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Colonization, Pathogenicity, Host Susceptibility and Therapeutics for *Staphylococcus aureus*: What is the Clinical Relevance?[1]

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Abstract

Staphylococcus aureus is a human commensal that can also cause a broad spectrum of clinical disease. Factors associated with clinical disease are myriad and dynamic and include pathogen virulence, antimicrobial resistance and host susceptibility. Additionally, infection control measures aimed at the environmental niches of *S. aureus* and therapeutic advances continue to impact upon the incidence and outcomes of staphylococcal infections. This review article focuses on the clinical relevance of advances in our understanding of staphylococcal colonization, virulence, host susceptibility and therapeutics.

Over the past decade key developments have arisen. First, rates of nosocomial methicillin-resistant *S. aureus* (MRSA) infections have significantly declined in many countries. Second, we have made great strides in our understanding of the molecular pathogenesis of *S. aureus* in general and community-associated MRSA in particular. Third, host risk factors for invasive staphylococcal infections, such as advancing age, increasing numbers of invasive medical interventions, and a growing proportion of patients with healthcare contact, remain dynamic. Finally, several new antimicrobial agents active against MRSA have become available for clinical use.

Humans and *S. aureus* co-exist and the dynamic interface between host, pathogen and our attempts to influence these interactions will continue to rapidly change. Although progress has been made in the past decade, we are likely to face further surprises such as the recent waves of community-associated MRSA.

Keywords

Staphylococcus aureus; methicillin-resistant; MRSA; epidemiology; pathogenesis; treatment

Background

The epidemiology and clinical manifestations of any infectious disease are influenced by several factors including the pathogen, the host, the environment and therapeutic advances.

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This article is published as part of the Special Issue on Staphylococcal Infections.

The story of *Staphylococcus aureus*, an incredibly versatile organism that is normally a human commensal, demonstrates how these factors result in a dynamic and rapidly shifting landscape. This article will begin by defining key concepts relevant to *S. aureus*, followed by a review of recent developments in the interactions between an organism that is becoming more resistant to antibiotics, a host population that is undergoing more medical procedures, and the host population's attempts to change the environment and course of disease.

Clinical syndromes

S. aureus is a gram-positive coccus with numerous virulence factors and the ability to acquire antibiotic resistance determinants [1]. Skin and skin structure infections (SSSI) represent approximately 90% of all *S. aureus* infections and thus the major burden of staphylococcal disease [2–6]. However, infections of the bloodstream, respiratory tract, bone and joint, surgical wounds, and increasingly medical devices are particularly feared due to the high morbidity and mortality and prolonged treatment required. More recently, severe manifestations of community-associated disease such as fulminant sepsis [7], the Waterhouse-Friderichsen syndrome [8] and necrotizing pneumonia [9,10] have become prevalent. The rise of antibiotic resistance has further compromised effectiveness of existing antimicrobial agents. Thus, despite improvements in medical care, mortality from *S. aureus* bacteremia continues to be 20–30% in the developed world [11].

Antibiotic resistance

Following the introduction of penicillin in the mid-1940s, penicillin-resistant strains of *S. aureus* were soon reported [12] and this was followed by a pandemic of penicillin-resistant *S. aureus* [13]. Although initially prevalent only in hospitals, penicillin-resistance is now present in over 90% of community isolates. These strains produce a plasmid-encoded penicillinase that disrupts the β-lactam ring of penicillin. Methicillin, a penicillinase stable β-lactam, was introduced in the late 1950s; however, reports of methicillin-resistance rapidly appeared [14]. The mechanism of resistance to methicillin involves an altered and low affinity penicillin-binding protein (PBP2a) that is encoded by the *mec*A gene, which confers a broad resistance against all members of the β-lactam antibiotics. The *mec*A gene is carried on the mobile genetic element staphylococcal chromosome cassette (SCC) with the entire complex termed the SCC*mec* element. There are now 11 defined variants of SCC*mec* (types I to XI) (see http://www.sccmec.org/) that differ in size and composition of antimicrobial resistance elements. Typically, multi-resistant nosocomial strains of MRSA harbor SCC*mec*II and SCC*mec*III, which are larger and include multiple resistance determinants. On the other hand, the more recent community-associated MRSA strains harbor the smaller SCC*mec*IV, which carry fewer resistance elements and thus often retain susceptibility to macrolides, quinolones, tetracyclines, trimethoprim-sulfamethoxazole and lincosamides. Moreover, the smaller size of SCC*mec*IV has been postulated to allow it to be more mobile and supportive evidence of this is the fact that SCC*mec*IV has been inserted into multiple lineages of *S. aureus* whereas SCC*mec*II and SCC*mec*III have only been found in three and two lineages respectively [15].

