Abstract

ABSTRACT

Staphylococcus aureus establishes successful infection of host by a plethora of structural and secreted virulence factors that include cell wall associated proteins such as clumping factor A/B, Protein A, secreted toxins such as hemolysin, leukocidin, hyaluronidase, toxic shock syndrome toxin and enterotoxins acquired from several pathogenicity islands (SaPIs) and prophages. The expression of these multifactorial pathogenicity determinants are regulated and coordinated by complex interplay between several global regulator systems, transcriptional regulatory proteins as well as several small regulatory noncoding RNAs (ncRNAs). Although a huge number of ncRNAs have been reported in *S. aureu*s, only a few of them have been characterized for their role in regulatory networks. Several ncRNAs such as SprC, RsaA, SSR42, RsaE, SprA1 are reported to have major influence on pathogenecity of *S. aureus*. *Staphylococcus aureus* strain Newman has been extensively used in studying the staphylococcal disease in animal models due to its virulence phenotypes. The present study is aimed at investigating the functional role of selected small regulatory RNAs, expressed from staphylococcal pathogenicity island (SaPI) in the clinical isolate methicillin-susceptible *Staphylococcus aureus* Newman (MSSA) strain.

Several functionally uncharacterised ncRNAs encoded in the staphylococcal pathogenicity islands (SaPIs) of Methicillin Susceptible *Staphylococcus aureus* (MSSA) strain Newman were bioinformatically analysed for their putative regulation on virulent targets. Two ncRNAs SprX and SprB were selected for further study. These were characterised for their putative promoters and transcription terminators as well as multiple mRNA targets associated with virulent factors by *in silico* analyses. SprX that has been shown to influence antibiotic resistance previously, positively regulated the expression of virulence genes specially the cell wall associated clumping factor B and delta hemolysin in this study. Both overexpression and chromosomal disruption of *sprX1* agreed with the scheme of upregulation of ClfB and Hld. Altered expression of SprX1 altered the levels of Hld and ClfB mRNAs, clumping of cells and biofilm formation as observed by plate adhesion studies and confocal microscopic analysis. These results were corroborated by infection pathology of modified strains in mice models, which revealed increase in the level of pathophysiological markers such as BUN, CK, SGOT, SGPT and also multiple organ disintegration with high bacterial load. The expression of other bioinformatically

predicted targets immunoglobulin binding protein (*sbi*), staphylocoagulase (*coa*) and staphylokinase (*sak*) did not show correlation in their expression with respect to SprX1 levels in real time PCRs. Interaction of *clfB* and *hld* mRNAs directly with SprX1 was confirmed by *in vitro* RNA interaction studies. In contrast, another sRNA SprB, when overexpressed, reduced the expression levels of *clfB*, *clfA* and *coa* in this study. The increased SprB levels in *S. aureus* Newman displayed reduced biofilm formation and staphyloxanthin pigment production and showed increased resistance to beta-lactam antibiotics. However, SprB showed negligible impact on pathogenicity in mice model of infection. Taken together, these results give an insight into the role of the two noncoding RNAs, SprX and SprB in the pathogenicity of *S. aureus* strain Newman.