International Journal of Medical Microbiology 306 (2016) 367–380

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

A unique SaeS allele overrides cell-density dependent expression of *saeR* and *lukSF-PV* in the ST30-SCC*mec*IV lineage of CA-MRSA

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 6 May 2016
Accepted 8 May 2016

Keywords:
lukSF-PV
saeRS
CA-MRSA
USA1100
ST30-SCC*mec*IV
Panton-Valentine leucocidin

ABSTRACT

ST30 (CC30)-SCC*mec* IV (USA1100) is one of the most common community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) lineages. ST30 isolates typically carry *lukSF-PV* genes encoding the Panton-Valentine leukocidin (PVL) and are responsible for outbreaks of invasive infections worldwide. In this study, twenty CC30 isolates were analyzed. All were very susceptible to non-β-lactam antimicrobials, 18/20 harbored the *lukSF-PV* genes, only 1/20 exhibited *agr-rnaIII* dysfunction, and the majority was not able to form biofilm on inert surfaces. Analysis of *lukSF-PV* temporal regulation revealed that opposite to other CA-MRSA isolates, these genes were more highly expressed in early log phase than in stationary phase. This inverted *lukSF-PV* temporal expression was associated with a similar pattern of *saeRS* expression in the ST30 isolates, namely high level expression in log phase and reduced expression in stationary phase. Reduced *saeRS* expression in stationary phase was associated with low expression levels of the *sae* regulators, *agr* and *agr*-upregulator *sarA*, which activate the stationary phase *sae*-P1 promoter and overexpression of *agr*-RNAIII restored the levels of *saeR* and *lukSF-PV* trancripts in stationary phase. Altered SaeRS activity in the ST30 isolates was attributed to amino acid substitutions (N227S, E268K and S351T) in the HTPase_c domain of SaeS (termed SaeS^{SKT}). Complementation of a USA300 *saeS* mutant with the *saeS^{SKT}* and *saeS* alleles under the direction of the log phase *sae*-P3 promoter revealed that *saeR* and *lukSF-PV* transcription levels were more significantly activated by *saeS^{SKT}* than *saeS*. In summary our data identify a unique *saeS* allele (*saeS^{SKT}*) which appears to override cell-density dependent SaeR and PVL expression in ST30 CA-MRSA isolates. Further studies to determine the contribution of *saeS^{SKT}* allele to the pathogenesis of infections caused by ST30 isolates are merited.

Abbreviations: Agr, accessory gene regulator; BLAST, Basic Local Alignment Search Tool; BU, biofilm unit; CA, community-acquired; CC, clonal complexes; CLSI, Clinical and Laboratory Standards Institute; Ct, threshold cycle; ΔΔCt, comparative Ct method; EMRSA-16, epidemic MRSA 16; FnBPA and B, fibronectin binding proteins A and B; HA, hospital-acquired; Hla, α-hemolysin; MLSBi, inducible macrolide lincosamide and streptogramin B resistance; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; MSCRAMMS, microbial surface components recognizing adhesive matrix molecules; OSP/SWP, Oceania Southwest Pacific/Southwest Pacific; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; P-SaeR, phosphorylated SaeR; PSMα3, phenol-soluble modulin α3; PVL, Panton-Valentine leukocidin; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; RM, restriction modification; RQ, relative quantification; SCC*mec*, staphylococcal cassette chromosome *mec*; SaeS^{SKT}, SaeS protein of the CC30 isolates containing three amino acid substitution N227S, E268K and S351T; SarA, staphylococcal accessory regulator A; Spa, staphylococcal protein A; SSTI, skin/soft tissue infections; TCS, two-component system; TSST-I, toxic shock syndrome toxin I.

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http://dx.doi.org/10.1016/j.ijmm.2016.05.001
1438-4221/

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates remain an important cause of community- (CA) and hospital-acquired (HA) infections (Figueiredo and Ferreira, 2014). CA-MRSA isolates cause significant infections in children and young adults, who do not present with classical risk factors for nosocomial diseases (David and Daum, 2010). These isolates frequently display distinct characteristics such as increased susceptibility to antimicrobial drugs and the presence of the staphylococcal cassette chromosome *mec* type IV or V (SCC*mec* type IV or V) and *lukSF-PV* genes, which encode for the S and F subunits of the Panton-Valentine leukocidin (PVL). In contrast, hospital-associated MRSA (HA-MRSA) isolates generally do not carry *lukSF-PV* and display antimicrobial multiresistant profiles (David and Daum, 2010; Vandenesch et al., 2003). Although most of the CA-MRSA-associated diseases involve skin/soft-tissue infections (SSTI), some cases are complicated by severe manifestations, including necrotizing fasciitis, pneumonia, blood stream infections, endocarditis, and generalized osteomyelitis (David and Daum, 2010; De Araujo et al., 2010). It has been suggested that PVL has cytolytic effects on human neutrophils (Diep et al., 2010). However, there is a continuing debate over the role of PVL in staphylococcal virulence. Notably, studies using recombinant PVL demonstrated a concentration-dependent effect leading to either apoptosis or the necrosis of neutrophils, depending on the toxin concentrations (Genestier et al., 2005). Studies by Labandeira-Rey et al. (2007), also using recombinant PVL, demonstrated that only PVL could induce pneumonia in mice. However, a large-scale epidemiological study published by Bae et al. (2009) found no correlation between the presence of PVL-encoding genes and the clinical outcome of complicated SSTI such as large abscesses. In addition, the epidemiological evidence is consistent with experimental data indicating that PVL is not likely to play an essential role in the pathogenesis of skin infections (Li et al., 2010). In fact, the first CA-MRSA strain described in the literature, the WA-1 MRSA, which caused infections among aborigines in Australia, did not carry *lukSF-PV* genes (Udo et al., 1993). Despite this, PVL seems to be involved in the pathogenesis of the more infrequent types of CA-MRSA diseases such as necrotizing pneumonia and osteomyelitis. Rabbit models of CA-MRSA bacteremia, pneumonia, and osteomyelitis have demonstrated a significant impact by *lukSF-PV* on the development of these diseases (Crémieux et al., 2009; Labandeira-Rey et al., 2007; Lina et al., 1999).