Molecular genotypes

Early studies using phage typing established the utility of discriminating different strains or lineages of *S. aureus* [16]. Currently, the most commonly used techniques for molecular genotyping of *S. aureus* are pulsed-field gel electrophoresis (PFGE) and sequence based methods such as multilocus sequence typing (MLST) and *spa* typing. PFGE involves separating *Sma*I-digested DNA fragments of the genome by size in agarose gel and provides a very fine level of resolution. PFGE is limited by difficulties with inter-laboratory standardizations and portability, although it continues to be widely used in the United States,

and the Centers for Disease Control and Prevention (CDC) has developed a national *S. aureus* PFGE database [17]. Sequence based approaches have the advantage of producing unambiguous and reproducible results that can be compared on web-based databases. MLST involves the sequencing of 450–500bp fragments of seven housekeeping genes [18]. By assigning sequences for each fragment to different alleles, the combination of alleles can be designated to a unique sequence type (ST). There are now over 2000 STs on the *S. aureus* MLST database (http://saureus.mlst.net/). Sequencing the *spa* locus, a rapidly evolving hypervariable region of the genome, is simpler, because it only requires the sequence from one gene, and in general produces results concordant with MLST [19]. More recently, whole genome sequencing with next generation sequencing technologies has become an incredibly powerful means of determining the genetic make-up and relationships between *S. aureus* isolates [20].

The widespread uptake of MLST has allowed a much deeper understanding of the global population structure of *S. aureus*. It appears that there are distinct lineages or clonal complexes (CCs) of *S. aureus* and that these tend to evolve through point mutation rather than recombination [21,22]. However, it is also clear that virulence and resistance genes are frequently acquired through horizontal gene transfer onto what is a relatively stable genetic background [23].

Genotypic and epidemiologic definitions

Although molecular genotyping techniques are useful in determining the molecular epidemiology of *S. aureus*, such techniques are rarely available in standard diagnostic laboratories or sufficiently rapidly performed to guide clinicians in real time. Thus definitions based on epidemiological and resistance phenotype patterns are more commonly used. The CDC definition divides infections into nosocomial (onset of infection >48 hours after hospital admission), community-onset healthcare-associated (onset of infection in the community or <48 hours after hospital admission and the presence of ≥ 1 of the following risk factors: a history of hospitalization, surgery, dialysis, or residence in a long-term care facility within one year before the culture date; or the presence of a permanent indwelling catheter or percutaneous medical device at the time of culture; or previous isolation of MRSA), and community-associated (onset of infection in the community or <48 hours after hospital admission with none of the above risk factors) [2]. Resistance phenotype definitions group isolates as multiresistant (resistant to ≥ 3 non β-lactam classes of antibiotics) or non-multiresistant (resistant to <3 non β-lactam classes of antibiotics) [24]. There is obvious overlap between the genotypic, epidemiologic and resistance phenotypic definitions, particularly with the blurring of boundaries and encroachment of initially community-associated MRSA strains, such as USA300 into hospital environments, and also the increase in breadth of resistance of certain strains [25].

The environment: colonization and interventions to change the environment

S. aureus is a common human commensal. Approximately 30% of healthy adults are colonized with the anterior nares being the typical site of carriage. However, extra-nasal sites of *S. aureus* colonization include the skin, perineum, gastrointestinal tract and the throat. Longitudinal studies have revealed that individuals can be non-carriers, intermittent carriers and persistent carriers [26,27]. The risk of developing a healthcare-associated staphylococcal infection is three to six times increased among nasal carriers with a large bacterial load compared to non-carriers or those with a low bacterial load [28,29] with nasal colonizing strains usually being the source of infection [30,31]. More recently, it has become apparent that the patterns of carriage of community-associated MRSA may differ

from that previously recognized for healthcare-associated MRSA. A substantial proportion of those colonized with community-associated MRSA appear to be colonized at body sites outside of the anterior nares. For example, one study found that 23% of patients colonized with community-associated MRSA were colonized at non-nasal sites (predominantly inguinal regions) [32] and in children with SSSI the rectum was found to be the key site of colonization [33]. Young, healthy individuals appear to be at higher risk of exclusive throat carriage. Of healthy blood donors colonized with *S. aureus*, 30% carried *S. aureus* in the throat only [34]. Furthermore, the household environment has also been found as a reservoir [35]. Even in the Intensive Care Unit (ICU) setting, the throat and rectum are important sites of carriage of MRSA [36].

The clinical importance of understanding the sites, patterns and methods of detection of colonization lies in the ability to intervene through infection control programs and to prevent clinical infections in colonized hosts. For example, various interventions involving nasal decolonization have been shown to be effective in reducing rates of *S. aureus* bacteremia (SAB) in hemodialysis patients [37], MRSA wound infections post cardiothoracic surgery [38] and surgical site infections [39]. Indeed, for the first time since the global epidemic of MRSA in hospitals, the past five years has seen notable successes in reducing rates of MRSA infection. In three Illinois hospitals between 2003–2007, universal screening of all admissions for MRSA followed by topical decolonization and contact isolation of MRSA colonized patients resulted in a 70% reduction in rates of hospital-associated MRSA infections [40]. In the United States, the Veterans Affairs hospitals universally implemented a “MRSA bundle”, consisting of universal active surveillance, contact precautions for those colonized or infected with MRSA, a hand hygiene campaign and programs to stimulate institutional culture change, resulting in a 62% reduction in the incidence of MRSA-related healthcare-associated infections from 1.64 infections per 1000 patient-days to 0.62 per 1000 patient days [41]. The incidence of central line-associated bloodstream infections (CLABSI) due to MRSA in ICUs has significantly reduced from 2001– 2007, together with an even greater reduction in CLABSI due to methicillin-susceptible *S. aureus* (MSSA) [42]. Strikingly, over the period 2005–2008 the incidence of invasive healthcare-associated MRSA infections decreased by 9.4% per year [43].

