis in rats. Antimicrob Agents Chemother 49(11):4789–4792

Fenton M, Ross P, McAuliffe O, O'Mahony J, Coffey A (2010) Recombinant bacteriophage lysins as antibacterials. Bioeng Bugs 1(1):9–16

Fischetti VA (2010) Bacteriophage endolysins: a novel anti-infective to control gram-positive pathogens. Int J Med Microbiol 300(6):357–362

Fischetti VA, Nelson D, Schuch R (2006) Reinventing phage therapy: are the parts greater than the sum? Nat Biotechnol 24(12):1508–1511

Garcia P, Garcia E, Ronda C, Tomasz A, Lopez R (1983) Inhibition of lysis by antibody against phage-associated lysin and requirement of choline residues in the cell wall for progeny phage release in *Streptococcus pneumoniae*. Curr Microbiol 8:137–140

Gilmer DB, Schmitz JE, Euler CW, Fischetti VA (2013) Novel bacteriophage lysin with broad lytic activity protects against mixed infection by *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS (1998) Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA J Am Med Assoc 279(8):593–598

Huang YT, Hsiao CH, Liao CH, Lee CW, Hsueh PR (2008) Bacteremia and infective endocarditis caused by a non-daptomycin-susceptible, vancomycin-intermediate, and methicillin-resistant *Staphylococcus aureus* strain in Taiwan. J Clin Microbiol 46(3):1132–1136

Jervis EJ, Haynes CA, Kilburn DG (1997) Surface diffusion of cellulases and their isolated binding domains on cellulose. J Biol Chem 272(38):24016–24023

Klein E, Smith DL, Laxminarayan R (2007) Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg Infect Dis 13 (12):1840–1846

Lai MJ, Lin NT, Hu A, Soo PC, Chen LK, Chen LH, Chang KC (2011) Antibacterial activity of *Acinetobacter baumannii* phage varphiAB2 endolysin (LysAB2) against both gram-positive and gram-negative bacteria. Appl Microbiol Biotechnol 90(2):529–539

Laupland KB, Ross T, Gregson DB (2008) *Staphylococcus aureus* bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000–2006. J Infect Dis 198(3):336

Li M, Diep BA, Villaruz AE, Braughton KR, Jiang X, DeLeo FR, Chambers HF, Lu Y, Otto M (2009) Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci USA 106(14):5883–5888

Loeffler JM, Fischetti VA (2003) Synergistic lethal effect of a combination of phage lytic enzymes with different activities on penicillin-sensitive and -resistant *Streptococcus pneumoniae* strains. Antimicrob Agents Chemother 47:375–377

Loeffler JM, Nelson D, Fischetti VA (2001) Rapid killing of *Streptococcus pneumoniae* with a bacteriophage cell wall hydrolase. Science 294(5549):2170–2172

Loeffler JM, Djurkovic S, Fischetti VA (2003) Phage lytic enzyme Cpl-1 as a novel antimicrobial for pneumococcal bacteremia. Infect Immun 71(11):6199–6204

Loessner MJ, Maier SK, Daubek-Puza H, Wendlinger G, Scherer S (1997) Three *Bacillus cereus* bacteriophage endolysins are unrelated but reveal high homology to cell wall hydrolases from different bacilli. J Bacteriol 179(9):2845–2851

Loessner MJ, Kramer K, Ebel F, Scherer S (2002) C-terminal domains of Listeria monocytogenes bacteriophage murein hydrolases determine specific recognition and high-affinity binding to bacterial cell wall carbohydrates. Mol Microbiol 44(2):335–349

Lood R, Winer BY, Pelzek AJ, Diez-Martinez R, Thandar M, Euler CW, Schuch R, Fischetti VA (2015) Novel phage lysin capable of killing the multidrug-resistant gram-negative bacterium *Acinetobacter baumannii* in a mouse bacteremia model. Antimicrob Agents Chemother 59 (4):1983–1991

