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Staphylococcus aureus: A pathogen with still unresolved issues

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ABSTRACT

Staphylococcus aureus is a major human pathogen, and considerable research efforts have been put forward to improve our understanding of its complex pathogenesis. In spite of these efforts, the burden of staphylococcal infections is still on the rise. This review focuses on a selected set of crucial unresolved questions regarding this pathogen, namely: (i) the nature of the driving forces behind the rise and decline of methicillin-resistant *S. aureus* (MRSA) clones; (ii) the mechanisms by which a commensal becomes a pathogen; (iii) the molecular underpinnings of toxin overexpression in hypervirulent MRSA clones such as USA300; and (iv) the repeated failures of anti-*S. aureus* vaccine approaches.

1. Introduction

Staphylococcus aureus is one of the most important human pathogens both in the hospital and the community (Lowy, 1998). Since the beginning of the antibiotics era, *S. aureus* has been a privileged target of therapeutics research, and numerous antimicrobial agents, as well as vaccine attempts, were specifically designed to combat this bacterium (Lowy, 2003; Proctor, 2012). The overall amount of research efforts aimed at understanding *S. aureus* is still on the rise: searching the PubMed database with the "*S. aureus*" keywords over the 2007–2012 period retrieved 21060 articles, a 51.4% increase as compared to the 13907 articles published during the 2002–2007 period. Such efforts in both the fields of basic science and clinical research could have been expected, from a clinical standpoint, to result in dramatic improvements in cure rates of *S. aureus*-infected patients as well as in a global decrease of the incidence of *S. aureus* infections. However, the history of the last decades clearly shows that these expectations were not met. In 2005, methicillin-resistant *S. aureus* infection has become a more common cause of death in US patients than HIV infection (Centers for Disease Control and Prevention, 2005; Klevens et al., 2007). Besides this striking US-specific figure lies a more global threat: the increase of MRSA infection incidence in several regions of the world, not only in the hospital but also in the community (Boucher and Corey, 2008). Although our understanding of the lifestyle and the pathogenic strategies of *S. aureus* is constantly increasing, several important questions have remained unresolved. This review will focus on these questions, which could be major obstacles against our efforts to translate the scientific achievements on *S. aureus* into clinical successes.

2. The rise and decline of MRSA clones

MRSA is resistant to all currently available beta-lactam antibiotics. Although this pathogen emerged rapidly after the introduction of methicillin in clinical practice in the early 1960s, it only became a public health threat during the 1980s. Its expansion was accompanied by the acquisition of resistance determinants to non-beta-lactam antibiotics, which led some authors to suggest that beta-lactam resistance alone was not sufficient to promote MRSA dissemination in the hospital (Knight et al., 2012). The spread of MRSA worldwide was due to a limited number of successful clones as determined using molecular typing techniques such as pulsed-field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST) (Maiden et al., 1998; Mcdougal et al., 2003). MRSA is polyphyletic, meaning that the MRSA clones responsible for the past and current epidemics do not share a common MRSA ancestor. Indeed, MRSA emerged from methicillin-susceptible *S. aureus* (MSSA) through the horizontal transfer of a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). Contrasting with previous studies favoring a clonal origin of methicillin resistance in *S. aureus* (Kreiswirth et al., 1993), more recent phylogenetic studies indicated that SCC*mec* acquisition was a frequent event in *S. aureus* evolution (Nubel et al., 2008), with MRSA subgroups such as ST36 or ST80 originating from genetically distinct MSSA lineages (Deurenberg et al., 2007; Rasigade et al., 2010). A

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consequence of this polyphyletic nature of MRSA is that it can hardly be studied as a whole because different MRSA lineages often retain the different genotypic and phenotypic traits of their respective MSSA ancestors, and acquire additional features that reflect their specific evolutionary history. As a consequence, MRSA clones show striking differences in terms of fitness, virulence and the spectrum of resistance (Diep et al., 2006; Li et al., 2010).

MRSA emergence and spread in the hospital and more recently in the community, is the result of consecutive and often intermingled epidemic waves due to different clones. Early MRSA strains belonged to the ST250 lineage, which is part of the highly successful clonal complex (CC) 8. This Archaic clone virtually disappeared from hospital in the late 1970s and was gradually replaced by other MRSA lineages. Some of these new lineages were genetically related to the Archaic clone but harbored different allotypes of the SCC*mec* resistance determinant, such as the ST247 Iberian clone or the ST239 EMRSA-1 clone, while some other lineages were genetically unrelated to CC8, such as the ST5 USA100 clone or the ST36 EMRSA-16 clone. The molecular epidemiology of MRSA has remained extremely dynamic, and dramatic changes in the relative abundance of each clone have occurred over the last two decades with, for instance, the replacement of the ST36 EMRSA-16 clone by the ST22 EMRSA-15 clone as the major cause of MRSA infection in UK hospitals (Wyllie et al., 2011; Knight et al., 2012), or the emergence of highly epidemic clones of community-acquired (CA-) MRSA such as ST8 USA300 in the US, which is currently replacing the ST5 USA100 clone in the hospital setting (Gonzalez et al., 2006; Maree et al., 2007; Patel et al., 2008).