These reductions in incidence of MRSA infections have not been limited to the United States. In England, following widespread efforts and interventions to combat MRSA, there has been a 57% reduction in MRSA bacteremia from 2004–2008 [44]. In France, sustained reductions over the past decade have also been evident [45], and the Netherlands continues to maintain an incredibly low incidence of MRSA infections [46]. Similarly, in Australia, concerted efforts to improve hand hygiene compliance resulted in significant reductions in rates of MRSA infections and bacteremias on a statewide level [47].

These encouraging indications from the above observational studies need to be tempered by the results of two large and well-designed clinical trials that found no statistically significant reduction in MRSA rates with the use of MRSA nasal surveillance and isolation precautions for MRSA carriers [48,49]. Therefore, it remains unresolved as to which interventions are effective, since multiple interventions targeting MRSA are typically implemented during observational studies. In addition, the publication and implementation of guidelines to prevent CLABSIs and ventilator-associated pneumonias may have occurred concurrently. Edgeworth argues that the decolonization of patients with mupirocin and skin antisepsis agents has likely played an under-recognized role in the success of these programs [44]. Despite these caveats, the balance of evidence indicates that the bundled interventions being administered are proving effective in reducing rates of MRSA in hospitals.

However, an even greater challenge will be to find interventions that are successful in the community where community-associated MRSA appears to be adding to the general burden of staphylococcal disease [50] and where high risk populations may be acting as reservoir and vectors for the amplification and wide-dissemination of community-associated MRSA strains [51,6]. Moreover, traditional approaches to surveillance and decolonization may not apply to community-associated MRSA due to its preponderance for colonization of extra-nasal sites such as the throat and rectum. Attempts to reduce rates of MRSA infections by screening and nasal decolonization of MRSA carriers in the setting of military and sporting communities have so far proved unsuccessful [52,53].

The pathogen: Organism related determinants of outcome

S. aureus has a large arsenal of virulence factors that allow it to evade host immune responses and to cause clinical disease. These comprise cell surface proteins, extracellular enzymes and exotoxins. Although *in vitro* studies and the use of animal models have provided many insights into the role of these various factors, there has been little to correlate the presence and expression of such factors with clinical disease and outcomes. Indeed, few therapeutic interventions are currently available that have applied this knowledge. Due to the epidemic of USA300 community-associated MRSA, much of the recent research described here has focused on factors associated with USA300, and findings from USA300 may not be applicable to other strains of *S. aureus*. It will be important for similar studies to be conducted with other strains of *S. aureus*.

The Panton-Valentine leukocidin controversy

One of the initial observations regarding community-associated MRSA was that the majority of strains harbored the genes encoding for Panton-Valentine leukocidin (PVL). PVL is a bi-component toxin that forms pores in neutrophils. Early epidemiological studies highlighted the association between PVL and necrotizing pneumonia, furunculosis and severe bone and joint infections [54,9,55]. An early paper described an association between PVL and lethal necrotizing pneumonia in young adults but the overall numbers were small with only 16 cases of PVL+ pneumonia of which 8 were recruited retrospectively [9]. Further cases of necrotizing pneumonia associated with PVL, in both MRSA and MSSA infections and often associated with influenza and poor outcomes, have been reported [56,10,57–59]. In Australia, where non-PVL lineages of community-associated MRSA from clonal complex 1 circulate [60], and also where the majority of PVL+ isolates were found to be MSSA in northern Australia [61], PVL has consistently been linked to furunculosis [61–63], but not to poorer outcomes [61,64]. Recent studies have failed to find an association between the presence of PVL and poorer outcomes in complicated skin and skin structure infections (cSSSI) [65,66] (Tong et al., Abstract C2-1287 at 50th Interscience Conference for Antimicrobial Agents and Chemotherapy, Boston, September 12–15, 2010), hospital acquired pneumonia [67] or invasive disease [68].

Conflicting results from experimental studies have also led to a significant degree of controversy regarding the pathogenic role of PVL. In one study using a mouse model, it was reported that PVL was directly causative of necrotizing pneumonia [69]. However, it was subsequently determined that an unintended point mutation in the *agr* promoter of the *S. aureus* isolate used resulted in defective virulence gene regulation and explained the observed phenotype [70]. Furthermore, mammalian neutrophils from different species differ in their susceptibility to PVL. Mouse neutrophils are more resistant than human and rabbit neutrophils to PVL [71–74]. Therefore mouse models may not be appropriate for determining the role of PVL in human disease and could explain the negative findings from several studies [75,76]. However, even the use of rabbit models (rabbit neutrophils are susceptible to PVL), has not resolved the controversy. A rabbit model of necrotizing

pneumonia compared the virulence of a USA300 wild-type strain with that of an isogenic PVL-deletion mutant and found that expression of PVL resulted in increased pathogenicity [77]. Two recent papers using a rabbit SSSI model came to conflicting conclusions about the role of PVL [78,79]. To complicate matters further, Yoong and Pier found in a low-inoculum murine skin abscess model that PVL stimulates a protective host immune response that is abrogated by antibodies to PVL [80]. However, epidemiological evidence linking a history of past PVL infection (using the history of a previous furuncle as a surrogate) with protection against death in PVL-associated pneumonia has also been reported [81]. Thus, the results of a Phase 1–2 vaccine trial of a vaccine containing a PVL component are eagerly awaited (ClinicalTrials.gov NCT01011335, study completed in March 2011).

