2. PRESENT STUDY

Small RNAs have emerged as important regulators involved in fine tuning of various physiological processes in bacteria such as oxidative stress, central metabolism, antibiotic resistance, biofilm formation, and secretion of various virulence factors. The small RNAs of *S. aureus* reported in the involvement of regulation of virulence include, RNAIII, which control the expression of multiple virulence factors such as α-hemolysin, autolysins, staphylococcal protein A; SprD, which negatively regulates the expression of the immune-evasion molecule Sbi; RsaA that coordinates the expression of global regulator MgrA and acts as virulence suppressor, SprC, which attenuates bacterial virulence and host cell phagocytosis and SprX, which is involved in the regulation of virulence factors as mentioned below.

The present study is focused on the small RNA SprX (Small pathogenicity island RNA X), 156 nucleotide long sRNA in *S. aureus*. Enhanced expression of SprX has been reported in different environments encountered during an infection such as high salt and temperature conditions and cell wall antimicrobials. Additionally, overexpression of SprX1 enhanced the pathogenicity and severity of virulence of *S. aureus* Newman in mice model of infection with multiple abscesses in kidneys, lungs, heart, and liver, and increased bacterial load in previous reports, which suggested its significance in the regulation of *S. aureus* virulence. However, at the time of undertaking the present study only a few targets (sporulation protein SpoVG, δ-hemolysin, and clumping factor B) of SprX were reported, and studied in detail. Therefore, the present study was aimed to identify the potential targets of SprX and analyze their role in virulence regulation.

The objective of the study

1. To study the influence of ncRNA or sRNA SprX on protein expression of *S. aureus* and selection of differentially regulated proteins.
2. Analysis of the influence of SprX on transcription, functional expression of selected targets, and the associated physiology.
3. Assessment of direct / specific interaction of SprX with selected target mRNA

The work presented here has identified *isaA*, a potential target identified using 2D-proteomics and its regulator *walR* mRNAs as new targets of sRNA SprX. This study shows that SprX regulates the autolysin expression by directly interacting with the mRNA

of the autolysin regulator WalR. The effect of altered levels of SprX on autolysins and WalR was examined by transcriptional analyses, physiological assays and *in vitro* SprX-mRNA interactions. The findings presented herein highlight a new mechanism in which SprX modulates the *S. aureus* pathogenicity by regulating the regulator of autolysins in cell wall metabolism and as virulence factors.