The present investigation

Chapter-2

2. THE PRESENT INVESTIGATION

2.1. Definition of the problem

Staphylococcus aureus, a versatile human pathogen has the ability to cause a wide variety of diseases, ranging from superficial abscesses and wound infections to life threatening deep and systemic infections such as osteomyelitis, endocarditis and septicaemia (Plata *et al.*, 2009). *S. aureus* is one of the main causes of hospital and community acquired infections and the success of this pathogen in establishing disease depends on a wide array of virulence factors. It is equipped with a great variety of multifactorial pathogenicity determinants including a large number of extracellular proteins such as cytolytic toxins (α, β, γ and δ- hemolysin), toxic shock syndrome toxin-I (TSST-1), enterotoxins, immunoglobulin binding protein A, coagulase and the bacterial surface components that include surface associated adhesions, capsular polysaccharides (Costa *et al.*, 2013) that are involved in the infection process. The multitude of gene expression is controlled by several global regulatory systems such as *agr, sarA, rot, mgrA, sae,* sigma B factor and small noncoding RNAs in a tightly coordinated manner that is synchronized with the biological cycle of *S. aureus* (Pragman *et al.*, 2004; Costa *et al.*, 2013; Caldelari *et al.*, 2013).

Regulatory RNAs are now recognized as important mediators in many physiological and adaptive responses in pathogenic bacteria. The switching on or off of pathogenic life style of *S. aureus* is determined by these regulatory factors. Small regulatory RNAs in bacteria have emerged as major elements in regulatory hierarchy of gene expression, mediating diverse biological events including bacterial quorum sensing, biofilm formation, oxidative stress, antibiotic resistance and most significantly the expression of virulence factors (Ortega *et al.*, 2014). The mechanism of regulation of ncRNA is linked with the modulation of transcription, mRNA stability, mRNA decay and translational activation or repression by base pairing with multiple mRNAs as targets or proteins (Argaman *et al.*, 2001, Wassarman *et al.*, 2001, Storz *et al.*, 2005).

Although, a burgeoning list of several hundreds of small RNAs in *S. aureus* have been identified in the last decade (Sassi *et al.*, 2015), only a few of them have been characterized for their regulatory functions. Moreover, much is known about the actions of these ncRNAs on various virulence regulators *in vitro*, fewer studies have been done

on the role of these regulators in *in vivo* models of infection. It is reported that the ncRNAs encoded in the staphylococcal pathogenicity islands (SaPIs) are involved in the regulation of staphylococcal virulence (Pichon and Felden, 2005; Felden *et al.,* 2011). For example, ncRNAs SprD (Chabelskaya *et al*., 2010), SprA1/ $SprA1_{AS}$ (Sayed *et al*., 2011), SprG1/F1 (Pinel-Marie *et al*., 2014) and SprC (Le Pabic *et al.,* 2015) expressed from the pathogenicity island of *S. aureus* have significant impact on the virulence of staphylococcus. Therefore, the current work aims to analyse the reported ncRNAs for regulating targets associated with pathogenecity by *in silico* prediction, selecting and characterising a few ncRNAs for their involvement of regulation of such targets expression under the modulated expression of ncRNAs and subsequent influence on animal model of infection.

Two small noncoding RNAs SprX and SprB, expressed from the pathogenicity islands in *S. aureus* Newman were selected for the present study which were expected to give an understanding on their regulation of virulence factors. Precise knowledge of these regulatory mechanisms and how they control virulence factor expression would open up new perspectives for combating *S. aureus* infections using small RNAs as anti-infective agents.

2.2. Objectives of the study

1. Bioinformatic analysis and characterisation of ncRNAs present in *Staphylococcus aureus* strain Newman.
2. Strain modification by altering the levels of selected ncRNAs by overexpression/ knockdown/ disruption.
3. Molecular and physiological analysis of expression of target virulent genes under the influence of altered levels of ncRNA.
4. The involvement of altered levels of ncRNAs in establishing *Staphylococcus aureus* pathogenecity in mice model of infection.

2.3. The present study

Methicillin Susceptible *Staphylococcus aureus* Newman (MSSA) strain, a clinical isolate was used as the model organism to functionally characterize the role of small noncoding RNAs in the regulation of pathogenicity factors *in vitro* and *in vivo.*

Computational characterization of a few uncharacterized ncRNAs namely SprA/B/C/D/E/F/G/X (Pichon and Felden, 2005; Bohn *et al.,* 2010) present in the pathogenicity island was done for finding putative promoter, transcriptional terminator and its potential virulence factors as targets using online softwares such as Softberry BPROM, Erpin and TargetRNA, TargetRNA2, RNApredator, IntaRNA, RNAup respectively. Bioinformatic analysis also included the determination of copy number, location and coordinates of ncRNA in the chromosome of Newman strain. Among the several ncRNAs screened, two small noncoding RNAs SprX1 and SprB were selected for the study for their functional significance. The altered expression of SprX1 and SprB was achieved by overexpression, knockdown and disruption.

The influence of altered levels of expression of ncRNA on candidate virulent targets such as clumping factor B (*clfB*), delta hemolysin (*hld*), immunoglobulin binding protein G (*sbi*), staphylocoagulase (*coa*), staphylokinase (*sak*) by SprX1 and clumping factor B (*clfB*), clumping factor A (*clfA*), staphylocoagulase (*coa*) by SprB was studied by molecular, physiological assays and their corroboration with animal model of infection. In addition to the transcriptional analysis of above genes and the studies of their interaction with the regulatory ncRNA, the phenotypic characteristics were measured that include hemolysis of RBC, biofilm formation, production of staphyloxanthin pigment and antibiotic susceptibility. Thus the present study aimed to investigate the functional role of small regulatory RNAs, SprX and SprB, in influencing the various phenotypes and pathobiology of *S. aureus* Newman.