Apart from PVL, phenol-soluble-modulins (PSMs) and alpha-toxins have been investigated as factors important for lysis of neutrophils. Alpha-toxin is another pore-forming toxin that has been found to be an important virulence factor in murine pneumonia and skin infection models [82,83]. PSMs are amphipathic, alpha-helical peptides that have cytolytic activity. PSMs have been shown to contribute significantly to community-associated MRSA virulence [84] and to enhance the activity of PVL in lysis of human neutrophils [73]. Notably, a recent study has used a rabbit skin infection model to compare the relative virulence of a wildtype USA300 isolate with isogenic deletion mutants for PVL, alpha-toxin and PSMα. These investigators found that alpha-toxin and PSMα played more important roles than PVL as virulence determinants for this USA300 strain [79]. A novel bi-component leukotoxin named LukGH has recently been identified through cell surface proteomics of USA300 strain LAC [85]. LukGH was found to have cytolytic activity towards neutrophils and is potentially a novel virulence factor for USA300.

In summary, there is strong evidence for an epidemiological link between PVL and furunculosis and necrotizing pneumonia. The outcomes from cSSSI and hospital acquired pneumonia appear to not be different for PVL+ and PVL- disease. This may not be the case with community-acquired PVL+ necrotizing pneumonia, which is often associated with influenza and frequently results in poor outcomes. There continues to be conflicting evidence of the role of PVL in animal models of SSSI and pneumonia but it is becoming clearer that PVL is not the sole or dominant factor contributing to virulence of *S. aureus*.

Nonetheless, some clinicians are now moving towards treating severe PVL+ disease with therapies targeted at the PVL toxin. Published recommendations from France suggest that for severe SSTI, severe bone and joint infections, and necrotizing pneumonia, that antitoxin agents that inhibit protein synthesis such as clindamycin, rifampicin or linezolid should be added to standard bactericidal agents and that intravenous immunoglobulin (IVIg) be considered for necrotizing pneumonia and refractory SSSI [86]. There is evidence that inhibitors of protein synthesis do reduce the production of PVL [87,88], however there is a cautionary note that the production of PSMs may be increased with sub-inhibitory concentrations of such agents [89]. For necrotizing pneumonia, the recommendations acknowledge that the use of IVIg is only supported by *in vitro* data and a handful of case reports [90,91].

Staphyloxanthin

Staphylococcus aureus is named as such due to the golden color of colonies in growth media (aureus = “golden”, Latin). Recent work has demonstrated the importance of the carotenoid pigment, staphyloxanthin, to the ability of *S. aureus* to resist killing by neutrophils. Loss of pigmentation results in reduced virulence in murine skin and sepsis models [92]. Additionally, inhibition of staphyloxanthin biosynthesis resulted in improved clearance of *S. aureus* in a murine intra-peritoneal sepsis model, although no mention is made of an impact on murine survival [93]. Interestingly, an isolate from an early-branching and

phylogenetically divergent *S. aureus* lineage, clonal complex 75, has been shown to lack the genes necessary for synthesis of staphyloxanthin and 126 isolates from this lineage were found to all be non-pigmented [94]. Whether the acquisition of genes for staphyloxanthin biosynthesis was a significant event in the evolution of *S. aureus* as a virulent species remains to be determined.

Underlying genetic background

A further question of interest is whether *S. aureus* strains of distinct lineages differ in their virulence and the clinical diseases caused. Earlier studies using MLST data demonstrated that there was no difference in the populations of *S. aureus* that caused asymptomatic nasal carriage compared to a diverse range of severe disease [95,96]. Similarly, using amplified fragment length polymorphism analysis, it was shown that all lineages found in carriage isolates were also present in invasive isolates. However, some lineages appeared to be more frequently associated with impetigo isolates [97]. Focused studies have implicated CC5 and CC30 as more likely to cause bacteremia with hematogenous complications [98]. CC30 was also more likely to be a cause of persistent bacteremia [99] and over-represented in isolates from infective endocarditis as compared to SSSIs [100]. The reasons for why CC30 might be over-represented in these severe forms of infection are unknown. Recent evidence demonstrating that the same CC30 isolates that are associated with an increased risk for endocarditis in humans are also paradoxically less likely to cause septic death in two *in vivo* models implies that these contemporary CC30 isolates may have evolved an increased tendency to bind to host tissues at the expense of specific toxin production (Sharma et al., Abstract B-060/60 at 51st Interscience Conference for Antimicrobial Agents and Chemotherapy, Chicago, September 17–20, 2011). A fascinating study from Deleo and colleagues provides support for this potential explanation. In their work, they elegantly demonstrate how contemporary CC30 isolates, when compared to isolates of the historical hypervirulent 80/81 clone from the 1950s and 60s, contained non-synonymous SNPs in genes encoding accessory gene regulator C (*agrC*) and alpha hemolysin (*hla*) and had reduced virulence *in vivo* (DeLeo et al., Proc Natl Acad Sci USA 2011 in press).