Lowy FD (1998) *Staphylococcus aureus* infections. N Engl J Med 339(8):520–532

Manoharadas S, Witte A, Blasi U (2009) Antimicrobial activity of a chimeric enzybiotic towards *Staphylococcus aureus*. J Biotechnol 139(1):118–123

Matsuzaki S, Rashel M, Uchiyama J, Sakurai S, Ujihara T, Kuroda M, Ikeuchi M, Tani T, Fujieda M, Wakiguchi H, Imai S (2005) Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 11(5):211–219

Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B (2005) Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 352(14):1445–1453

Nelson D, Loomis L, Fischetti VA (2001) Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. Proc Natl Acad Sci USA 98(7):4107–4112

O'Flaherty S, Coffey A, Meaney W, Fitzgerald GF, Ross RP (2005) The recombinant phage lysin LysK has a broad spectrum of lytic activity against clinically relevant staphylococci, including methicillin-resistant *Staphylococcus aureus*. J Bacteriol 187(20):7161–7164

O'Flaherty S, Ross RP, Coffey A (2009) Bacteriophage and their lysins for elimination of infectious bacteria. FEMS Microbiol Rev 33(4):801–819

Rashel M, Uchiyama J, Ujihara T, Uehara Y, Kuramoto S, Sugihara S, Yagyu K, Muraoka A, Sugai M, Hiramatsu K, Honke K, Matsuzaki S (2007) Efficient elimination of multidrug-resistant *Staphylococcus aureus* by cloned lysin derived from bacteriophage phi MR11. J Infect Dis 196(8):1237–1247

Schmelcher M, Powell AM, Camp MJ, Pohl CS, Donovan DM (2015) Synergistic streptococcal phage lambdaSA2 and B30 endolysins kill streptococci in cow milk and in a mouse model of mastitis. Appl Microbiol Biotechnol

Schuch R, Nelson D, Fischetti VA (2002a) A bacteriolytic agent that detects and kills *Bacillus anthracis*. Nature 418(6900):884–889

Schuch R, Pelzek AJ, Raz A, Euler CW, Ryan PA, Winer BY, Farnsworth A, Bhaskaran SS, Stebbins CE, Xu Y, Clifford A, Bearss DJ, Vankayalapati H, Goldberg AR, Fischetti VA (2013) Use of a bacteriophage lysin to identify a novel target for antimicrobial development. PLoS ONE 8(4):e60754

Schuch R, Lee HM, Schneider BC, Sauve KL, Law C, Khan BK, Rotolo JA, Horiuchi Y, Couto DE, Raz A, Fischetti VA, Huang DB, Nowinski RC, Wittekind M (2014) Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus aureus*-Induced murine bacteremia. J Infect Dis 209 (9):1469–1478

Sonstein SA, Hammel JM, Bondi A (1971) Staphylococcal bacteriophage-associated lysin: a lytic agent active against *Staphylococcus aureus*. J Bacteriol 107(2):499–504

Tiemersma EW, Bronzwaer S, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundman H (2004) Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg Infect Dis 10(9):1627–1634

Wang IN, Smith DL, Young R (2000) Holins: the protein clocks of bacteriophage infections. Annu Rev Microbiol 54:799–825

Wang IN, Deaton J, Young R (2003) Sizing the holin lesion with an endolysin-beta-galactosidase fusion. J Bacteriol 185(3):779–787

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL (2005) The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 5 (12):751–762

White A, Smith J (1963) Nasal reservoir as the source of extranasal staphylococci. Antimicrob Agents Chemother (Bethesda) 161:679–683

Woodford N, Ellington MJ (2007) The emergence of antibiotic resistance by mutation. Clin Microbiol Infect 13(1):5–18

Yoong P, Nelson D, Schuch R, Fischetti VA (2004) Identification of a broadly active phage lytic enzyme with lethal activity against antibiotic-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Bacteriol 186:4808–4812