It is well documented that a number of *S. aureus* virulence factors are acting in cooperation under the coordination of an intricate and complex net of gene regulators. *In vivo* data have also underlined the role of staphylococcal protein A (Spa) as an important proinflammatory molecule in pneumonia (Gómez et al., 2004). Labandeira-Rey et al. (2007) have proposed that the increased production of Spa—associated with the ability of PVL to lyse polymorphonuclear leucocytes and macrophages—may result in enhanced cell recruitment, lysis, and the release of inflammatory mediators, leading to significant tissue inflammation and necrosis. The evolution of CA-MRSA is certainly a multifaceted process that originated primarily from genetic events that occurred occasionally during the evolutionary journey of some MRSA lineages. Despite recent advances in our understanding of the evolution of MRSA, the driving forces toward the selection of more fit (well-adapted) bacterial clones remain to be clarified (Figueiredo and Ferreira, 2014; Rasigade and Vandenesch, 2013).

The Agr (accessory gene regulator) effector molecule RNAIII is a negative regulator of a number of *S. aureus* MSCRAMMS (microbial surface components recognizing adhesive matrix molecules), including Spa and fibronectin binding proteins A and B (FnBPA and B). Conversely, RNAIII indirectly upregulates (via *rot*-inhibition) a number of extracellular proteins such as α-hemolysin (Hla), toxic-shock syndrome toxin I (TSST-I), and some proteases (Novick et al., 2000). In addition to Agr, various regulatory systems modulate the virulence of *S. aureus* (Cheung et al., 2004). The Staphylococcal accessory regulator A (SarA) family of transcriptional regulators include important components of the *S. aureus* virulence regulatory network. The SarA protein upregulates RNAIII expression and mediates the expression of different virulence factors in either an *agr*-dependent or −independent fashion. Studies by Montgomery et al. (2012) with a *codY* knockout derived from CA-MRSA strain 923 (USA300) have suggested that the transcriptional regulator CodY is involved in the negative regulation of *lukSF-PV*.

The two-component system (TCS) SaeRS is also an important *S. aureus* global virulence regulator that controls a number of virulence factors including hemolysins, leukocidins and proteases (Cue et al., 2015; Mrak et al., 2012). A *saeRS* knockout derived from USA300 had reduced *lukSF-PV* expression in comparison with the wild-type strain, indicating that the Sae TCS is a positive regulator of *lukSF-PV* (Montgomery et al., 2010). The *sae* operon is composed of four open reading frames (*saeP*, *saeQ*, *saeR*, and *saeS*), with the *saeS* gene encoding the histidine kinase sensor (SaeS), and *saeR* encoding the response regulator (SaeR). It has been proposed that *saePQ* encode two proteins that form a complex with SaeS, which activates the phosphatase domain of the sensor kinase (Jeong et al., 2012). In the presence of a signal (e.g. H_2O_2 and α-defensin) SaeS promotes the activation of SaeR by phosphorylation (Geiger et al., 2008). Two promoters, *sae*-P1 and *sae*-P3, coordinate the transcription of the operon (Adhikari and Novick, 2008). The *sae*-P3 promoter is active during the early-log phase and coordinates the transcription of *saeRS* (Novick and Jiang, 2003). The activity of the *sae* operon is autoregulated by phosphorylated SaeR (P-SaeR), which represses the transcription activity of the log-phase promoter, *sae*-P3, and activates the transcription of the stationary-phase promoter, *sae*-P1 (Geiger et al., 2008). The *sae*-P1 promoter, which is more efficient than *sae*-P3, is also activated by RNAIII and SarA and repressed by the regulators SigB and Rot (Geiger et al., 2008; Giraudo et al., 2003; Li and Cheung, 2008; Novick and Jiang, 2003).

The emergence of a PVL producer (PVL^+) CA-MRSA clone—called Oceania Southwest Pacific, Southwest Pacific or USA1100 (OSP/SWP/USA1100)—from the ST30-SCC*mec*IV lineage was first reported in Brazil in 2005 (Ribeiro et al., 2005). This clone was initially described in Australia in the 1990s (Udo et al., 1993), and it was later detected in San Francisco, CA, USA (Carleton et al., 2004). Currently, ST30-SCC*mec*IV isolates are spread worldwide (Figueiredo and Ferreira, 2014). The primary aim of this work was to investigate the mechanisms responsible for the differential expression of *lukSF*-PV observed in ST30 isolates. The resistance and virulence profiles of clinical isolates belonging to this CA-MRSA lineage were also characterized.

2. Materials and methods

2.1. Bacterial isolates, constructions, and plasmid

Twenty MRSA clinical isolates with the characteristics of CA-MRSA (presence of SCC*mec* IV and susceptibility to non-β lactam drugs) were included in this study. These isolates were collected between 2007 and 2010 from inpatients, outpatients, and emergency patients in a public general hospital in Rio de Janeiro, Brazil. The clinical data were unavailable for six isolates, but the data from the remaining fourteen are described in Table 1. The *S. aureus* identification was confirmed by the routine methods of Gram staining and catalase and coagulase tests. *Staphylococcus aureus* ATCC 25923 was used for quality control in the antimicrobial susceptibility tests, and the isolate WB69 (ST30-SCC*mec*IV; belonging to the