Other studies have corroborated this surprising finding that even within monophyletic lineages significant differences in virulence may exist. In a genomic study of 10 USA300 isolates, isolates that were very closely related on a genome level demonstrated substantial differences in their virulence as determined in a murine sepsis model [101]. Similarly, single amino acid polymorphisms in the fibronectin binding protein A of *S. aureus* can result in an increased force of binding between *S. aureus* cells and fibronectin (Lower et al., Proc Natl Acad Sci USA 2011 in press). These single amino acid polymorphisms were found to be more common in SAB isolates from patients with infected cardiac devices compared to those from patients with uninfected cardiac devices. Collectively, these studies suggest that for *S. aureus*, as for other pathogens [102], determining variation at the single nucleotide level across the whole genome will be necessary to truly understand and elucidate associations with virulence in clinical disease.

The human host: Host related determinants of outcome

Perhaps of greater importance than pathogen virulence factors, many characteristics of the human host, whether intrinsic or acquired, have a major influence on clinical outcomes – ranging from establishing *S. aureus* as an asymptomatic colonizer to deleterious and fulminant invasive staphylococcal infection.

For some time now, researchers and clinicians alike have known that *S. aureus* infections may be associated with specific racial, social and physical attributes of the human host. Age, sex and racial origin are three important characteristics that determine risk of *S. aureus*

colonization and infection. For example, a secondary analysis of the 2001–2002 National Health and Nutrition Examination Survey (NHANES) in which 9,622 participants contributed microbiologic data showed women and patients aged 65 years and older were more likely to have MRSA colonization [103]. In addition to risk of colonization, older age group was also a risk factor for higher mortality if a patient became infected with *S. aureus*. A recent study of patients with *S. aureus* bacteremia found that patients aged 80 or older had substantially higher mortality rates [104]. The authors of the study went on to conclude that specific interventions to optimize clinical care practices in elderly patients with *S. aureus* bacteremia are essential given the compelling data on the disproportionately high rates of morbidity and mortality.

Racial origin is also a major determinant of risk of *S. aureus* colonization and infection at the population level. In the same aforementioned NHANES study, white persons were found to have statistically higher risks of *S. aureus* colonization than Hispanic or Black persons [103]. Despite this lower rate of colonization, African Americans were significantly more likely to develop invasive MRSA infections than whites [43,105]. Indigenous populations of different continents also appear to be at high risk for the acquisition and infection with community-associated MRSA as seen in the Native American, Pacific Islander, and Australian Aboriginal populations [51]. The reasons for these higher infection rates in racially distinct populations are unknown, and likely complex. At least some of these differences in infection rates may relate to sociocultural rather than genetic issues. For example, culturally unique behaviors among different populations may be more relevant in the pathogenesis of some forms of *S. aureus* infection. Other economic factors such as living in crowded conditions, lack of access to healthcare and suboptimal personal hygiene practices, are also likely to be relevant. In support of the role of these lifestyle issues is the observation that crowding, communal living conditions and sharing of personal items are thought to be important contributors to community-associated MRSA outbreaks among incarcerated inmates [106], military recruits [107] and professional athletes [108].

Skin and mucosal protection against *S. aureus*

There is no greater barrier against *S. aureus* than our skin and mucosal defenses. Data clearly show that *S. aureus* infections occur more frequently when the normal protective defenses of human skin are weakened or if they are breached, especially if there is a high burden of *S. aureus* colonization or if personal hygiene practices are lacking. Early evidence of the protective importance of human skin came from patients who had skin conditions and high rates of *S. aureus* colonization. For instance, a point-prevalence study of 54 patients with atopic dermatitis attending the dermatology clinic of the Children's Hospital of Philadelphia showed that greater than 80% were colonized with MRSA [109] – a colonization rate that is substantially higher than what is expected in the general population (~3%).

Within the community setting, protective skin barriers may be breached as a result of simple trauma during shaving of facial or body hair or from more serious injuries sustained during sports and training. Interestingly, the simple act of shaving body hair was identified as a specific risk factor associated with MRSA infections among college football players during an outbreak in Connecticut [110]. "Turf burns" and other more serious skin abrasions sustained during sports are much more obvious portals of entry for *S. aureus* colonizing the skin. An outbreak of community-associated MRSA SSSI among St. Louis Ram's football players demonstrated that infections often occurred at the sites of skin abrasions and turf burns [111]. The authors of the report also suggested that appropriate treatment and coverage of such skin abrasions may have helped to prevent community-associated MRSA infections seen in the outbreak.

Two other activities in the community setting are associated with high risks of *S. aureus* infection, namely tattooing and injection drug use. Both such activities may occur with contaminated sharps that can introduce *S. aureus* directly into soft tissue and the vascular system, respectively. For example, a 2006 report from the CDC revealed that 34 cases of community-associated MRSA infections were reported in patients who received tattoos from unlicensed tattooists who did not routinely perform hand hygiene, skin antisepsis or adequate disinfection of equipment and surfaces [112].