A thorough understanding of the driving forces that rule the expansion and decline of these different clones is clearly needed to help improve infection control strategies and contain the MRSA epidemics. However, our knowledge of these driving forces is still limited. The most obvious contributing factor to MRSA dynamics is antibiotics use and overuse. Nevertheless, several other important factors likely contribute to MRSA epidemics. For instance, high rates of MRSA have been recorded from remote populations with limited access to the healthcare system or antibiotics prescription (Mcdonald et al., 2006), thus indicating that antibiotics selection pressure is not sufficient to explain MRSA emergence. Similarly, infection control enforcement do not always correlate with the decline of a given MRSA clone: in the UK, the decline of the ST36 EMRSA-16 lineage appeared to have begun before that new infection control policies took place (Wyllie et al., 2011). During the 1990s, the decline in France of the gentamicin-resistant MRSA clone, which was replaced by the gentamicin-susceptible MRSA Lyon clone, was not explained by a reduction of gentamicin consumption (Lelievre et al., 1999), but maybe by a differential fitness of the two lineages (Laurent et al., 2001). Indeed, several clone-specific features could be linked with the emergence or decline of a peculiar lineage. Bacterial competition could occur between clones that share the same niche, similar to what has been demonstrated for *S. aureus* and *S. epidermidis* (Iwase et al., 2010). As mentioned above (Laurent et al., 2001), differences in bacterial fitness such as those observed between the highly successful ST22 EMRSA-15 clone and the currently declining CC30 MRSA are also potentially involved (Knight et al., 2012). Alternatively, the acquisition of immunity in the community in contact with a given clone could play a role in the subsequent decline of this clone (Gupta et al., 1998). Finally, lineage-specific features such as chlorhexidine resistance have been shown to influence MRSA dynamics in settings where infection control guidelines encourage the use of this antiseptic (Batra et al., 2010; Edgeworth, 2011). Overall, MRSA dynamics appear to be ruled by a complex interplay between human interventions, bacterial evolution and clone-specific biological behaviors, the consequence of which being our current inability to predict the epidemic potential of newly emerging clones.

3. What turns commensal strains into invasive strains?

S. aureus is carried without damage in 30% of humans, mostly in the nose but also in the throat, gut and skin folds (Sivaraman et al., 2009; Faden et al., 2010; Yang et al., 2010). Carriage increases the risk of developing a staphylococcal infection, and healthcare-associated infections are usually caused by the strains carried in the patient nose (Luzar et al., 1990; Kluytmans et al., 1995; Von Eiff et al., 2001; Wertheim et al., 2004). An important yet still unanswered question is whether genotypic differences exist between harmless carriage isolates and those that cause invasive infections. Several molecular typing studies using either MLST or amplified fragment length polymorphism failed to demonstrate such a difference (Day et al., 2001, 2002; Melles et al., 2004), and there is no evidence that lineages exist that are specifically associated with either colonization or disease. Indeed, although the presence of specific genes has been undoubtedly associated with specific clinical entities, such as the exfoliative toxins with staphylococcal scalded skin syndrome, or superantigens such as TSST-1 with toxic shock syndromes, isolates harboring such toxins are also found in healthy carriers (Mccormick et al., 2001; Nishifuji et al., 2008; Lozano et al., 2011; Piechowicz et al., 2011).

Another unanswered question is whether *S. aureus* colonization is beneficial or detrimental to the carrier. On the one hand, as stated above, colonized sites can act as a reservoir for healthcare-associated infections in compromised patients. Carriage is thus righteously considered detrimental in patients at risk for staphylococcal nosocomial infection, and decolonization measures have proven efficient at reducing the risk of staphylococcal infection in colonized patients undergoing hemodialysis or surgery (Tacconelli et al., 2003; Bode et al., 2010). On the other hand, *S. aureus* carriers that develop *S. aureus* bacteremia have a lower risk of death than non-carriers (Wertheim et al., 2004), suggesting that *S. aureus* colonization can induce some form of immunological adaptation beneficial for the host in terms of outcome if an invasive infection occurs.

4. Toxin expression regulation in *S. aureus*

The molecular basis of virulence in *S. aureus* has been an active field of research for several decades, but such research has further intensified and become decisive with the recent emergence of highly pathogenic CA-MRSA strains that combined antibiotics resistance, rapid spreading ability and exceptional virulence (Chambers, 2005). The last decade has seen the identification of yet unrecognized *S. aureus* virulence factors such as the phenol-soluble modulins (Wang et al., 2007), as well as the characterization of the pathogenic role of long-known toxins such as the Panton-Valentine leukocidin (Gillet et al., 2002; Labandeira-Rey et al., 2007; Diep et al., 2010). Vast amounts of work have been necessary to gain understanding of the complex multifactorial nature of CA-MRSA virulence and to outline which genetic features make one *S. aureus* strain more virulent than another. A clear distinction has been made between, on the one hand, virulence gained through the acquisition of new toxin genes by horizontal transfer such as the phage-borne *pvl* genes, and on the other hand, virulence gained through the overexpression of core genome-encoded toxins such as PSMs or alpha-toxin (Wang et al., 2007; Li et al., 2010; Otto, 2010). An additional layer of complexity has also added to this already intricate scheme with the observation that the acquisition of new genes harbored by mobile genetic elements (MGEs) correlated with modifications in the expression level of core genome-encoded toxin genes (Kaito et al., 2008, 2011). Gaining a deeper understanding of the molecular pathogenic mechanisms of CA-MRSA is crucial as a prerequisite to the design of dedicated therapeutic strategies,

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