Immune Deficiencies

Immune competency is also an important part of the body's defense against *S. aureus*. There are inherited and acquired immunodeficiency states that are well associated with *S. aureus* infections. Inherited defects in white blood cell function or immune responses such as present in chronic granulomatous disease (CGD), Job's syndrome, Chediak-Higashi syndrome, and Wiskott-Aldrich syndrome all predispose to recurrent staphylococcal infections [113]. The key role of Il-1 and Il-17 in the immune response to *S. aureus* infections of the skin has recently been reviewed [114]. On the other hand, defects in specific cellular immunity are generally not closely associated with infections with *S. aureus.* Even though some studies have shown that HIV-infected patients are at higher risk of *S. aureus* infections, much of the link between *S. aureus* infection in HIV-infected patients may be attributable to higher frequency of healthcare contact, more antibiotic consumption, high proportion of indigent or incarcerated patients, and histories of substance abuse and high-risk behaviors. Interestingly, regardless of HIV-infection status, men who have sex with men have emerged as an important and independent risk factor for development of community-associated MRSA infections over the last decade [115].

Medical comorbidities and Healthcare Contact

Many medical comorbidities have been found to be important and manageable risk factors for *S. aureus* colonization and infection, including peritoneal dialysis or hemodialysis, diabetes mellitus, and rheumatoid arthritis [116]. There are many thoughts as to how these comorbidities increase risk of *S. aureus* infection. First, some of these comorbidities are associated with functional defects of the immune system. For instance, poorly-controlled diabetes mellitus is known to be associated with decreased neutrophil function and decreased cellular chemotaxis [117]. Similarly, patients with uremia or who are receiving dialysis have a degree of neutrophil dysfunction as well as diminished antibody production and opsonic capabilities.

Second, many of the aforementioned comorbidities may result in regular healthcare contact, more hospitalizations and frequent invasive procedures – all of which are well-understood major risk factors for *S. aureus* colonization and invasive disease [118,119]. In particular, breaches of the skin and mucosal defense with intravascular lines and hemodialysis needling are key risk factors. Furthermore, frequent healthcare contact often culminates in antibiotic use (whether appropriate or not), which is also is independently associated with acquisition of MRSA [120].

Finally, comorbid conditions can result in reduced functional status and an inability to independently perform personal hygiene and skin care, leading to higher rates of *S. aureus* colonization and infection. A case-control study at 7 hospitals in the southeastern region of the United States showed that poor functional status (defined as requiring assistance with any activity of daily living) was highly associated with infection with MRSA, particularly surgical site infections [121]. Additionally, if patients with multiple comorbidities and poor functional status were admitted to long-term care facilities (LTCF), the risk of MRSA colonization and infection also increases [122]. Specific characteristics in LTCFs have been

found as significant risk factors for MRSA colonization and infection: low ratio of nurses to patient beds, location of facility in a deprived area, and a stay of more than 6 months in the LTCF.

It is important to remember that modern medicine can offer new diagnostic and therapeutic options through the use of indwelling lines and other invasive procedures. Such opportunities come at a great risk of causing debilitating or even, lethal, *S. aureus* infections. Indeed, there has been a notable shift in the epidemiology of infective endocarditis (IE) over the past 20 years with *S. aureus* now the leading cause of IE in many regions of the world and much of this being healthcare-associated [123]. This sobering reminder underscores the need for increased vigilance and the urgency for a collective and aggressive response to reduce the threat of *S. aureus* infection.

Therapeutics: attempts to change outcomes

There are several overarching principles in the management of *S. aureus* infections. Two such important principles are grounded in basic science and studies on pathogenesis of staphylococci and the conclusions correlate well with data and outcomes from clinical studies.

S. aureus can form biofilm which facilitates its survival and multiplication on infected surfaces and prosthetic. In addition, the biofilm can provide protection against both host immune defenses as well as antibiotic agents, potentially prolonging the duration of organism exposure to antibiotics and promoting the possible opportunity for transfer of antibiotic resistance genes between organisms [124]. Thus, one of the most important management principles for invasive *S. aureus* infection is to completely remove infected tissue and/or prosthetic material [125] – to minimize the ability for *S. aureus* to persist and relapse.

Direct drainage of an abscess is another tenet in the management of *S. aureus* infections [126]. Models of staphylococcal abscess clearly demonstrate the ability of *S. aureus* to replicate in the center of an abscess, separated from surrounding immune cells and protected from high levels of antibiotics [127]. In fact, many smaller *S. aureus* abscesses may be adequately treated with drainage alone; the converse is also true, that treatment failure frequently occurs if antibiotics are used while the staphylococcal abscess remains undrained.

Over the last 30 years, oxacillins (nafcillin and flucloxacillin) and vancomycin have been the stalwarts of antibiotic therapy against MSSA and MRSA respectively. Recently, several new anti-staphylococcal antibiotics have become available. Furthermore, the quest for an effective vaccine to prevent staphylococcal infections continues. The following is a brief overview of recent drug and vaccine developments.

Linezolid

Linezolid is a synthetic oxazolidinone that is bacteriostatic against *S. aureus*. It acts by binding to the 23S portion of the 50S subunit of bacterial ribosomes and inhibits protein synthesis. The antimicrobial activity of linezolid is predicted by the 24-hour area under the time-concentration curve to minimum inhibitory concentration (MIC). Interestingly, the drug also displays moderate post-antibiotic effect against *S. aureus* [128].

Linezolid has been approved by the Food and Drug Administration (FDA) for the treatment of nosocomial pneumonia and cSSSI, including those due to MRSA [129]. The retrospective analysis of two randomized controlled trials (RCTs) claimed that linezolid was associated with improved survival and clinical cure compared to vancomycin for nosocomial

pneumonia due to MRSA [130]. However, these findings have been seriously questioned due to flaws in study design and a recent meta-analysis of RCTs involving 1641 patients found that linezolid was not superior to glycopeptide antibiotics [131]. This issue is hoped to be resolved when the results of a RCT of linezolid versus vancomycin for MRSA ventilator-associated pneumonia are published (ClinicalTrials.gov NCT00084266).

There are three important pharmacologic features of linezolid that require caution with prescription. Linezolid has been associated with dose-dependent myelosuppression that is usually reversible. Thrombocytopenia is the commonest pattern of haematologic change. Renal impairment, low baseline platelet count and prolonged use of linezolid beyond 14 days are all major risk factors for linezolid-associated thrombocytopenia. Thus, weekly complete blood counts should be monitored in patients receiving linezolid for more than 7–10 days [132]. Second, manifestations of mitochondrial dysfunction, such as neuropathy and lactic acidosis, have also been infrequently reported with linezolid use. Interestingly, some reports indicated that neuropathy tended to persist even after cessation of linezolid [133]. Third, linezolid is a weak inhibitor of monoamine oxidase (MAO) and can precipitate serotonin toxicity when administered with a non-selective MAO-Inhibitors such as a serotonin-reuptake inhibitor (SSRI) or a serotonin-noradrenergic reuptake inhibitor (SNRI) [134].

Although resistance to linezolid appears to be rare [135], possibly because its high cost limits widespread use, documented mechanisms include the horizontal acquisition of a *cfr* rRNA methyltransferase [136] and point mutations at the target site of 23S rRNA [137] and *rlmN* that encodes for a conserved RNA methyltransferase [138].

Finally, linezolid is available in both oral and intravenous formulations and the bioavailability of the oral form approaches 100%, which should be used whenever possible.

Tigecycline

Tigecycline is a glycylcycline antibiotic with acts on the 30S ribosomal subunit and prevents amino acid incorporation into bacterial peptide chains. Tigecycline is structurally related to minocycline and tetracycline, however, studies show that it remains active against most *S. aureus* isolates that are tetracycline resistant [139]. In addition to effective coverage against MRSA, tigecycline has broad gram-negative activity except for *Pseudomonas* and *Proteus spp.*. Currently, tigecycline has FDA approval for three common indications: treatment of cSSSI, complicated intra-abdominal infections and community-acquired pneumonia (CAP).

Tigecycline has several important pharmacological properties that require particular caution and consideration in prescription. First, tigecycline distributes extensively into lung and biliary tissue, however drugs levels are often low in serum, bone and joints and in the cerebrospinal fluid (CSF). Second, tigecycline has a high proportion of gastrointestinal adverse effects; nausea and vomiting have consistently been more frequent in the tigecycline compared to the comparator arms of phase III studies [140]. Finally, tigecycline has a Pregnancy Class D rating which is based on animal studies showing delayed bone development and increased incidence of fetal loss. Thus, it should not be used in pregnant women unless potential benefits clearly outweigh the potential risks to the mother and/or the fetus.

Ceftaroline

Ceftaroline is a novel cephalosporin with high affinity for the modified penicillin-binding protein (PBP 2A) and thus has *in vitro* activity against MRSA. Ceftaroline is different to recent new antibiotics with anti-MRSA coverage. First, ceftaroline also has activity against a narrow spectrum of gram-negative organisms; however, there is no intrinsic activity against

Pseudomonas spp., or other gram-negative pathogens that produce beta-lactamases, including AmpCs, extended-spectrum beta-lactamases and *Klebsiella pneumoniae* carbapenemases [141]. Second, there are some emerging data to suggest that ceftaroline might be more efficacious for invasive infections. For example, a recent *in vivo* study indicated that ceftaroline is superior to daptomycin in sterilizing cardiac vegetations infected with *S. aureus* [142].

Two pairs of large RCTs have studied ceftaroline for treatment of cSSSI and CAP. CANVAS 1 & 2 trials were parallel phase III studies that found ceftaroline monotherapy to be non-inferior to the combination of vancomycin plus aztreonam in the treatment of cSSSI, including those infections caused by MRSA [143]. Although FOCUS 1 & 2 studies found ceftaroline to be non-inferior to ceftriaxone for treatment of moderately-severe CAP not requiring intensive care therapy, efficacy of ceftaroline on CAP due to MRSA was not specifically examined [144]. In these randomized studies, ceftaroline was well tolerated and there were no excess adverse effects compared to controls.

Daptomycin

Daptomycin is a cyclic lipopeptide with marketing approval for the treatment of cSSSIs and for SAB and right-sided infective endocarditis. There have been two international phase III studies of daptomycin at a dose of 4mg/kg intravenously (IV) for 7–14 days compared to vancomycin or penicillinase-resistant penicillins for cSSSIs [145]. Of clinically evaluable outcomes, daptomycin and comparator arms demonstrated successful treatment in 83% and 84% of patients respectively. Further analyses demonstrated that daptomycin treated patients required a shorter duration of IV therapy with an earlier clinical response [145,146].

In a RCT of daptomycin 6mg/kg IV compared to either, vancomycin or a β-lactam, plus an aminoglycoside, for the first four days of therapy, daptomycin demonstrated non-inferiority for treatment of SAB and right-sided endocarditis [147]. For MRSA bacteremia, the success rate was higher for the daptomycin treated patients (44% versus 32% for daptomycin and standard therapy respectively).

These data suggest that daptomycin is now clearly part of the armamentarium for the treatment of cSSSIs and SAB. Importantly, daptomycin should not be used for treatment of staphylococcal pneumonia as pulmonary surfactant inhibits the action of daptomycin [148] and creatine kinase levels should be monitored weekly during therapy. Also notable, and of significant concern, is that in the registrational multinational trial of daptomycin for SAB, treatment emergent resistance occurred in ~5% of daptomycin treated patients [147]. Others have also reported daptomycin resistance that appears related to heterogenously vancomycin intermediate *S. aureus* (hVISA) and VISA strains [149,150].

Telavancin

Telavancin is a lipoglycopeptide that disrupts peptidoglycan synthesis. Phase III clinical trials have been conducted for cSSSI and hospital-acquired pneumonia. The ATLAS studies enrolled 1867 patients with cSSSI and compared telavancin to vancomycin [151]. Of clinically evaluable patients 88% and 87% were cured with televancin and vancomycin respectively and for those with MRSA infections eradication rates were 90% and 85% respectively. Recently, the ATTAIN study found that telavancin was non-inferior to vancomycin for treatment of hospital-acquired pneumonia due to gram-positive pathogens with respective cure rates of 59% and 59% respectively [152].

Vaccines

Given the large burden of disease due to *S. aureus*, concerns about increasing antimicrobial resistance and the growing numbers of patients with risks for invasive staphylococcal infections, there is a great need for an effective vaccine. However, to date, there have only been disappointing results in clinical trials for staphylococcal vaccines. The general topic of vaccines for *S. aureus* has been thoroughly addressed elsewhere [153–155] and here we briefly comment on human clinical trials conducted in the past 10 years.

StaphVAX®, produced by Nabi Biopharmaceuticals, is a polysaccharide conjugate vaccine that contains purified capsular antigens CP5 and CP8. A phase III clinical trial of StaphVAX® in hemodialysis patients showed no protection against bacteremia between 3 and 54 weeks, but a post-hoc analysis demonstrated efficacy up to 40 weeks post vaccination [156]. In a second unpublished study involving 3600 hemodialysis patients no protection was observed. There are now studies progressing with the use of a capsular polysaccharide conjugate vaccine (PentaStaph®, that has been purchased by GlaxoSmithKline from Nabi) that also contains antigens to teichoic acid and the secreted toxins alpha-toxin and PVL [155].

Merck's V710® vaccine contains IsdB, one of many microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) of *S. aureus*. Use of V710 in a phase I study elicited promising immune responses [157] however a phase II/III trial in elective cardiothoracic surgery patients to prevent serious *S. aureus* infections has recently been terminated. Although full results have not yet been released, it is understood that the Data Monitoring Committee recommended termination of the study due to the observation that V710 was unlikely to demonstrate a statistically significant clinical benefit as well as safety concerns (http://www.merck.com/newsroom/news-release-archive/research-and-development/2011_0608.html). There is an ongoing trial of V710 in hemodialysis patients.

Passive immunization approaches also have not been successful in clinical trials. INH-A21 (Veronate) is a pooled human immunoglobulin preparation with high antibody titres against clumping factor A (ClfA) that was tested in a phase III trial involving low birth weight infants. It failed to reduce late onset sepsis and SAB in this cohort [158]. Other products now through phase II studies include tefibazumab (monoclonal antibodies to ClfA) [159], Altastaph® (antibodies to CP5 and CP8) [160,161] pagibaximab (monoclonal antibodies to lipoteichoic acid) [162] and Aurograb® (antibodies to ATP-binding cassette). Pagibaximab has proceeded to a phase 2b/3 trial.

All these results demonstrate the great difficulties in developing a vaccine against *S. aureus*. Questions remain as to the best combination of antigens, and also which patient population should be targeted for the use of vaccines or passive immunotherapy. The conundrum is that high-risk patients such as those on hemodialysis may have relatively poor immune responses to a vaccine.

Conclusions

As long as *S. aureus* remains a human commensal, humans will continue to be at risk for developing staphylococcal infections. With increases in patient age, comorbidities, healthcare contact, and use of invasive medical procedures, the number of patients at risk of invasive staphylococcal infections will almost certainly increase. Furthermore, *S. aureus* has proven remarkably adaptable to challenges posed through the introduction of antibiotics and we have witnessed multiple waves of resistant *S. aureus* to β-lactams and reports of resistance to new antimicrobial agents continue unabated. Studies into the virulence and

pathogenicity of *S. aureus* have deepened our understanding of these mechanisms and it is hoped that this will translate into new therapeutics that specifically target such virulence factors (although it is likely that the organism will once again adapt to such agents). The good news is that efforts to reduce the incidence of healthcare-associated staphylococcal infections have recently achieved some success and highlights the importance of prevention of what are often devastating and costly infections.

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